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Vascular Smooth Muscle Growth: Autocrine Growth Mechanisms

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I. Introduction

Vascular smooth muscle cells (VSMC) are among the most plastic of all cells in their ability to respond to different growth factors. Specifically, VSMC may proliferate (hyperplasia with an increase in cell number), hypertrophy (an increase in cell size without change in DNA content), endoreplication (an increase in DNA content and usually size), and undergo apoptosis. Among the mechanisms utilized by VSMC to mediate these varying cellular responses are autocrine and paracrine growth pathways. An autocrine growth mechanism is one in which the individual cell, in response to a growth factor, synthesizes and/or secretes a substance that stimulates the same cell type to undergo a growth response. A paracrine growth mechanism is one in which the individual cells responding to the growth factor synthesize and/or secrete a substance that stimulates neighboring cells of another cell type.

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V. Conclusions: Angiotensin II-Mediated Events as a Paradigm for Autocrine Growth Mechanisms

Berk, Bradford C. Vascular Smooth Muscle Growth: Autocrine Growth Mechanisms. Physiol Rev 81: 999–1030, 2001.—Vascular smooth muscle cells (VSMC) exhibit several growth responses to agonists that regulate their function, including proliferation (hyperplasia with an increase in cell number), hypertrophy (an increase in cell size without change in DNA content), endoreplication (an increase in DNA content and usually size), and apoptosis. Both autocrine growth mechanisms (in which the individual cell synthesizes and/or secretes a substance that stimulates the same cell type to undergo a growth response) and paracrine growth mechanisms (in which the individual cells responding to the growth factor synthesize and/or secrete a substance that stimulates neighboring cells of another cell type) are important in VSMC growth. In this review I discuss the autocrine and paracrine growth factors important for VSMC growth in culture and in vessels. Four mechanisms by which individual agonists signal are described: direct effects of agonists on their receptors, transactivation of tyrosine kinase-coupled receptors, generation of reactive oxygen species, and induction/secretion of other growth and survival factors. Additional growth effects mediated by changes in cell matrix are discussed. The temporal and spatial coordination of these events are shown to modulate the environment in which other growth factors initiate cell cycle events. Finally, the heterogeneous nature of VSMC developmental origin provides another level of complexity in VSMC growth mechanisms.
cells of another cell type. In many situations, autocrine and paracrine growth mechanisms occur simultaneously. It is very difficult to separate these pathways in vivo, so in this review the focus is on VSMC autocrine growth mechanisms. When there is solid evidence for interactions between autocrine and paracrine mechanisms, an effort will be made to delineate the separate and specific roles.

The concept of VSMC auto/paracrine growth was first proposed in the late 1970s as a result of work in the laboratories of Gospodarowicz et al. (101), Harker and Ross (120), Karnovsky and co-workers (41), and Chanley-Campbell et al. (43). Dzau (70) and Nilsson et al. (230) were the first to use the term autocrine growth to describe increased expression of VSMC growth factors by VSMC. It has now become clear that almost all VSMC growth factors elicit auto/paracrine growth pathways. However, recent data indicate that many other stimuli that modulate VSMC function including extracellular matrix, biomechanical forces, reactive oxygen species (ROS), lipids, and other proteins alter VSMC growth by inducing auto/paracrine growth mechanisms. The major questions that will be addressed in this review are as follows: 1) Why do VSMC utilize auto/paracrine growth mechanisms? 2) Why are so many growth factors induced by a single stimulus (in other words, what is the reason for redundant growth mechanisms)? To answer these questions the following issues are addressed below. First, the physiological processes that require VSMC growth are discussed to provide insight into how these differing situations may have influenced the development of autocrine growth. The concept to be advanced is that temporal, spatial, and pathophysiological specific situations have mandated a coordinated and complex series of growth responses. Second, the “plastic” nature of VSMC growth is presented to illustrate the diversity of these responses. The concept to be discussed is that there is a correlation between the multiple auto/paracrine growth mechanisms, the presence of VSMC heterogeneity, and the varied nature of VSMC growth responses. Third, the individual growth factors that have been identified as mediating auto/paracrine growth are discussed. Fourth, the stimuli that elicit the synthesis and/or release of these factors are presented. Finally, an integrated analysis of the autocrine mechanisms utilized by angiotensin II are discussed as a model that places the relative roles of different factors into a pathophysiologically important context.

II. PHYSIOLOGICAL PROCESSES THAT REQUIRE VASCULAR SMOOTH MUSCLE CELL GROWTH

The concept to be developed in this section is that temporal, spatial, and pathophysiological specific situations have mandated a coordinated and complex series of VSMC growth responses. The ability of VSMC to be plastic in their growth responses is a key mechanism by which the vasculature responds to hemodynamic, developmental, and injurious stimuli. Fundamental to our understanding of the role of auto/paracrine growth mechanisms in VSMC growth is relating VSMC growth to important biological functions of the blood vessel. Examples of biological processes during which VSMC would be expected to grow include vessel development, the vascular response to tissue injury, and vessel remodeling in response to changes in tissue demand. Pathological examples include atherosclerosis, hypertension, restenosis postangioplasty, and vasculitis. In all situations it is clear that interactions between endothelial cells and VSMC as well as between VSMC and other cells (e.g., fibroblasts, dendritic cells, and inflammatory cells) within the vessel wall determine the nature of the growth response.

A. Development

The process of vessel development, growth, and remodeling provides important insights into mechanisms that regulate vessel function and VSMC growth in the adult. The process of blood vessel formation in the embryo is termed angiogenesis, which involves the differentiation of angioblasts into endothelial cells (EC) that assemble into a primitive vascular network. Subsequently, growth and remodeling of the network occurs, a process termed angiogenesis. In the adult, three processes can be used to form new vessels: vasculogenesis (rarely), angiogenesis, and arteriogenesis. Arteriogenesis has frequently been termed collateral vessel growth and refers to enlargement of small arterioles into larger vessels. Because these processes have been extensively reviewed (40), this section focuses primarily on VSMC developmental features.

The first cell responsible for the formation of the primordial blood vessel tube is the EC (130). Once the primitive EC tubes are formed, the endothelium secretes factors that lead to the recruitment and/or induction of primordial smooth muscle, a process termed vascular myogenesis. Several recent reviews have carefully documented the current state of knowledge regarding the differentiation and growth of VSMC to form the tunica media (40, 130). This may occur by 1) angiopoietin-1-mediated production of VSMC inducing factor(s) by EC that causes differentiation from the mesoderm; 2) an autocrine mechanism in which angiopoietin-1 causes EC to differentiate into VSMC (transdifferentiation) (61) as well as transdifferentiation from bone marrow precursors or macrophages; 3) transformation of epicardial cells to form the coronary VSMC (130, 328); and 4) differentiation of the mesoectoderm of the neural crest into VSMC (18, 264). It is
important to note that VSMC have a complex origin depending on their location. For example, VSMC of coronary veins are derived from atrial myocardium while VSMC of coronary arteries are derived from epicardium (62). This suggests that individual growth factors and their receptors will have different effects on VSMC growth and differentiation in specific vascular beds. This is an important caveat for many of the discussions of autocrine VSMC growth below.

The process by which VSMC contribute to vessel formation may be divided into three components: differentiation, recruitment and growth, and remodeling. Differentiation of VSMC involves transcriptional events mediated by the serum response factor, Prg-1 and Prg-2, CRP2/SmlIM, and members of the HOX, MEF2, and GATA family. Factors that stimulate VSMC from mesoderm have been best studied, and candidate mediators include platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β (98, 130). Factors that act as chemoattractants for VSMC include PDGF-BB and epidermal growth factor (EGF). Studies in mice lacking PDGF-BB and PDGFR-β (124) suggest that PDGFR-β expressing VSMC progenitors form around certain vessels by a process independent of PDGF-BB. These cells then undergo angiogenic sprouting and vessel enlargement in a process that is both PDGF-BB dependent and independent depending on tissue context. The nature of the growth factors secreted by embryonic EC that stimulate VSMC proliferation remain to be identified. In addition, it is possible that VSMC themselves, upon interacting with embryonic EC, activate auto/paracrine pathways that lead to VSMC hyperplasia. Important roles for TGF-β1 and endoglin (an endothelial TGF-β binding protein) have been established in that they stimulate VSMC differentiation and extracellular matrix deposition and strengthen EC-VSMC interactions (63, 177). Endothelin (ET)-1 appears to have an important role in migration and differentiation of VSMC from neural crest cells (342). Other growth factors with important roles in differentiation and growth include tissue factor, heparin binding EGF-like factor (HBEGF), and the Eph-Ephrin system. Remodeling during development involves transcription, growth factors, and physical forces. Aortic arch abnormalities as an example of defective remodeling have been demonstrated in knockout mice that include MFH-1, dHand or Msx1, pax-3, P1x, retinoic receptors, the neurofibromatosis type-p1 gene product, Wnt-1, connexin 43, and ET-1. Finally, physical forces, notably the initiation of blood flow, may have important effects to stimulate the primitive vessel to remodel especially via regulation of nitric oxide production. In summary, it is clear that multiple transcriptional and growth factor-related events participate in the process by which VSMC create the vascular media; many of these same processes occur in the adult during arteriogenesis and angiogenesis.

B. Injury

Perhaps the best studied situation in which VSMC growth occurs is after injury to the blood vessel. While the rat carotid balloon injury model has been investigated extensively for many years (49), the pattern of events that leads to vessel repair and intimal thickening appears similar in other species (pig, mouse, nonhuman primate, and human) and other arteries (aorta, iliac, femoral, and brachial). Many candidate molecules that regulate VSMC growth have been studied in the rat carotid injury model by use of pharmacological and gene therapy approaches. Results suggest important roles for the renin-angiotensin system, catecholamines, ET-1, natriuretic peptides, thrombin, PDGF, TGF-β and other activins (242), fibroblast growth factor (FGF), and oxidative stress among other stimuli (100, 163). Recent results with transgenic knockout mice provide further support for these molecules as regulators of VSMC growth after injury as well as nitric oxide (269) and the estrogen receptor (134). Despite this long history, the exact origin of the cell type that leads to formation of the neointima (dedifferentiated VSMC, VSMC progenitor cell, or myofibroblast) remains unknown. The mechanisms by which VSMC growth is halted and cell number regulated remain unclear. Much progress has been made in mechanisms of VSMC apoptosis, but how the size of blood vessels and the media in particular are regulated remains to be defined. Finally, it is important to note that both autocrine and paracrine growth mechanisms are essential for formation of the neointima. In this review emphasis is on autocrine VSMC growth mechanisms, acknowledging important paracrine contributions from endothelial cells, monocyte/macrophages, fibroblasts, dendritic cells, and polymorphonuclear leukocytes.

C. Remodeling

Vascular remodeling (Fig. 1) is a physiological response to alterations in flow, pressure, and atherosclerosis. Remodeling involves changes in VSMC growth and migration as well as alterations in vessel matrix (214). Remodeling may be classified as proposed by Mulvany based on the nature of changes in vessel diameter (inward or outward) and by changes in mass (increased = hypertrophic, decreased = atrophic, no change = eutrophic) (214). As an example “eutrophic outward” remodeling would be an increase in lumen diameter without change in amount or characteristics of the vessel such as may occur with increased flow and atherosclerosis. In contrast, “hypertrophic inward” remodeling would be defined as a decrease in lumen diameter with increased wall thickness such as may occur with increased pressure. It has been best studied in resistance vessels during hyper-
tension. During chronic hypertension, there is an increase in vessel wall thickness hypothesized to normalize wall stress. Physical forces (wall stress and cell stretch), autocrine growth mechanisms, and paracrine growth mechanisms (EC actions on VSMC) stimulated by the hypertensive environment appear causative.

In response to changes in blood flow, remodeling appears to be fundamentally dependent on the presence of an intact endothelium as shown by Langille and co-workers (173, 174) and by Kohler et al. (155). Because flow-induced remodeling would be expected teleologically to be mediated by changes in vessel tone and hence diameter, candidate mediators are vasoactive molecules. Among these, nitric oxide [produced by endothelial nitric oxide synthase (eNOS)] appears to play a predominant role. Recent studies show that ~70% of flow-dependent outward remodeling is due to EC nitric oxide production as determined by inhibiting production of nitric oxide with eNOS inhibitors (317). During inward remodeling in response to decreased flow, there is a coordination of increased VSMC apoptosis and decreased VSMC proliferation to effect the decrease in vessel wall mass that occurs (47).

An important role for monocytes has been elucidated in remodeling, especially in response to ischemia such as occurs after occlusion of a supply artery (277). In response to increases in flow, EC express monocyte chemotactic peptide-1 (MCP-1) and monocyte adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1). The monocytes are recruited to the vessel and infiltrate and digest the media. The EC are activated by monocytes and express basic FGF (bFGF), PDGF-BB, and TGF-β. These growth factors then lead to VSMC growth and vessel enlargement.

In response to increased pressure, remodeling appears to be due to activation of autocrine mechanisms that stimulate VSMC growth and changes in vessel wall matrix (123, 213, 215). As discussed in greater detail in section vi, many VSMC growth factors have been implicated in the growth and remodeling of hypertensive vessels including PDGF (227, 274), TGF-β, insulin-like growth factor I (IGF-I) and the IGF-I binding proteins (7), and hepatocyte growth factor (221). Paracrine mechanisms that are important in hypertension include increased production of ET-1 and angiotensin II by the endothelium.

III. DIFFERENT TYPES OF VASCULAR SMOOTH MUSCLE CELL GROWTH

The concept to be discussed is that there is a correlation between the multiple autocrine growth mechanisms, the presence of VSMC heterogeneity, and the diverse nature of VSMC growth responses. VSMC, like other mesenchymal cells, differentiate and then exist in a G0 growth-arrested state. In general, VSMC have resembled most other cell types in the mechanisms for cell cycle entry, progression, and arrest. Several recent reviews have discussed the mechanisms by which VSMC exit the G0 state and enter the cell cycle (33). The reader is referred to these reviews for further information.

A. VSMC Heterogeneity

Although many investigators assume that smooth muscle cells in the vessel wall are morphologically similar, it has become clear that they are phenotypically and functionally heterogeneous, which has obvious conse-
quences for responses to various growth factors. A basic question is whether this is due to differences in origin or to spatiotemporal heterogeneity in expression of differentiation markers due to local environmental and hormonal factors. As discussed below, both developmental and environmental factors influence VSMC heterogeneity.

It is important to note that while the medial layer of the vessel is highly enriched in VSMC, other cell types may coexist in this layer. This has important implications since migration and growth of medial cells to form a neointima is an important pathological process in atherosclerosis and restenosis. By implication, not all cells that are present in the neointima may be VSMC. For example, Frid et al. (84) were able to isolate at least four phenotype unique cell subpopulations from the inner, middle, and outer compartments of the arterial media. Differences in cell phenotype were demonstrated by morphological appearance and by differential expression of muscle-specific proteins. The isolated cell subpopulations exhibited markedly different growth capabilities. Two SMC subpopulations grew slowly in 10% serum and were quiescent in plasma-based medium. The other two cell subpopulations, exhibiting nonmuscle characteristics, grew rapidly in 10% serum and proliferated in plasma-based medium. These differences in growth were subsequently related to production of autocrine growth factors (85). Similar VSMC heterogeneity was observed for human VSMC (17). Two morphological phenotypes of VSMC are usually defined, namely, the epithelioid and the spindle-shaped cell (29). Functionally these phenotypes have been suggested to correlate with the synthetic and contractile cell types, respectively (43). Contractile VSMC express high levels of contractile proteins including myosin and low levels of α-actin. In contrast, synthetic VSMC express high levels of α-actin, extracellular matrix proteins, and low levels of myosin. In general, the spindle-shaped, contractile VSMC are not proliferating or migrating, whereas the epithelioid, synthetic VSMC have entered the cell cycle and are proliferating. How do these different cell populations coexist and maintain their specificity?

The first answer is that developmentally the vessel media layer includes cells of different embryonic origins and/or VSMC stem cells may differentiate into cells with varying morphology and function. In addition, other cell types such as the adventitial cell, the SMC stem cell, the myofibroblast, and the dendritic cell may participate in formation of the vessel wall and even assume VSMC-like appearances. Examples of the role of cell origin in terms of growth properties are shown by the differences in TGF-β responses of neural crest-derived VSMC compared with mesoderm-derived VSMC (312, 313). Treatment of VSMC derived from neural crest (ectoderm) with TGF-β increased DNA synthesis, whereas treatment of cultures of VSMC derived from mesenchyme (mesoderm) inhibited DNA synthesis (313). Thus autocrine growth responses will be highly dependent on the nature of VSMC heterogeneity. Based on techniques used for cell isolation and growth, there may be enrichment of particular subpopulations of VSMC that may explain some of the different results that have been reported for in vitro studies of autocrine growth mechanisms.

With the identification of genes whose expression is specific for VSMC (thereby enabling localization in situ by mRNA or protein detection), it has become clear that upon development of intimal thickening (e.g., during atherosclerosis, restenosis, or closure of the ductus arteriosus), there is reexpression of fetal genes. These findings suggest that there is significant plasticity in VSMC function. There may also be embryonic cells ("progenitors") left from development (285) similar to those isolated from fetal animals. For example, Schwartz and colleagues (191) have shown that proliferating smooth muscle cells isolated from the aorta express unique cytochrome P-450 enzymes that are typical of embryonic smooth muscle cells. Also, the myofibroblast has been proposed to transdifferentiate into an endothelial-like cell as well as into synthetic phenotype VSMC during intimal thickening (292, 333). Finally, there is increasing evidence that differentiated cells can transdifferentiate into other cell types (61). With the use of DNA microarrays (278) it will be possible to more accurately assign the origin of cell types found in the vessel wall.

Second, heterogeneity within the vessel wall may be related to alterations in the local environment. To take three examples: 1) variations in the hemodynamic environments may modify local gradients in substances (e.g., increased residence time of lipids) or local metabolic requirements (e.g., increased energy metabolism or altered cytoskeleton arrangements) (54, 55, 171). The normal blood flow pattern may be described as pulsatile and laminar. This ensures that fluid shear stress (the dragging frictional force of blood on the vessel luminal surface) is maintained within the narrow range of 10–20 dyn/cm². When the blood flow pattern is no longer laminar, it may be described as turbulent, and as a consequence pulsatility may be lost resulting in oscillatory flow patterns. Intimal proliferation occurs most commonly in these areas of turbulent and oscillatory flow such as the human carotid bulb. One explanation for intimal proliferation at these sites is related to alterations in EC-derived factors; specifically, there may be a decrease in factors that inhibit VSMC growth and an increase in factors that stimulate VSMC growth (316). Another explanation is that bloodborne factors are better able to influence VSMC in these regions of disturbed flow. This has been shown to be the situation for low-density lipoproteins, which show increased accumulation in these regions (60, 346). 2) Variation in matrix composition may be important, as illustrated by the fact that fibronectin is thought to be growth promoting and laminin growth inhibiting (128, 273, 306).
There are clear interactions between matrix and the ability of VSMC to respond to growth factors (336). Specifically, Wilson et al. (336) showed that mechanical strain increased VSMC DNA synthesis when cells were grown on collagen, fibronectin, or vitronectin, but not on elastin or laminin. When strain was applied on matrices containing both laminin and vitronectin, the mitogenic response to strain depended on the vitronectin content of the matrix. In addition, the assembly of the matrix molecule determines the nature and magnitude of the growth response. For example, VSMC are arrested in the G1 phase of the cell cycle on polymerized type I collagen fibrils, while monomer collagen supports smooth muscle cell proliferation (165). Monovalent blocking antibodies to α2-integrins, integrins that mediate adhesion to both forms of collagen, mimic these effects on monomer collagen. In addition, the cdk2 inhibitors p27Kip1 and p21Cip1/Waf1 are increased in cells grown on polymerized collagen compared with monomer collagen. Thus fibrillar collagen specifically regulates early integrin signaling that may lead to upregulation of cdk2 inhibitors and inhibition of SMC proliferation. 3) Variations in physical forces at a particular site as a consequence of vessel architecture and flow pattern may modulate VSMC function (99, 172, 346). While fluid shear stress is likely to be the major force that influences EC function, mechanical strain may be more important for VSMC. Changes in mechanical strain have been shown to induce many VSMC growth factors including PDGF, bFGF, IGF-I, and TGF-β (37, 46, 129, 178, 293, 299, 335, 337, 348). In addition, mechanical strain may make VSMC more sensitive to the mitogenic actions of other factors such as angiotensin II (299). In conclusion, VSMC heterogeneity is a fundamental feature of the vessel wall. This heterogeneity is a consequence of both developmental and environmental factors.

B. Hyperplasia

Hyperplasia as used in this review refers to an increase in VSMC cell number associated with DNA synthesis. As discussed above, entry of VSMC into the cell cycle and proliferation appears to be governed by many of the same mechanisms common to all cells. It is important to note that VSMC hyperplasia occurs in response both to agonists that stimulate G protein-coupled receptors and those that stimulate tyrosine kinase-coupled receptors. Hyperplasia is an important component of hypertension as shown by a significant increase in smooth muscle cell proliferation rate and the number of cell layers in the media of vessels from animals with chronic hypertension (115, 175, 237). It should be noted that hyperplasia is characteristic of intermediate and large arterioles, whereas small vessels undergo remodeling. Hyperplasia also occurs in many other vascular diseases including atherosclerosis, restenosis, and the response to vascular injury. Hyperplasia is a slow process in chronic human hypertension. Normal rat aortic smooth muscle cell growth is 0.01%/day (310) In hypertensive models, this increases to a maximum of 1%/day. Simple calculations indicate that if this rate persisted, an arteriole 30 μm in diameter would occlude in 40 days, based on a medial thickness of 20 μm and cell diameter of 5 μm. These calculations suggest several scenarios: 1) only a certain percentage of cells may be able to replicate (smooth muscle cell heterogeneity); 2) there must be only brief periods of proliferation; and 3) cell growth is inhibited or cells undergo death (necrosis and/or apoptosis). In fact, all three scenarios are likely to occur to varying extents in vivo depending on the nature of the stimulus and the vascular bed. In summary, VSMC proliferation is a common response to mechanical stress and injury.  

C. Hypertrophy

Similar to cardiac myocytes and skeletal muscle myocytes, VSMC share the ability to undergo hypertrophy. Hypertrophy is a particularly “valuable” growth response because it is reversible (by unknown mechanisms). In this review hypertrophy will refer to increases in smooth muscle cell size whether there is DNA synthesis (endoreduplication) or not. Hypertrophy with increased DNA content (endoreduplication) is a common feature of hypertension that has been little studied (27, 176, 238). However, the dominant VSMC hypertrophic mechanism is one in which the cell enlarges without change in DNA content. Increases in cell volume are a consequence both of increased intracellular protein and intracellular water. Increased protein content occurs both by stimulation of protein synthesis and inhibition of protein degradation (25, 95). However, changes in the membrane proteins that regulate transmembrane movement of ions and water are critical to maintain increased cell volume. Thus hypertrophic stimuli such as angiotensin II also increase protein expression of specific molecules such as the Na+/K+-ATPase and the Na+/K+2Cl− cotransporter (170, 318), but not the Na+/H+ exchanger (259). Hypertrophy is a reversible mechanism when unaccompanied by endoreduplication. However, when DNA synthesis occurs, the change in cell size is probably irreversible. In general, hypertrophy has been a property relatively unique to agonists that stimulate G protein-coupled receptors (with the exception of TGF-β), rather than tyrosine kinase-coupled receptors (22, 25, 94, 95, 97, 169, 239, 240, 320, 322, 345). However, it should be noted that in many situations, G protein-coupled receptor agonists (e.g., angiotensin II) stimulate hypertrophy by autocrine growth mechanisms that involve tyrosine kinase-coupled receptor agonists (e.g., PDGF) (22). One potential common pathway may be
generation of ROS by a membrane NAD(P)H oxidase that has been shown to be stimulated by VSMC agonists and associated with VSMC hypertrophy (104, 320, 322, 345). Another mechanism may be the ability of G protein agonists to activate tyrosine kinase receptors by direct intracellular mechanisms, a process called transactivation. Examples include angiotensin II and thrombin-mediated tyrosine phosphorylation of the EGF and PDGF receptors (19, 137, 184, 186, 210, 347). In conclusion, hypertrophy is a relatively unique property of muscle that confers significant advantages in terms of physiological responses because of its reversible nature.

D. Antiapoptotic Effects

In addition to stimulating cell proliferation, most growth factors are also able to prevent cell death by activating survival pathways. This ability will be referred to as antiapoptotic effects in this review because most studies have focused on morphological and biochemical characterization of apoptotic cell death. Because of the complexity of apoptotic mechanisms in VSMC, this review only addresses several general issues. Although it is clear that many of the same growth factors that stimulate hypertrophy and hyperplasia are also antiapoptotic, two important points should be noted. First, the mechanisms for their antiapoptotic effects differ from their growth effects. Recent data from our laboratory investigating the functional domains of the angiotensin receptor (AT1R) highlight these differences (110). Second, there are many factors that may stimulate both antiapoptotic and proapoptotic autocrine pathways [e.g., Gas6-Axl, ROS, and atrial natriuretic peptide (ANP)-ANP receptor]. Finally, many autocrine mechanisms that prevent VSMC apoptosis involve secretion of factors such as bFGF (83), heat shock proteins, and cyclophilins (181). Thus multiple autocrine mechanisms mediate the antiapoptotic effects observed in VSMC.

IV. AUTOCRINE GROWTH FACTORS AND RECEPTORS

In the discussion below, factors that have been shown to be induced and participate in VSMC autocrine growth pathways are discussed alphabetically based on their mechanism of action.

A. Secreted Factors Coupled to Tyrosine Kinase Receptors

Many molecules act both as stimuli for autocrine growth mechanisms and as growth factors themselves. Therefore, to organize the presentation of autocrine growth mechanisms, the discussion focuses first on the factors that act as stimuli of autocrine growth mechanisms. Then the role of these same molecules as mediators (regardless of which stimuli induce expression of the factor) is presented. For each autocrine factor, the discussion includes a description of the ligand and its receptor, known interactions with other growth factors, and primary functional role based on in vitro and in vivo studies (hyperplasia, hypertrophy, migration, and antiapoptosis). Finally, an integrated model for VSMC autocrine growth is detailed based on angiotensin II-stimulated growth effects.

1. EGF-like proteins and receptors: epiregulin and HBEGF with ErbB1-ErbB4

EGF and the EGF receptor (EGF-R) are members of a large family of homologous peptides and relatively specific receptors. The six known EGF receptor ligands include EGF, TGF-α, amphiregulin, HBEGF, betacellulin, and epiregulin, as well as the related molecule heregulin-α. These peptides bind with varying affinity to the EGF receptor-related receptor tyrosine kinases [ErbB1 through 4 (also termed HER1–4), approximate molecular weight of 180 kDa]. Among these molecules, the ones that have been shown to stimulate VSMC growth include EGF (20), epiregulin (308), HBEGF (66, 226, 243), and the ErbB1 (EGF-R) and ErbB4 receptors (208). To date, no role for EGF as an autocrine stimulus has been identified (137), so the discussion will focus on epiregulin and HBEGF.

Epiregulin was originally purified from conditioned medium of the mouse fibroblast-derived tumor cell line NIH3T3/clone T7, suggesting a role as an endogenous autocrine factor (315). In conditioned medium, epiregulin was present as a 46-amino acid single-chain polypeptide. Epiregulin has 24–50% amino acid sequence identity with sequences of other EGF-related growth factors. Epiregulin exhibited bifunctional regulatory properties; it inhibited the growth of several epithelial tumor cells and stimulated the growth of fibroblasts and various other types of cells (315). When the human epiregulin gene was cloned, it was found to encode a 163-residue putative transmembrane precursor containing an EGF-like domain in the internal segment, and the structural organization was similar to that of other members of the EGF family that bind to EGF receptors. Expression of full-length epiregulin in mammalian cells showed that human epiregulin was secreted as a soluble form of ~5 kDa that was biologically active on the basis of the stimulation of DNA synthesis (314). Recent studies suggest that proteolysis of the precursor may be mediated by members of the matrix metalloprotease family (MMPs) (251). Epiregulin directly bound to both the EGF-R (ErbB-1) and ErbB-4, but not to ErbB-2 and ErbB-3 (162). Epiregulin has been shown to
have the broadest binding properties of any EGF-like ligand so far characterized; not only does it stimulate homodimers of both ErbB-1 and ErbB-4, but it also activates all possible heterodimeric ErbB complexes (290). Despite having an affinity that is ~100-fold lower than the affinity of ligands with more stringent selectivity (including EGF), epiregulin is a more potent mitogen than EGF. This discrepancy between binding affinity and bioactivity was related to decreased EGF-R downregulation, which resulted in a weak, but prolonged, state of receptor activation (290).

Epiregulin was first shown to be involved as a mediator of autocrine VSMC growth when it was isolated as a major mitogenic protein present in angiotensin II-stimulated rat aortic smooth muscle cell conditioned medium (308). Angiotensin II, as well as ET-1 and thrombin, stimulated expression of two epiregulin transcripts (the known 4.8-kb transcript and a novel transcript of ~1.2 kb). Recombinant rat epiregulin was strongly mitogenic for rat aortic VSMC, stimulating DNA synthesis to levels similar to those induced by serum and approximately threefold above that observed with saturating concentrations of EGF. To date, there are no data regarding expression of epiregulin in vascular injury or atherosclerosis.

HBEGF is a 22-kDa heparin-binding member of the EGF family that was initially identified in the conditioned medium of human macrophages as a potent mitogen and chemotactic factor for fibroblasts and smooth muscle cells (but not endothelial cells) (126). Like epiregulin, soluble mature HBEGF is proteolytically processed from a larger membrane-anchored precursor (251). HBEGF activates two EGF receptor subtypes, Erb-1 and Erb-4, and binds to cell surface heparan sulfate proteoglycans. Interestingly, the receptor for HBEGF is also the receptor for diphtheria toxin. Recent data discussed below suggest that proteolytic processing of HBEGF by MMPs is highly regulated by G protein receptor-coupled agonists and may explain transactivation of tyrosine kinase receptors by peptides such as angiotensin II and thrombin (251). HBEGF gene expression is highly regulated, for example, by cytokines, growth factors, and transcription factors such as MyoD. HBEGF is a far more potent mitogen for VSMC than is EGF, suggesting that it is a particularly important autocrine factor.

HBEGF is typical of many autocrine factors in that it regulates its own expression; HBEGF mRNA levels were shown to increase ~10-fold by addition of HBEGF itself (66). HBEGF is induced by thrombin (224, 309), ROS (150), bFGF (260), and PDGF (66). All these factors are present during vascular injury, suggesting that HBEGF is important in the proliferative response of VSMC to arterial injury. Northern blot analysis showed that the transcript levels of HBEGF increased ~12-fold within 2 h after balloon injury and remained 3-fold elevated at 14 days. In situ hybridization analysis demonstrated that the transcript of HBEGF remained strongly expressed in the neointima, especially near the luminal surface, at 14 days after injury (135). A role for HBEGF in human atherosclerosis has been suggested on the basis of immunohistochemical localization in human coronary arteries obtained at autopsy (226). In atherosclerotic plaques of coronary arteries with eccentric intimal thickening, both VSMC and macrophages in and around the core lesions, in addition to the intimal and medial VSMC located adjacent to the plaque, produced HBEGF protein. Strong immunostaining for EGF receptors was observed in these VSMC, suggesting a close association of HBEGF and EGF receptor expression (226). However, another study failed to show HBEGF localized to VSMC in atherosclerosis (260). Although HBEGF mRNA was detected in all atherosclerotic tissue examined, RT-PCR, in situ hybridization, and immunohistochemistry revealed expression of HBEGF only in small portions of diseased arteries that colocalized with macrophages (260). In conclusion, HBEGF is a potent mitogen for VSMC that is highly regulated by both induction of expression and processing to an active form. The relative expression of HBEGF by macrophages and VSMC in human pathogenic conditions remains to be established.

2. FGF (HBGF2)/FGF-R

The acidic fibroblast growth factor (aFGF) and bFGF proteins are potent VSMC mitogens that are expressed by endothelial cells and VSMC, respectively. Thus bFGF plays an autocrine growth role and aFGF a paracrine growth role for VSMC. A role for bFGF was first reported by Maciag’s group (338) who termed the protein HBGF for heparin binding growth factor because of its strong affinity for heparin sepharose (338). There are now four human bFGF isoforms (24, 22, 21, and 18 kDa), three of which are targeted to the nucleus (24, 22, and 21 kDa). Although the intracellular isoforms may play a role in autocrine growth, the 18-kDa isoform is the only secreted isoform and will be the focus of this discussion. Among the four major types of FGFR (110–130 kDa), FGF receptor 1 is the major form of FGF receptor mRNA expressed by proliferating human arterial VSMC (340). Both FGFR-1 and FGFR-2 mRNAs have been found to be present in VSMC of vessels. There is uncertainty regarding the role of bFGF in vivo as a VSMC mitogen (34). In human atherosclerotic plaques, bFGF appears to be present at low levels and does not exhibit differences in the level of expression compared with normal vessels. In contrast, aFGF mRNA was detected by Northern blot in 100% of atherosclerotic samples tested, but only 20% of control arteries were positive for aFGF. Immunohistochemistry showed high expression of aFGF in plaques especially in neovascularized and macrophage-rich regions of plaque. Thus, in human atherosclerosis, bFGF has an unclear role...
in VSMC growth. In rat vessels bFGF also does not appear to be an important mediator of the vessel response to injury based on studies with anti-bFGF antibody (236). However, the FGFR-1 receptor was reported to be induced by vascular injury (204), suggesting that increased receptor number may be important. Alternatively, intracellular bFGF may mediate autocrine growth effects not detectable by immunohistochemistry and neutralizing antibody techniques.

Many factors have been shown to stimulate bFGF expression including bFGF itself (2). Recently, it has become clear that secretion of endogenous FGF is required to prevent apoptosis of VSMC (83, 206). One of the first reports that demonstrated autocrine bFGF expression was the induction of bFGF in human saphenous vein VSMC by interleukin (IL)-1α and IL-1β (92). Other factors reported to stimulate bFGF expression and release include angiotensin II (140), ET-1 (244), HBEGF (243), and oxidized low-density lipoprotein (42). Of great interest is the ability of mechanical forces to stimulate FGF-dependent VSMC growth. Transient compression (5-min duration) of VSMC grown in a three-dimensional collagen gel system led to delayed 3.3-fold increases in [3H]thymidine incorporation. Serum-free media conditioned by transiently compressed gel cultures induced DNA synthesis in control, unstimulated VSMC, suggesting the release of growth factors by transient compression. Neutralizing antibodies against bFGF, but not against PDGF, significantly inhibited DNA synthesis caused by media conditioned by transiently compressed gels. These data suggest that bFGF is an important mediator of VSMC growth induced by mechanical forces (46). However, in a cyclic strain model, no release of bFGF was observed (335), suggesting model-dependent effects. In summary, bFGF is a potent autocrine VSMC mitogen that mediates effects both by secretion of an 18-kDa isoform and by intranuclear accumulation of three other isoforms. Future work will be required to determine the importance of intranuclear FGF in vivo.

3. Gas6/Axl

Gas6 is a 75-kDa secreted protein that bears significant homology at the amino acid level to Protein S, a negative regulator of the coagulation cascade. Gas6, which encodes the protein (growth arrest-specific gene 6), was named by virtue of the initial finding that the gene is highly expressed in growth-arrested cells (195, 280). The receptor for Gas6 is a 140-kDa transmembrane tyrosine kinase named Axl (also called UFO, ARK, and Tyro7) originally identified as a transforming gene in human leukemias (143, 232). Gas6 was first shown to have effects on VSMC when Nakano et al. (223) identified it as a factor released from VSMC. After extensive investigation, the regulator(s) of Gas6 expression in VSMC has yet to be identified (M. G. Melaragno and B. C. Berk, unpublished data). In cultured VSMC, only the G protein-coupled receptor agonists angiotensin II and thrombin caused significant upregulation of Axl mRNA and protein (202). However, both Axl and Gas6 are significantly up-regulated in the rat carotid balloon injury model. Northern blotting for Gas6 revealed increased mRNA expression beginning between 6 h and 3 days after injury, with continued elevation for at least 4 wk (202). Immunohistochemistry indicated that Gas6 protein levels were increased in the vessel wall as early as 24 h after injury with highest expression in the most luminal medial VSMC and adventitia at early time points, and in the neointima at later times (Melaragno and Berk, unpublished observations; Ref. 202). Axl expression followed a different time course with significant increases only at the 7- and 14-day time points. The results of these studies established that expression of Axl and Gas6 is increased in the injured rat carotid, with a time course paralleling that of neointima formation.

Two studies using vascular cells point to a role for Gas6/Axl in limiting apoptosis. Nakano et al. (223), who used only very high concentrations of Gas6, reported prevention of serum deprivation-induced cell death in rat VSMC, while O’Donnell et al. (233) found that a much lower amount of Gas6 rescued human umbilical vein endothelial cells from serum deprivation- and tumor necrosis factor-α-induced apoptosis. The role of Axl/Gas6 as a mitogen for VSMC remains unresolved. Gas6 alone is not mitogenic and was only mitogenic when added to cells simultaneously with G protein-coupled receptor agonists (223). In fact, other investigators have found no effect of Gas6 on proliferation even in the presence of other agonists (86). Thus, based on the available evidence, it appears likely that Gas6/Axl acts as an autocrine mediator primarily by inhibiting apoptosis of VSMC.

4. Hepatocyte growth factor (also termed scatter factor) and c-met

Hepatocyte growth factor (HGF) is a mitogen and angiogenesis factor (263) that is identical to scatter factor, a fibroblast-derived cytokine characterized by its ability to convert nonmotile epithelial cells to a motile fibroblast-like phenotype. HGF is a heterodimeric glycoprotein that is homologous to plasminogen and other blood coagulation proteases but lacks proteolytic activity. Its receptor is the c-met protooncogene product, a 140-kDa tyrosine kinase-coupled transmembrane receptor. HGF is synthesized and secreted by both VSMC and EC, but appears to act primarily on EC. HGF is broadly angiogenic as it stimulates migration, protease production, invasion, proliferation, and differentiation into capillary-like tubes in vitro (220). Specifically, exogenously added human recombinant HGF stimulated EC growth, but not VSMC...
growth in a dose-dependent manner (221). However, HGF has been proposed to stimulate VSMC migration after vascular injury (8). Interestingly, both angiotensin II and TGF-β may act as negative regulators of HGF expression (222). Results from this study suggest that downregulation of the local vascular HGF system by TGF-β and angiotensin II may play a role in the pathogenesis of cardiovascular diseases especially after arterial injury. Thus HGF may be part of an autocrine/paracrine system in which stimulation of VSMC by agonists such as angiotensin II inhibits HGF production, thereby decreasing EC growth and migration, resulting in increased intimal VSMC proliferation (222).

5. IGF-I and IGF-R

IGF-I is a 12-kDa peptide hormone secreted by multiple cells that has been shown to mediate autocrine, paracrine, and endocrine growth. The biological effects of IGF-I are regulated by a series of IGF binding proteins (IGFBPs) that determine the ability of IGF-I to interact with its physiological receptors which include both the IGF-R (130 kDa) and the insulin receptor itself. Among the IGF binding proteins, IGFBP-3, IGFBP-4, and IGFBP-5 are the most important. IGFBP-3 is the main circulating carrier of IGF-I, IGFBP-4 is the main IGFBP produced by VSMC in vitro, and IGFBP-5 is highly regulated by VSMC factors including IGF-I. All three components of the IGF-I, IGFBP, and IGF-R system are highly regulated.

IGF-I is regulated positively in VSMC by IGF-I and insulin and negatively by serum, bFGF, and PDGF-BB (30). IGF-I is also regulated by ROS as shown by increased IGF-I mRNA in response to xanthine/xanthine oxidase and H₂O₂. Importantly, xanthine/xanthine oxidase- and H₂O₂-stimulated DNA synthesis was completely inhibited by a neutralizing anti-IGF-I antibody (58). IGF-I secretion from VSMC is stimulated by cyclic stretch (1 Hz at 120% resting length for 48 h). The 40% increase in thymidine incorporation seen in stretched cells was completely blocked upon addition of anti-IGF-I antibody, demonstrating an essential role in VSMC proliferation (293).

The IGFBPs can act as both positive and negative regulators of the growth effects of IGF-I. When added together with IGF-I, exogenous IGFBP-4 inhibited IGF-I-induced DNA synthesis in a concentration-dependent manner. IGFBP-5, on the other hand, potentiated the effect of IGF-I. Treatment of VSMC with exogenous IGF-I increases IGFBP-5 mRNA levels selectively (68). Therefore, IGFBP-4 and IGFBP-5 appear to be differentially regulated by autocrine IGF-I through distinct mechanisms (68). These two proteins, in turn, play opposing roles in modulating IGF-I action in stimulating VSMC proliferation (68). Less is known regarding the effect of changes in circulating IGFBP-3 on VSMC growth.

Multiple hormones including PDGF, bFGF, and angiotensin II have been shown to stimulate IGF-I receptor expression (326). While exposure of quiescent VSMC to all three growth factors caused a 1.5- to 2.0-fold increase in IGF-I receptors per cell, only FGF treatment caused a marked increase in the mitogenic response to IGF-I, suggesting that bFGF is the primary regulator of IGF-I receptors. There are few data available that address the role of IGF-I interactions with the insulin receptor on VSMC growth.

The strong in vitro data for a role of IGF-I in VSMC growth are supported by several in vivo studies. After arterial injury, there is increased IGF-I expression, although it appears to be primarily in endothelial cells (118). However, after rat carotid artery denudation, semi-quantitative PCR analysis demonstrated a significant elevation of IGF-I concomitantly with the induction of VSMC proliferation and intimal thickening (122). Administration of a d-analog of IGF-I that is biologically inactive reduced intimal VSMC replication by 60–70%. These results suggest that IGF-I/IGF-I receptor interaction is a rate-limiting step for smooth muscle cell replication after vessel injury (122). There are few data for the role of IGF-I in atherosclerosis. However, a potential role in hypertension is suggested by the findings that IGFBP-4 mRNA levels rapidly increased in the hypertensive aorta (7), and cyclic stretch stimulated IGF-I production (293), suggesting an important role for IGF-I in hypertension.

6. PDGF and PDGF-R

PDGF exists as a dimer composed of two homologous but distinct peptides termed PDGF-A (17 kDa) and PDGF-B (14 kDa) chains and may exist as AA, AB, and BB dimers. Two related PDGF receptors also exist termed PDGF-Rα (170 kDa) and PDGF-Rβ (190 kDa). VSMC express both PDGF-A and PDGF-B chains in a growth- and hormone-dependent manner. One of the earliest reports (230) suggested that autocrine production of PDGF by VSMC explained the transition from nonproliferating contractile phenotype to the proliferating synthetic phenotype in culture. In the intact aortic media, where the cells are in a contractile phenotype, only minute amounts of PDGF-A and no PDGF-B mRNA were detected (291). Upon placement in tissue culture, and modulation of the cells into a synthetic phenotype, PDGF-A gene was expressed, whereas PDGF-B gene remained unexpressed. Cells kept in serum-free medium on a substrate of plasma fibronectin showed high levels of PDGF-A mRNA and high PDGF receptor activity, but did not secrete detectable amounts of PDGF-like mitogen. After exposure to PDGF, which is itself sufficient to initiate DNA synthesis and mitosis in these cells, a PDGF-like mitogen was released into the extracellular medium, suggesting that PDGF stimulates its own expression and/or release (291). More recent work has demonstrated additional complex-
ity of these initial observations. Another mechanism for PDGF autocrine growth effects is PDGF-dependent synthesis and secretion of thrombospondin, a glycoprotein component of the VSMC extracellular matrix in vitro (188). Although thrombospondin is not itself a mitogen, coadministration of thrombospondin and EGF synergistically stimulate DNA (188). Finally, PDGF has been reported to stimulate expression of ET-1 (113) and HBEGF (66, 150).

Stimulation of PDGF expression is among the most common autocrine VSMC growth mechanisms (265, 267). Autocrine growth events include changes in both PDGF-A and PDGF-B chain and PDGF-R expression. One of the earliest and best examples of this complex regulation was described for the autocrine growth effects of TGF-β (13). TGF-β autocrine growth effects are discussed comprehensively below. While TGF-β acts primarily as a VSMC growth inhibitor, it can stimulate proliferation at low concentrations (1–2 fg/cell of TGF-β) in a PDGF-dependent manner. At low concentrations of TGF-β, there was a 12-h delay in DNA synthesis compared with that elicited by PDGF, suggesting synthesis and/or release of a growth factor. In fact, PDGF-A was detected in the culture medium at 24 h, and anti-PDGF IgG blocked DNA synthesis. At higher concentrations, TGF-β decreased expression of PDGF-Rα subunits. Hence, TGF-β induces VSMC proliferation at low concentrations by stimulating autocrine PDGF-A secretion, while at higher concentrations of TGF-β, growth is decreased by downregulation of PDGF-Rα (and perhaps by direct growth inhibition). Other growth stimuli that stimulate synthesis and release of PDGF-A chains include angiotensin II (1, 22, 140, 330), ET-1, FGF (37), IL-6 (136), oxidized low-density lipoprotein (294, 349), thrombin (224, 298), TGF-β, and uric acid (257). PDGF-A chain also appears to be released from VSMC exposed to cyclic mechanical stretch (335). In conclusion, PDGF-A and PDGF-B are among the most prevalent and frequently induced VSMC mitogens.

PDGF has been suggested to play a role both in VSMC migration and proliferation after vascular injury (31, 79, 80, 266). With the use of a rat specific PDGF-B cDNA, it was found that only a distinct population of luminal VSMC (7–10%) in the developing neointima after balloon injury expressed PDGF-B mRNA. Very few luminal VSMC still expressed PDGF-B (0.5%) when the lesion had stopped growing (183). Primary VSMC cultures revealed expression of PDGF-B mRNA in 1.6% of VSMC derived from normal media and in 11% of VSMC derived from the neointima. These data demonstrate that VSMC in the injured vessel wall are heterogeneous with regard to PDGF-B expression and that subculturing of these cells will give rise to cultures that exhibit varying PDGF-B expression. In a balloon overstretch injury of porcine coronary arteries, PDGF was suggested to play a role in proliferation of adventitial cells that had morphology consistent with myocytes and hence termed “myofibroblasts” (286). Immunohistochemistry showed that 3 days after injury, a large number of proliferating cells were located in the adventitia, with significantly fewer positive cells found in the media and lumen. Seven days after injury, proliferating cells were found primarily in the neointima, extending along the luminal surface. In situ hybridization for PDGF-A-chain and PDGF-β-receptor mRNAs showed significant expression, which closely correlated with the sites of proliferation at each time point (286). In atherosclerosis, PDGF appears particularly important for proliferation of macrophages (268).

A role for PDGF in vascular assembly and remodeling has been suggested (93, 154, 167, 168, 209). A number of factors associated with normal and pathological artery wall remodeling are induced by shear stress in endothelial cells including PDGF (205). The effects of reduced blood flow on endothelial cell PDGF expression and proliferation in the rat carotid artery were studied after carotid ligation (209). PDGF-B expression increased in the endothelium of the reduced flow artery within 48 h and persisted at 72 h. PDGF-A expression was similarly increased in the reduced flow endothelium. In contrast, expression of PDGF-Rα and PDGF-Rβ was undetectable in the endothelium at all times. On the basis of these findings, it appears that endothelial cell PDGF ligand expression is induced by reduced shear stress in vivo and may play an important role in flow-mediated remodeling and atherogenesis. In summary, PDGF is a critical autocrine and paracrine factor for VSMC mediating hyperplasia, hypertrophy, and remodeling.

B. Secreted Factors Coupled to G Protein-Coupled Receptors

1. Angiotensin II

Angiotensin II is an eight-amino acid peptide (1 kDa) that binds to a family of receptors that include both the angiotensin type 1 receptor (AT₁R, 60 kDa) and type II receptor (AT₂R, 60 kDa). Both receptors play a role in VSMC growth, although they differ markedly in their effects, with AT₁R being associated with proliferation, hypertrophy, and antia apoptotic effects, whereas the AT₂R is associated with proapoptotic effects (218, 247, 341). Angiotensin II is one of the most studied autocrine growth factors for several reasons; perhaps most prominent is that it exhibits all the complexities present in growth regulation. Specifically, there is strong evidence for tissue-specific regulation of angiotensin II production, the receptors for angiotensin II, the intracellular signal transduction mechanisms, and stimulation of other autocrine growth mechanisms by angiotensin II including both pro-
duction of growth factors and transactivation of growth factor receptors (97, 140, 247).

It has become clear that the many components of the renin-angiotensin system are present within the vessel wall and can function as an autocrine growth mechanism for VSMC (71). For example, angiotensinogen mRNA is present in the endothelium, medial VSMC, and periendothelial fat of normal rat arteries (217), suggesting that several cell types in the vessel can synthesize angiotensinogen (72). After balloon injury, the ratio of medial to adventitial angiotensinogen mRNA increases, implying increased production of this angiotensin II precursor in the media. Our laboratory showed that VSMC can express angiotensin converting enzyme (ACE) and that ACE expression was dramatically upregulated by injury in an FGF-dependent manner (81). The presence of renin in the vessel wall is controversial; although several studies have shown renin activity in vessels (70, 194, 327), it is not clear whether the renin is synthesized locally or taken up from the circulation. Nonetheless, renin present in the vessel wall can cleave angiotensinogen to angiotensin I. ACE then generates the vasoconstrictor and growth factor angiotensin II. Furthermore, because ACE is highly regulated by multiple factors (53) including bFGF (81), increased expression of ACE by endothelial and smooth muscle cells may increase the amounts of angiotensin II present locally in the vessel wall.

The many factors whose expression is regulated by angiotensin II include the following: ET-1 (113), PDGF-A and PDGF-B (1, 22, 140, 330), bFGF (140), epiregulin (308), IL-6 (116), IGF-I (59), TGF-β, HBEGF (150, 309), and activin A (208, 242).

Angiotensin II has been reported to stimulate hyperplasia, hypertrophy, and both proapoptotic and antiapoptotic effects on VSMC (105). The magnitude and types of growth factors induced by angiotensin II determine the relative balance of these growth effects. It has been suggested that angiotensin II exerts its growth effects in part through stimulation of PDGF-A mRNA and protein production (21, 114). This has been supported most strongly by experiments in which transfection into VSMC of antisense PDGF-A oligomers inhibited angiotensin II-stimulated protein synthesis by more than 50% (141, 156). Angiotensin II also induces TGF-β mRNA in VSMC. Gibbons and colleagues (141, 156) observed that, in the presence of a neutralizing antibody to TGF-β, angiotensin II stimulated DNA synthesis and cell division of VSMC from normotenive rats. Based on this finding they hypothesized that angiotensin II was a bifunctional growth factor. Angiotensin II stimulated hyperplasia when PDGF-A was the dominant growth factor expressed, whereas angiotensin II stimulated cell hypertrophy when TGF-β was dominant (141, 156). Buhler’s group (112) obtained similar findings regarding PDGF-A and TGF-β induction by angiotensin II in cultured VSMC from the SHR. However, other investigators (295) have found that angiotensin II induction of TGF-β was associated with enhanced PDGF-stimulated mitogenesis. Although most investigators agree that PDGF-A is a weak mitogen for VSMC by itself, it appears to be critical to the hypertrophic response stimulated by angiotensin II in normotensive VSMC (22). In addition, bFGF plays a role in angiotensin II-stimulated growth as shown by antisense FGF oligonucleotides (140). Recently changes in cell redox state mediated by NAD(P)H oxidase were shown to play an important role in angiotensin II-stimulated hypertrophy, since antisense to the p22 phox subunit of the oxidase prevented the increase in protein synthesis (106, 320). Another hypertrophic mechanism that will be discussed below is the transactivation of tyrosine kinase-coupled receptors (e.g., EGF-R and PDGF-R) by G protein receptor-coupled agonists such as angiotensin II, ET-1, and thrombin (19, 137, 184, 186, 210, 347).

Angiotensin II exerts powerful cell survival signals via the AT1R including activation of the kinase Akt (also termed protein kinase B) (247, 300). When angiotensin II binds to the AT2R it stimulates proapoptotic effects by unknown mechanisms, although the MAP kinase phosphatase-1 may be involved (341). By transfecting an AT2R expression vector into the balloon-injured rat carotid artery, Nakajima et al. (218) observed that overexpression of the AT2R attenuated neointimal formation. In cultured VSMC, AT2R transfection reduced proliferation and inhibited mitogen-activated protein kinase activity. These results suggest that the AT2R exerts an antiproliferative effect, counteracting the growth action of AT1R. Gibbons and colleagues (247) have suggested that the antagonistic balance between vasoactive substances such as nitric oxide and angiotensin II regulates the control of VSMC apoptosis. They postulated that the cellular signaling pathways involved in regulating vessel tone are also coupled to the regulation of programmed cell death. In cultured VSMC, these investigators showed that addition of nitric oxide donor molecules dose-dependently induced apoptosis. A critical role for the guanylate cyclase signaling pathway in nitric oxide-induced apoptosis was established. In contrast, angiotensin II directly antagonized nitric oxide donor- and cGMP analog-induced apoptosis via activation of the AT1R.

An important role for angiotensin II in vivo is suggested by the effects of ACE inhibitors (249) and AT1R blockers (323) on neointimal formation after balloon injury of the rat carotid. In addition, Griffin et al. (107) showed that the vascular effects of angiotensin II were independent of changes in pressure and reflected a direct change in VSMC growth. More recently, data have accumulated that suggest a role for angiotensin II in atherosclerosis. Epidemiologically, higher renin levels are associated with increased frequency of cardiovascular events. The HOPE study showed that chronic administration of
the ACE inhibitor ramipril significantly decreased cardiovascular events (344). In experimental animals, inhibiting ACE resulted in reduction in the extent of atherosclerosis including apoE −/− mice (121), hamsters (164), cholesterol-fed rabbits (38, 283), WHHL rabbits (48), and non-human primates (207). The extent to which these proatherosclerotic effects of angiotensin II are related to autocrine effects on VSMC, as opposed to proapoptotic effects on EC (65) and/or proinflammatory effects (4, 103, 105, 106, 111), remains to be defined. In summary, angiotensin II is a powerful VSMC growth factor that has been shown to mediate hypertrophy, hyperplasia, and both proapoptotic and antiapoptotic effects on VSMC.

2. Catecholamines (norepinephrine) and adrenergic receptors

Classic vasoconstrictors such as catecholamines are potent VSMC mitogens in certain settings (28, 219, 253). Norepinephrine is synthesized by the adrenal medulla, and also may be released from local sympathetic nerve terminals, indicating that it normally acts in an endocrine and/or paracrine manner. It has been shown that angiotensin II potentiates release of norepinephrine from sympathetic nerves (194, 199). Norepinephrine binds to the α1-adrenergic receptor (60 kDa) that is highly expressed in VSMC. In vitro, it has been shown that norepinephrine stimulates both VSMC endoreplication (253) and hyperplasia (28). In cardiomyotid models, Majesky et al. (190) showed that α1-adrenergic stimulation caused PDGF-A expression. The importance of this finding was emphasized by the discovery that α1-adrenergic receptor blockade with prazosin inhibited balloon injury-induced VSMC proliferation (324). On the basis of these findings, it appears that norepinephrine is part of an autocrine growth loop that involves angiotensin II and PDGF-A.

Luttrell et al. (187) have found evidence that α-adrenergic receptors transactivate the EGF-R. Stimulation of the G1-coupled α2A-adrenergic receptors in transfected COS cells was shown to stimulate tyrosine phosphorylation of the Shc adapter protein. Shc then associated with activated phosphorylated proteins of ~130 and 180 kDa, as well as Grb2. The 180-kDa Shc-associated tyrosine phosphoprotein band was found to contain both the EGF-R and p185 (neu). EGF-R, but not p185 (neu), showed a three- to fivefold increase in tyrosine phosphorylation after α2A-receptor stimulation.

3. Calcitonin gene-related peptide family

Calcitonin gene-related peptides (CGRPs) together with calcitonin, amylin, and adrenomedullin are members of a supergene family. CGRPs are peptides (parent molecule is CGRP1–37) that act as vasodilators by binding to CGRP receptors, two of which have been cloned; CGRP-1 receptor (56 kDa) and CGRP-2 receptor (56 kDa) (16). The CGRP receptors are seven transmembrane spanning G protein-coupled receptors. Adrenomedullin is a 52-amino acid vasorelaxant peptide that was originally isolated from human pheochromocytoma (152). Adrenomedullin has 24% amino acid homology with CGRP and is synthesized and secreted by both endothelial cells (302, 303) and VSMC (303).

Because the CGRP family promotes vasodilation via increases in cAMP and cGMP, it is likely that these molecules will inhibit VSMC growth (and/or promote VSMC apoptosis) while they will prevent apoptosis in endothelial cells (149). Adrenomedullin probably inhibits proliferation of VSMC by increasing cAMP (12, 146). A current working model for these peptides is that CGRP-(8–37), a truncated version of CGRP, acts as an adrenomedullin receptor antagonist since both peptides bind to the adrenomedullin and CGRP-1 receptors (12, 16, 73, 148). This model suggests that the relative balance of CGRP peptides, adrenomedullin, and the nature of receptor expression will determine the effect of CGRP family members on vascular structure. In endothelial cells, adrenomedullin suppresses serum deprivation-induced apoptosis via a cAMP-independent mechanism (149). Similar to other vasoactive G protein-coupled receptors, CGRP induced time- and concentration-dependent increases in Shc tyrosine phosphorylation, Shc-Grb2 association, and ERK1/2 phosphorylation (assayed in a HEK 293 cell line that stably expresses the rabbit calcitonin receptor C1a isoform) (45). In conclusion, these data suggest that coupling of CGRP agonists to their receptors stimulates many of the same signals as other G protein-coupled receptors; however, the stimulation of cAMP or cGMP is associated with inhibition of VSMC growth. To date, no autocrine growth factors have been shown to be synthesized or released in response to CGRP.

4. ET-1 and ET receptors

ETs are a family of peptides with potent biological properties (200). Endothelial cells produce exclusively ET-1 (a 21-amino acid peptide, ~2.2 kDa) while other tissues produce ET-2 and ET-3. ET-1 is similar to angiotensin II in that it is regulated at the level of prohormone synthesis and proteolysis, receptor level and isoform expression, and intracellular signal transduction. ET-1 is among the most potent vasoconstrictors (343). Pro-ET-1 is acted on by a furinlike enzyme to generate big ET-1, a 38-amino acid peptide, which is converted to the mature 21-amino acid peptide ET-1 by ET-converting enzyme (ECE) in endothelial cells, both intracellularly and on the cell membrane, and on the surface of underlying smooth muscle cells. (76). ET-1 is primarily produced by endothelial cells (343) and is downregulated by fluid shear stress (193, 289). VSMC may also produce ET-1 (262). The levels of pro-ET are regulated in VSMC by TGF-β, PDGF-A chain
Two mammalian ET receptors have been cloned: ET\textsubscript{A}-R (45–50 kDa) and ET\textsubscript{B}-R (45–50 kDa). All ET receptors are seven transmembrane spanning G protein-coupled receptors. In vascular tissue, ET\textsubscript{A} receptors are expressed on VSMC and responsible for vasoconstriction. ET\textsubscript{B} receptors are expressed on endothelium and linked to nitric oxide and prostacyclin release. Activation of these receptors explains the transient vasodilation observed with intraluminal application of ET. The role of the recently cloned ET\textsubscript{C} receptor in the vasculature is still uncertain.

ET-1 regulates expression of many factors including epiregulin (308), bFGF (244), PDGF-A chain (112), and TGF-\beta. Similar to angiotensin II, ET-1 causes a delayed mitogenesis of cultured VSMC that is related to the accumulation of growth factors such as epiregulin and TGF-\beta.

Addition of exogenous ET-1 may promote VSMC hyperplasia (127, 331) or hypertrophy. ET-1 is among the most potent VSMC mitogens with half-maximal stimulation of DNA synthesis occurring at 2 \times 10^{-10} M (127). However, increased secretion of endogenous ET-1 is associated with VSMC proliferation (3). Specifically, several VSMC lines expressing variable levels of ET-1 mRNA and biologically active ET-1 were used to show that the transfected VSMC line secreting the highest level of ET-1 had an enhanced growth rate when compared with untransfected or vector-alone transfected cells. The growth rate of this VSMC line was significantly reduced when the ET\textsubscript{A} receptor subtype-selective antagonist BQ-123 was included in the culture medium (3). ET-1 has also been proposed to play a pathogenic role in hypertension (67) and atherosclerosis (279). In summary, ET is an important VSMC mitogen that shares many properties with angiotensin II.

5. Thrombin

Thrombin is the VSMC mitogen “par excellence” involved in the response to injury. Thrombin (33.5 kDa) is generated by proteolytic cleavage of prothrombin activated during platelet activation at the sites of vascular damage. A single G protein-coupled thrombin receptor (55 kDa) has been identified in VSMC. The thrombin receptor is a member of an expanding family of receptors whose activation is dependent on proteolytic cleavage (PAR for protease-activated receptor) initiated by binding of the ligand (52). Prothrombin is synthesized by the liver cells, and no regulation of its expression by VSMC has been demonstrated. However, the thrombin receptor is highly regulated in vitro and in vivo by multiple factors (39, 228, 334).

Like angiotensin II and ET-1, thrombin regulates expression of multiple hormones as recently reviewed (297). Increased expression of PDGF A-chain (224, 298), HBEGF (224), epiregulin (308), bFGF (297), TGF-\beta, and activin A (242) have all been observed. Interestingly, thrombin dose-dependently decreased IGF-I mRNA levels and caused a delayed decrease in IGF-I secretion from VSMC (57). In contrast, thrombin doubled IGF-I receptor density on VSMC, and an anti-IGF-I antiserum markedly reduced thrombin-induced DNA synthesis, demonstrating that a functional IGF and IGF-I receptor pathway is essential for thrombin-induced mitogenic signaling (57).

Thrombin has been shown to stimulate VSMC hyperplasia (201) and hypertrophy (23, 24) and is likely anti-apoptotic. In addition, thrombin stimulates a delayed increase in DNA synthesis that is likely mediated by secretion of factors such as PDGF-A, epiregulin, and Gas6 (224, 298, 308).

An important role for thrombin in vivo has been suggested by the dramatic changes in receptor expression observed in atherosclerosis, vessel injury, and hypertension (39, 228, 334). In normal-appearing arteries, thrombin receptor was expressed almost exclusively in the endothelial layer (228). In contrast, in human atherosclerotic plaques, the receptor was widely expressed both in regions rich in macrophages and in regions rich in vascular smooth muscle cells and mesenchymal-appearing intimal cells of unknown origin. Thrombin receptor was expressed by human vascular endothelial cells and VSMC in culture and by macrophages obtained by bronchioalveolar lavage, thus demonstrating that all three cell types are indeed capable of expressing the thrombin receptor. Thrombin receptor mRNA was not detected in normal rat arteries by in situ hybridization and immunohistochemistry. In contrast, balloon injury increased thrombin mRNA expression in medial VSMC within 6 h (334). This increased thrombin receptor expression continued within the media and in neointimal cells throughout vascular lesion formation, predominantly in areas of active cell proliferation. Finally, in angiotensin II-mediated hypertension, there was an 11-fold increase in expression, which correlated with a 4-fold increase in thrombin-induced constriction in isolated endothelium-denuded aortic rings (39). In summary, thrombin is a powerful mitogen and hypertrophic factor for VSMC whose regulation is exquisitely controlled by the coagulation pathway.

C. Secreted Factors Coupled to Other Receptors

1. ILs

Both IL-1 (17.5 kDa) and IL-6 (20.5 kDa) have been reported to have autocrine growth effects on VSMC (136, 254). Cellular effects of interleukins are also regulated by levels of endogenous inhibitors of the IL-1 receptor (15) and by processing of the IL-1 precursor to mature hormone. The growth effects of the interleukins are some-
what controversial because other investigators have observed that IL-1 inhibited VSMC growth (51). However, cell lines constitutively expressing IL-1α precursor demonstrated metabolism to the mature peptide and increased growth (14).

Levels of IL-1 are regulated primarily by inflammatory cytokines such as TNF-α, which induces IL-1 mRNA in human endothelial cells and VSMC (182). IL-1 can also induce its own expression (14) and is upregulated by TGF-β and by hypoxia (50). Recently, the mechanism by which IL-1β is produced by VSMC has been elucidated (282). VSMC express the IL-1β precursor upon stimulation and the IL-1β-converting enzyme (ICE) constitutively, but do not produce mature IL-1β or express ICE activity. Libby and colleagues (282) showed that CD40 ligand, a mediator recently localized in human atherosclerotic plaques, increased IL-1β precursor as well as activated cell-associated ICE. In addition to the constitutively expressed 45- and 30-kDa immunoreactive ICE proteins, VSMC incubated with recombinant human CD40 ligand demonstrated increased expression of a 20-kDa immunoreactive ICE protein, and generation of an IL-1β precursor cleavage product of 17 kDa. These results suggest that binding of CD40 ligand to its receptor (CD40) is an important component in the generation of active IL-1β in vivo.

IL-1 has been reported to stimulate expression of PDGF-A chain (254), bFGF (92), and as described above IL-1 itself, while IL-6 induces PDGF-A chain (136). Other autocrine factors induced by IL-1 and IL-6 remain to be identified.

In vivo, both IL-1 and IL-6 would be anticipated to show increased expression in atherosclerosis and in injured vessels. The strongest data for a proatherogenic role of IL-1 have been presented for transplant atherosclerosis (272). Cytokines such as IL-1 and TNF-α have been proposed as primary mediators of the inflammatory component of atherosclerosis (182, 267) and can regulate the production of MCP-1, a potential signal for directed migration of monocytes into the intima. Cytokines can also regulate genes that encode other growth factors and cytokines themselves. TNF-α can induce IL-1 mRNA in human endothelial cells and VSMC. IL-1 and TNF-α can augment the production by vascular cells of macrophage colony stimulating factor, which may promote growth and activation of mononuclear phagocytes. Because these activated macrophages are powerful producers of ROS, this process may generate additional VSMC autocrine growth mechanisms.

More recently, another autocrine mechanism for IL-6 has been proposed that involves the release of 60-kDa heat shock protein (HSP60) from apoptotic VSMC. Libby and co-workers (158) found that either human or chlamydial HSP60 stimulated production of IL-6 from human VSMC. These results suggest a plausible mechanism by which chronic bacterial infection may lead to inflammatory activation of VSMC within the vessel wall.

2. Natriuretic peptides and the natriuretic receptors

The natriuretic peptides are vasodilators and inhibitors of VSMC growth because they increase intracellular cGMP levels by stimulating particulate guanylate cyclase. There are three natriuretic peptides and their cognate receptors: A type or ANP, which is produced by atrial myocardium; B type or BNP, which is produced mainly by the myocardium and also found in brain; and C type or CNP, which is produced by endothelial cells (160). The three atrial natriuretic peptide receptors that have been described are the A receptor, which binds ANP and BNP and contains intrinsic guanylate cyclase activity; the B receptor, which is structurally related to the A receptor but is activated by CNP; and the C or “clearance” receptor, which has no intrinsic cyclase activity and appears to be involved in clearance of circulating forms of natriuretic peptides (160, 225, 301). Autocrine production of both ANP and CNP has been demonstrated by RT-PCR (132).

ANP is a vasodilator and inhibits growth of cultured VSMC (211, 225, 301). In addition, ANP prevents the hypertrophy of cultured VSMC stimulated by angiotensin II and TGF-β. Because ANP activates guanylate cyclase, cGMP levels rise. As discussed earlier for nitric oxide, elevations in guanylate cyclase appear to be growth inhibitory, suggesting that ANP exerts its antiproliferative effects by increasing guanylate cyclase activity (91). When VSMC are placed in culture they rapidly lose guanylate cyclase activity, which may be one form of loss of growth inhibition. Because the intact vessel expresses ANP mRNA (90), it is possible that the family of atrial natriuretic peptides may be a local autocrine growth-regulating system analogous to the renin-angiotensin system. The potential importance of this system in hypertension is suggested by the demonstration that long-term infusion of low concentrations of ANP in the spontaneously hypertensive rat (SHR) (insufficient to lower blood pressure) decreased carotid artery media thickness and also inhibited VSMC hypertrophy (endoreduplication) as measured by nuclear size (212). BNP appears to mediate similar intracellular signal events as ANP.

It has been demonstrated that the normally low level of endothelial cell CNP expression is dramatically increased by TGF-β. Receptors for CNP have been demonstrated in both cultured VSMC (301) and aorta (161). Activation by CNP increases cGMP, suggesting a hormonally activated receptor that is functionally coupled. In vitro growth inhibition studies show that CNP may be more potent than ANP at inhibiting VSMC proliferation (248). In vivo CNP appears to be highly regulated by vessel injury (30). CNP was detected immunohistochemically in neointimal but not medial VSMC. No other natri-
uretic peptides were detected immunohistochemically. CNP transcripts were identified by RT-PCR in carotid segments that had been stripped of endothelium, but only once neointima had formed. Moreover, neointima expressed the C-type natriuretic peptide receptor at the same time as it synthesized CNP. Thus neointima develops an autocrine system for CNP that could regulate neointimal growth (36).

In summary, the natriuretic peptides may act as an autocrine growth inhibitor to “counterbalance” the growth-promoting effects of other G protein-coupled receptor agonists present at sites of vascular injury.

3. TGF-β and activins (ALK-R)

TGF-β is the prototypic member of a large family of structurally related proteins. Three vertebrate TGF-β isoforms have been identified and termed TGF-β1, TGF-β2, and TGF-β3. In addition, two receptors of the serine/threonine kinase family termed type I (also called ALK-5) and type II have also been identified. At least six TGF-like receptors (termed ALK1–6) have been discovered. VSMC express ALK-2, ALK-3, ALK-5, and ALK-6 based on RT-PCR (242). Among ligands for these receptors, VSMC autocrine growth effects have been proposed for TGF-β and activin.

Because TGF-β is synthesized and secreted in a latent form, storage of this latent molecule by matrix-bound receptors such as decorin (271) and activation of the latent molecule by proteases such as plasmin are critical regulatory steps. Most cell types express TGF-β as a large latent TGF-β complex that must be converted to an active form before TGF-β can interact with cell surface TGF-β receptors. This conversion involves the release of mature TGF-β from the complex by disrupting noncovalent interactions between mature TGF-β and its propeptide, latent TGF-β-associated peptide. Activation of the large latent TGF-β complex in the vessel wall is thought to occur through a plasmin-dependent mechanism that requires concentration of reactants on the cell surface and/or extracellular matrix.

The mechanism of latent TGF-β activation self-regulates through effectors of plasmin generation (231). Importantly, TGF-β itself stimulates production of the protease inhibitor plasminogen activator inhibitor-1 (275). Thus posttranslational regulation of TGF-β (activation, storage, and presentation) contributes significantly to its physiological effects in the vessel wall.

TGF-β is also an important stimulus for autocrine production of many factors including angiotensin II (140, 156, 295, 330), ET-1 (113), PDGF (66, 150), HEBGF (66, 150), bFGF (66), and thrombin (297). TGF-β is also a negative regulator of HGF expression (222). In vitro experiments suggest that TGF-β can be both growth promoting and growth inhibiting for VSMC. At low concentrations (<0.1 ng/ml), TGF-β is growth promoting, which is thought to be due to increased expression of PDGF-A and the PDGF-β-receptor (13, 108, 142), as well as thrombospondin (142, 189). At higher concentrations, TGF-β is growth inhibiting, which may be due to decreased PDGF-A and PDGF-β-receptor expression (13, 108). Other investigators have found that TGF-β induces a delayed increase in DNA synthesis associated primarily with cell hypertrophy (240). The complexity of TGF-β growth effects is compounded when combinations of growth factors are examined (e.g., PDGF and TGF-β or angiotensin II and TGF-β).

For example, VSMC from the SHR show different growth responses to angiotensin II than those of Wistar-Kyoto rats (WKY) (113, 295). In SHR VSMC, angiotensin II is mitogenic, and this correlates with relatively diminished TGF-β expression compared with WKY cells, where angiotensin II is primarily hypertrophic (113). As already discussed, TGF-β neutralizing antibody inhibited angiotensin II-induced increases in DNA synthesis (97, 156, 295). However, exogenous TGF-β, at concentrations similar to those induced by angiotensin II, failed to elicit a mitogenic response in the SHR (295). In one series of experiments (156), two separate VSMC cultures were examined, one in which angiotensin II induced hypertrophy and the other in which angiotensin II induced hyperplasia. Angiotensin II stimulated bFGF expression two- to fivefold in both cultures. In the culture that responded with hypertrophy, angiotensin II induced the expression of the active form of TGF-β two- to threefold. However, in the culture that responded with hyperplasia, no active TGF-β was detected either at baseline or after angiotensin II exposure. Interestingly, all the TGF-β present was in the inactive, latent form. In another series of experiments, antisense oligonucleotides were used to inhibit TGF-β production (140). Under these conditions, angiotensin II-mediated cell proliferation was enhanced, further supporting the role of TGF-β as an antiproliferative autocrine factor. A similar mechanism has been proposed for the inhibition of VSMC proliferation by fluid shear stress (319). TGF-β may also interact with inflammatory cytokines, including interleukin-1 and its receptor antagonist (IL-1Ra). Specifically, TGF-β was found to stimulate IL-1Ra immunoreactivity in the VSMC conditioned medium and cell lysates (64). TGF-β is likely to play an important role in the vessel response to injury. Majesky et al. (192) showed that TGF-β was rapidly increased following balloon injury of the rat carotid. Administration of neutralizing anti-TGF-β antibodies significantly reduced the size of the intimal lesions that developed after carotid balloon injury (339). Immunohistochemical staining showed that two TGF-β-induced extracellular matrix components, fibronectin and versican, were greatly increased in the untreated neointimal lesions, but were almost completely absent from the lesions of the anti-TGF-β. Injury of atherosclerotic rabbit vessels demonstrated a sustained expression of TGF-β compared with nonatherosclerotic vessels (102). On the
basis of these data it is clear that TGF-β is one of the most important autocrine growth factors produced by VSMC, with the ability to promote or inhibit growth depending on concentration- and species-specific differences.

Recently, it has become clear that activin A, a 14-kDa peptide related to TGF-β, is also an important VSMC autocrine factor (208). Activin A is normally present as disulfide-linked homodimers or heterodimers complexed with the inhibin βA and/or βB chains. Similar to TGF-β, which is bound to decorin, activin A is normally bound to follistatin, an activin-binding protein. It was found that follistatin mRNA was present in VSMC, and follistatin protein was secreted into conditioned medium (139). Furthermore, immunostaining and in situ hybridization of the atherosclerotic lesions showed that both activin A and follistatin were highly expressed in the diseased artery (75, 138, 139). Activin A was found to be a VSMC mitogen by Kojima et al. (157). While activin A stimulated production of IGF-I, it also modified the effects of exogenous IGF-I, suggesting complex regulation similar to TGF-β (157). In cultured VSMC, bioactivity of activin was increased in quiescent cells treated with fetal calf serum or PDGF, but not with angiotensin II or IGF-I (147). In contrast to these results, increased activin A expression was observed after treatment of VSMC with angiotensin II and thrombin (242), while PDGF-BB or serum caused only a minor induction of this protein. Although activin A alone only weakly stimulated DNA synthesis, it demonstrated a potent mitogenic effect in combination with either EGF or HBEGF. Furthermore, in a rat carotid injury model, activin A mRNA was upregulated within 6 h after injury followed by increases in immunoreactive protein in the neointima at 7 and 14 days (242). The controversy regarding the mechanisms of activin function are likely due to the complex interactions between follistatin, binding to different TGF-β receptors, and VSMC heterogeneity.

D. Other Proteins Involved in Autocrine Growth Mechanisms

1. α2-Macroglobulin

A single report (329) showed that the protease inhibitor and cytokine carrier α2-macroglobulin (α2-M) increased rat VSMC PDGF α-receptor expression. PDGF α-receptor mRNA levels increased threefold by 6 h in VSMC treated with methylamine-modified α2-M, a form of activated α2-M. Recombinant and proteolytic α2-M derivatives were used to demonstrate that α2-M increased PDGF α-receptor expression by binding VSMC-secreted cytokine(s) and interrupting an autocrine loop that ordinarily suppresses PDGF α-receptor expression in these cells (329).

2. Cholesteryl ester

A single report (166) showed that cholesteryl ester increased bFGF expression in VSMC, compared with control cells. Conditioned media from cholesteryl ester-enriched VSMC contained six times more mitogenic activity than conditioned media from control cells did. The mitogenic activity was neutralized by an antibody directed against bFGF but not by an antibody directed against PDGF. These results suggest that cholesteryl ester enrichment also enhances bFGF release.

3. Cyclophilins

Cyclophilins are a family of highly conserved and ubiquitous proteins termed immunophilins (196). Cyclophilin A (CyPA) (117, 119), cyclophilin B (252), and cyclophilin C (281) are the three known isoforms of small-molecular-mass (~20-kDa) cyclophilins. Our laboratory studied proteins secreted from VSMC stimulated by the O2− generator LY83583 (179). Among these proteins, cyclophilin A, a member of the immunophilin family, was identified as a secreted factor, responsible for LY83583-mediated ERK1/2 activation and VSMC growth (181). Specifically, immunodepletion of CyPA from conditioned medium using anti-CyPA antibody significantly inhibited ERK1/2 activity, and human recombinant CyPA stimulated ERK1/2 in a dose-dependent manner. LY83583 conditioned medium and recombinant CyPA increased VSMC DNA synthesis, suggesting that CyPA has mitogenic properties (181). Both conditioned medium and recombinant CyPA significantly inhibited VSMC apoptosis induced by 0.5 mM sodium nitroprusside. CyPA protein expression was dramatically upregulated in balloon-injured rat carotid with a time course that paralleled neointima formation. These findings identify CyPA as a VSMC autocrine growth factor, released in response to oxidative stress. Generation of other growth factors by CyPA remains to be determined.

4. Heat shock proteins

Heat shock proteins are a family of cellular proteins characterized by their upregulation in response to stress, the presence of a weak ATPase activity, and a high degree of sequence homology. Heat shock proteins were initially described as chaperones that facilitate the folding of other proteins. However, recent studies now indicate that heat shock proteins also play a role in cytoprotection, actin-cytoskeleton rearrangements, antiapoptosis, and signal transduction (32, 88, 109, 131, 158, 159, 181, 246, 250). Multiple species of heat shock proteins have been characterized in VSMC including HSP10, HSP27, HSP40, HSP60, HSP70, HSP75, HSP78, HSP90, and HSP110. HSPs are both constitutively expressed and transcriptionally
regulated. Early work showed that VSMC treated with either heat shock or arsenite (an oxidant stress) induced HSPs with molecular masses of 70, 90, and 110 kDa (153). In these studies, treatment of cells with norepinephrine or angiotensin II induced cellular hypertrophy without eliciting HSP expression (153). However, another study found that both HSP60 and HSP70 were strongly induced by growth factors including angiotensin II (241). Specifically, high-resolution two-dimensional gel electrophoresis and internal protein microsequencing showed that VSMC growth factors increased expression of HSP60, HSP70, protein disulfide isomerase, and protein disulfide isomerase isozyme Q-2, all involved in protein folding. Hypertrophic and hypertrophic growth were accompanied by similar changes in protein expression, suggesting that both types of growth require upregulation of the protein synthesis and folding machinery (241). Evidence that VSMC have receptors for HSPs comes from earlier work that showed addition of exogenous HSP70 to VSMC in vitro protected against toxins that may initiate necrosis (145). These investigators showed that exogenous HSP70 protects viability through interactions with the cell surface rather than via internalization (145). Both exogenous and endogenous HSP70 protected VSMC against serum-deprivation-induced apoptosis (144). In rat carotid arteries there was a significant increase in HSP70 expression after injury (151). Our laboratory recently identified HSP90-α as a VSMC growth factor secreted in response to oxidative stress (181). HSP90 was secreted in response to generators of superoxide anion and stimulated ERK1/2 activity. Recombinant HSP90 also stimulated VSMC growth, suggesting that HSP90 binds to a receptor that is coupled to cell proliferation.

More recently a role has been proposed for HSP60 in the response of endothelial cells and VSMC to chlamydia (158). Both human and chlamydia HSP60 were found to induce E-selectin, ICAM-1, and vascular cell adhesion molecule-1 expression on endothelial cells similar to levels induced by lipopolysaccharide. Both HSP60s also significantly induced IL-6 production by VSMC, further supporting a role for heat shock proteins in VSMC function (158). A possible receptor for HSP60 is CD14 (159), which stimulates p38 kinase in response to exogenous HSP60. Future studies will be required to define the role of specific HSPs and the receptors expressed on VSMC.

5. Extracellular matrix proteins

Matrix is important in determining cell growth and shape. The best-characterized interaction between cells and matrix is mediated by integrins (cell receptors) and their ligands (extracellular matrix proteins). Examples include the interactions of the α5β1 integrin heterodimer with vitronectin and the αvβ3 heterodimer with fibronectin (133). Integrin interactions with their ligands stimulate many signal transduction events that are required for cell growth and survival (203, 284). In fact, cell shape was found to govern whether individual cells grow or die, regardless of the type of matrix protein or antibody to integrin used to mediate adhesion (44). Numerous examples exist for matrix effects on VSMC growth responses. When VSMC were cultured on plastic, angiotensin II induced only a 1.6-fold increase in [3H]thymidine incorporation, but when cultured on fibronectin- or type I collagen-coated plastic, the response to angiotensin II was enhanced from two- to fourfold (299). In addition, angiotensin II is able to alter the matrix expressed by VSMC. Osteopontin is a matrix molecule whose expression is dramatically increased by angiotensin II (9, 56). Osteopontin has been shown to exert important effects on VSMC growth. With the use of immunohistochemistry and in situ hybridization, it was found that medial VSMC in uninjured arteries contained very low levels of osteopontin protein and mRNA (96). Injury to either the adult rat aorta or carotid artery using a balloon catheter initiated a qualitatively similar time-dependent increase in both osteopontin protein and mRNA in VSMC (9, 56). Expression was transient and highly localized to neointimal SMC during the proliferative and migratory phases of arterial injury, suggesting a possible role for osteopontin in these processes. In vitro, bFGF, TGF-β, and angiotensin II elevated osteopontin expression in VSMC (96). There was also a positive correlation between osteopontin containing VSMC and DNA replication (56). Osteopontin has also been shown to be essential for angiotensin II-stimulated DNA synthesis of cardiac fibroblasts, further supporting its role in VSMC growth (56). There are many other matrix molecules whose expression has been shown to be regulated by VSMC growth factors including fibronectin (291, 307), vitronectin, and type I collagen (82, 178, 229, 245).

The nature of matrix assembly (fibrils vs. monomers) is all critically important for VSMC growth. bFGF has been shown to exert powerful effects on assembly of type I collagen fibers (245), which may have important implications for VSMC growth since VSMC are arrested in the G1 phase of the cell cycle on polymerized type I collagen fibrils, while monomer collagen supports SMC proliferation (165). Specifically, fibrillar collagen regulates early integrin signaling that may lead to upregulation of cdk2 inhibitors and inhibition of VSMC proliferation (165).

Finally, it should be noted that VSMC have several inducible MMPs that may regulate the nature of the matrix (89). For example, VSMC stimulated with IL-1 or TNF-α synthesized de novo 92-kDa gelatinase, interstitial collagenase, and stromelysin. Together, the constitutive and the cytokine-induced enzymes can digest all the major components of the vascular matrix. In summary, extracellular matrix and the receptors for these proteins represent an important regulatory mechanism that modulates the nature of the VSMC growth response.
E. Nonprotein Stimuli That Activate VSMC Autocrine Growth Mechanisms

1. Hypoxia and hyperoxia

Hypoxia has been reported to cause two direct and distinct effects on VSMC growth (50). Exposure to moderately low O₂ tension induces VSMC proliferation, independent of IL-1, whereas exposure to very low O₂ tension induces production of IL-1α. Levels of IL-1α and IL-1β mRNA increased in VSMC after 48-h incubation in low O₂ compared with levels in normoxic cells. Both IL-1α and IL-1β decreased upon subsequent reoxygenation. Levels of cell-associated IL-1α also increased progressively after 48 h in low O₂; however, detectable IL-1α was not released from the cells in the media. In contrast to hypoxia, hyperoxia inhibits VSMC growth. It has been observed that hyperoxia increases levels of p21CIP1/waf (185, 288, 311), an inhibitor of cell cycle progression, but the mechanism for induction of p21CIP1/waf is unknown.

2. Injury

In response to arterial injury, many mechanical and hormonal events are stimulated that promote VSMC growth. Both VSMC proliferation and migration contribute to the development of neointima. Separating the relative contributions of individual growth factors to these two VSMC functions is not simple. In general, PDGF is involved primarily in chemotaxis, not in proliferation (261). Important roles in cell proliferation have been proposed for angiotensin II (249) and TGF-β, but not for bFGF (236). HBEFG (135) and activin A (242) are also significantly increased after injury, although their contribution to neointima formation remains to be determined. In contrast, growth arrest genes, such as gax, are downregulated in the injured vessel (332), except for Gas6 (202). For further discussion of VSMC growth mechanisms after injury, the reader is referred to recent reviews (6, 106, 261).

3. Mechanical forces: stretch, pressure, and shear stress

VSMC in the vessel wall are continuously exposed to mechanical forces that modulate function. It has become clear that in addition to regulating vessel tone these physical forces modulate vessel architecture by changing VSMC gene expression. It should be noted that many of the experimental models to study strain might not accurately reflect the in vivo situation. In fact, strain may be a very specific stimulus that alters gene expression to a large extent of only a small number of genes based on a recent study. Specifically, Feng et al. (78) observed using a microarray with 5,000 genes that only 3 transcripts were induced greater than 2.5-fold: cyclooxygenase-1, tenascin-C, and plasminogen activator inhibitor-1. Downregulated transcripts included MMP-1 and thrombomodulin. Of interest, 3,157 transcripts changed by <2-fold.

A) Stretch/strain. Autocrine growth factors reported to be regulated by strain include PDGF, angiotensin II, IGF-I, bFGF, and TGF-β. A common experimental model has been the FlexerCell in which cells are grown on silicone elastomer plates and subjected to cyclic strain (60 cycles/min) by application of a vacuum under the plates. An early study (335) showed that 48-h exposure to mechanical strain increased the basal rate of DNA synthesis by threefold and increased cell number by 40% compared with cells grown on stationary rubber plates. Strain also increased the rate of thymidine incorporation in response to α-thrombin (from 15- to 33-fold), but not to PDGF. Strain appeared to induce the production of an autocrine growth factor(s), since conditioned medium from cells subjected to strain induced a fourfold increase in DNA synthesis in control cells. Western blots of medium conditioned on the cells subjected to strain indicate that the cells secrete both PDGF-A and PDGF-B in response to strain. Finally, polyclonal antibodies to PDGF-A and PDGF-B significantly reduced DNA synthesis, while antibodies to bFGF had no effect. Thus the mechanism of strain-induced growth appears to involve the intermediary action of secreted PDGF. A more recent study failed to show significant increases in PDGF expression when the strain was a biaxial stimulus that more accurately reflects the in vivo mechanical force (78). Increased production of angiotensin II and increased cell responsiveness to angiotensin II also occur with mechanical strain (299). Specifically, angiotensin I increased DNA synthesis in VSMC, and this response was also enhanced by mechanical strain. Mitogenic activity of angiotensin I was blocked by ramiprilat, indicating that its mitogenic activity was via conversion to angiotensin II. The synergy between angiotensin II and strain was completely eliminated by neutralizing antibodies to PDGF-AB. Thus the synergy between angiotensin II and mechanical strain probably results from synergism between angiotensin II and PDGF secreted in response to strain. Using a similar model, Standley et al. (293) showed that cyclic stretch increased IGF-I secretion from stretched cells by 20- to 30-fold, and stretch-induced increases in growth were completely blocked by addition of anti-IGF-I antibody. Finally, in response to a single transient 5-min stretch, VSMC were found to release bFGF, which was responsible for DNA synthesis (46).

Stretching VSMC also increases collagen synthesis that was shown to be due to the actions of angiotensin II and TGF-β. A twofold increase in collagen synthesis and a concurrent increase in total protein synthesis were noted in stretched VSMC. The concentration of immunoreactive angiotensin II and TGF-β (both active and latent forms) was increased in the medium of stretched VSMC.
Saralasin inhibited the stretch-induced secretion of TGF-β from VSMC and collagen synthesis, suggesting that formation of angiotensin II was the initial autocrine growth event. The large number of VSMC autocrine factors released by mechanical strain suggests that this is an important process for vascular changes present in hypertension and volume-overload states such as congestive heart failure.

b) Pressure. Increased pressure has been suggested to stimulate both IGF and PDGF expression (77, 227). It has been suggested that pressure maintains a differentiated phenotype in culture (26) as shown by continued expression of high-molecular-mass caldesmon and filamin in the organ cultures of pressurized and stretched vessels. In vivo, increased pressure due to aortic coarctation was associated with enhanced IGF-I expression (77) and IGFBP-4 expression (7). Finally, it has been suggested that increases in levels of both PDGF-A mRNA and Sp1 in VSMC of SHR are associated with high blood pressure (227). In general, it appears that pressure stimulates fewer autocrine growth mechanisms that strain.

c) Shear stress. Although VSMC are not usually exposed to fluid shear stress, after vascular injury the developing neointima lacks an endothelium and is exposed to blood flow. Shear stress has been shown to regulate TGF-β expression (319) and PDGF-R phosphorylation (129). Exposure of VSMC to fluid flow for 24 h inhibited proliferation significantly in association with increased expression of TGF-β and tissue-type plasminogen activator (319). The levels of both latent and active forms of TGF-β in conditioned media of VSMC exposed to fluid flow increased significantly. In another study (129), shear stress was shown to rapidly induce phosphorylation of PDGFR-α, which was not inhibited by antibodies binding to all forms of PDGF, suggesting an effect independent of PDGF. In summary, mechanical forces are clearly important regulators of VSMC function. Alterations in gene expression appear to be highly specific and include several autocrine growth factors.

4. ROS

Recent evidence suggests an important role for ROS (which include O₂, H₂O₂, and OH⁻) in the control of VSMC proliferation both in vitro and in vivo, as recently reviewed by Alexander (4) and Griendling and Usui-Fukai (106). ROS increase cell proliferation, mediate hormone-induced hypertrophy, and in certain circumstances induce apoptosis (106). It has now become clear that ROS directly stimulate VSMC growth and also act as second messengers for more classic G protein-coupled and tyrosine kinase-coupled growth receptors.

a) Direct ROS-stimulated Autocrine Growth Effects. Direct effects of ROS to stimulate VSMC growth are mediated by activation of signal transduction events, increased expression and secretion of growth factors, and transactivation of tyrosine kinase-coupled receptors. Our laboratory was among the first to show that ROS stimulated VSMC growth and signal transduction (256). In this early study we found that stimulation of VSMC by products of xanthine metabolism (O₂, H₂O₂, and uric acid) increased VSMC number and DNA synthesis. Subsequently, we and others found that all three products exerted important growth effects as detailed below. Over the last 8 years it has become apparent that H₂O₂ has several properties (concentration, duration, ease of transit across cell membranes) that make it likely to be the most important ROS in vivo.

H₂O₂ is an important VSMC growth factor based on its stimulation of autocrine growth factors, protein kinases (e.g., mitogen-activated protein kinases), DNA synthesis, and cell number (10). Growth factors regulated by H₂O₂ include bFGF (125), IGF-I (58), EGF (270), HBEGF (150), CyPA (181), and HSP90 (181). Herbert et al. (125) showed that H₂O₂-induced VSMC proliferation was strongly and specifically inhibited by a neutralizing monoclonal antibody directed against bFGF but was not due to increased expression of bFGF or the bFGF receptor-1. Instead they found that H₂O₂ strongly increased the affinity of bFGF for its receptor at the surface of the SMC, therefore showing that the mitogenic effect of H₂O₂ might occur through a direct effect on the bFGF receptor (125). The relative roles of H₂O₂-induced growth factors in vivo remain to be defined.

Transactivation of tyrosine kinase-coupled receptors as a mechanism of action for H₂O₂ was first described by Rao (255). Specifically, he showed that H₂O₂ stimulated tyrosine phosphorylation of several proteins including the EGF-R in VSMC. After H₂O₂ treatment, the tyrosine-phosphorylated EGF-R formed a complex with SHC-Grb2-SOS (255). ROS have now been shown to activate many intracellular kinases including protein kinase C (258), ERK1/2 (10), and many tyrosine kinases (255).

b) ROS as second messengers for other growth factors. The role of ROS as second messengers for more classic VSMC growth factors has become well-established (106). VSMC growth factors that use intracellular ROS as mediators include angiotensin II, insulin, IL-1, PDGF, and TGF-β. Three lines of evidence support the concept that ROS act as autocrine mediators for growth factors. 1) PDGF and angiotensin II increase ROS production in VSMC (104, 305). 2) Most of the O₂ generated in VSMC appears to be produced by the intracellular NAD(P)H oxidase, which includes a novel p91 homolog termed Nox1 (104, 304). 3) Signal transduction by PDGF is inhibited when cells are transduced with superoxide dismutase or catalase, or after treatment with antioxidants (305). Sundaresan et al. (305) showed that PDGF transiently increased H₂O₂, which was required for PDGF-induced tyrosine phosphorylation and ERK1/2 activation. The in-
crease in H$_2$O$_2$ could be blunted by increasing the intracellular concentration of the scavenging enzyme catalase or by the chemical antioxidant N-acetylcysteine. The response of VSMC to PDGF, which included tyrosine phosphorylation, mitogen-activated protein kinase stimulation, DNA synthesis, and chemotaxis, was inhibited when the growth factor-stimulated rise in H$_2$O$_2$ concentration was blocked (305). In vessels, a plasma membrane NADH oxidase accounts for $\sim$90% of O$_2$ formation (88, 104). Of note, the NADH oxidase is regulated at the protein level and may be increased by hormonal stimuli. Griendling et al. (104) showed that angiotensin II caused a sustained increase in VSMC O$_2$. Importantly, angiotensin II hypertrophy was inhibited by transfection with antisense p22phox cDNA, a component of NADH oxidase (322). A role for angiotensin II-induced H$_2$O$_2$ production in VSMC was supported by findings that cell lines that overexpress catalase showed decreased hypertrophy (345). The intracellular signal events mediated by ROS in VSMC stimulated by angiotensin II occur via p38 (320) and via Akt/PKB (321). More recently, it has been shown that PDGF induces Nox1, which stimulates ROS production that leads to increased VSMC growth (304). Thus ROS (and H$_2$O$_2$ in particular) act as intracellular autocrine growth mediators for VSMC in response to both G protein and tyrosine kinase-coupled receptors.

Finally, uric acid, which is the by-product of xanthine oxidase-dependent xanthine metabolism, has also been reported to mediate autocrine growth (257). Uric acid stimulated VSMC DNA synthesis, as measured by $[^3]$H]thymidine incorporation. Exposure of VSMC to uric acid stimulated accumulation of PDGF-A mRNA and secretion of PDGF-like material in conditioned medium (>10-fold at 24 h). Uric acid-induced DNA synthesis was markedly inhibited by incubation with anti-PDGF-A antibodies. Thus uric acid stimulates VSMC growth via an autocrine mechanism involving PDGF-A.

Table 1. Growth factors and effects on VSMC growth

<table>
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<tr>
<th>Stimulus</th>
<th>Hyperplasia</th>
<th>Hypertrophy</th>
<th>Antiapoptosis</th>
<th>Growth Inhibition</th>
<th>Development</th>
<th>Injury</th>
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Table consists of appropriate reference numbers. CGRP, calcitonin gene-related peptide; EGF, epidermal growth factor; FGF, fibroblast growth factor; HBEGF, heparin binding EGF-like factor; HGF, hepatocyte growth factor; IGF-I, insulin-like growth factor I; IL, interleukin; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TGF-β, transforming growth factor-β.
In conclusion, ROS are powerful extracellular and intracellular mediators of VSMC growth in response to a variety of growth factors. Increasing evidence suggests important roles in VSMC hyperplasia, hypertrophy, and apoptosis, making ROS among the most important VSMC growth regulators.

V. CONCLUSIONS: ANGIOTENSIN II-MEDIATED EVENTS AS A PARADIGM FOR AUTOCRINE GROWTH MECHANISMS

An integrated analysis of the autocrine mechanisms utilized by angiotensin II will be discussed as a model that places the relative roles of different factors and pathways into context. The effect of angiotensin II on VSMC growth represents an excellent example of how autocrine growth mechanisms regulate VSMC. Angiotensin II has been found to stimulate hyperplasia, hypertrophy, and antiapoptotic effects depending on VSMC type. Although it appears probable that the effect of angiotensin II on VSMC growth depends on the relative magnitude of expression of autocrine growth factors, it is also a consequence of systemic factors and biomechanical forces that modulate VSMC growth. In addition, the nature of the VSMC state (heterogeneity and phenotype) also determines the nature of the VSMC response to angiotensin II.

Angiotensin II regulates VSMC growth via at least four different growth pathways as shown in Figure 2. For ease of discussion, these are presented in temporal sequence. 1) Direct effects of the AT$_1$R: upon binding of angiotensin II to the AT$_1$R, a conformation change occurs in the receptor that activates heterotrimeric G proteins. These stimulate phospholipase C-$\beta$ leading to hydrolysis of polyphosphoinositide bisphosphate and generation of two second messengers, inositol trisphosphate and diacylglycerol. Inositol trisphosphate stimulates release of intracellular calcium that activates many calcium-dependent intracellular kinases including the tyrosine kinase Pyk2. Diacylglycerol activates protein kinase C. Both of these signals are required for the growth-promoting effects of angiotensin II. Other direct signals that appear important for angiotensin II growth are stimulation of kinases such as raf-1 and ERK1/2 (69, 180). Furthermore, activation of phosphatidylinositol 3-kinase (PI3K) stimulates AKT, a serine/threonine kinase. Activation of PI3K and AKT are associated with growth stimulation by angiotensin II (276) and antiapoptotic effects (11). Recent studies have demonstrated direct activation of tyrosine kinases by the AT$_1$R including JAK2 and TYK2 (5, 197, 198, 325). These tyrosine kinases phosphorylate and activate the signal transduction and activators of transcription (STAT) family of transcription factors that are also required for VSMC growth (197). These rapid effects occur within 10 min and do not require de novo protein or gene synthesis.

Table 2. VSMC autocrine growth factors

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Activin</th>
<th>ANG II</th>
<th>Epiregulin</th>
<th>ET-1</th>
<th>PGGF HBEGF2</th>
<th>Gas6</th>
<th>HBEGF</th>
<th>HGF</th>
<th>IGF-I</th>
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Table consists of appropriate reference numbers. Definitions are as in Table 1.
mRNA synthesis. 2) Transactivation of EGF-R and PDGF-R: angiotensin II transactivates tyrosine kinase-coupled growth factor receptors including the EGF-R and PDGF-R leading to autophosphorylation and downstream signal transduction. To date, transactivation of EGF-R appears to be involved in angiotensin II-mediated growth (74, 137, 210, 216, 308). Possible mechanisms for transactivation include c-Src-mediated phosphorylation of EGF-R and PDGF-R, assembly of signal transduction complexes mediated by the adapter protein Shc (and recruitment of another tyrosine kinase), or changes in receptor dimerization. Alternatively, a recent report suggests that G protein-coupled receptors transactivate tyrosine kinase receptors via stimulation of matrix metalloproteinases that release HBEGF, which now binds to the EGF receptor (251). 3) Increased ROS generation: angiotensin II increases ROS generation via at least two mechanisms. First, it rapidly stimulates activity of the plasma membrane NAD(P)H oxidase leading to increased O₂⁻ and H₂O₂ formation. Second, by stimulating autocrine release of growth factors such as PDGF that stimulate ROS formation, angiotensin II indirectly increases ROS formation. Finally, angiotensin II increases protein and mRNA expression of critical components of the NAD(P)H including p22phox (87) and Nox1, a homolog of the catalytic subunit of the superoxide-generating NADPH oxidase of phagocytes gp91phox (304). 4) Induction of other growth factors: as discussed above, angiotensin II stimulates expression of multiple VSMC growth factors including PDGF-A and PDGF-B, ET-1, FGF, epiregulin, IL-6, IGF-1, TGF-β, HBEGF, and activin. Each of these growth factors may then induce and regulate other growth factors as detailed in Tables 1 and 2. In addition, angiotensin II regulates the levels of receptors for growth factors including PDGF-R and Axl. Additional growth effects of angiotensin II are mediated by changes in cell matrix that include increased expression of osteopontin and fibronectin. The temporal and spatial coordination of these events will modulate the environment in which the other growth factors initiate cell cycle events. Finally, the heterogeneous nature of VSMC developmental origin provides another level of complexity in the cell response to VSMC. Thus the plasticity of the VSMC growth response to angiotensin II (hypertrophy, hyperplasia, and inhibition of apoptosis) represents the interplay of direct effects, alterations in intracellular second messengers, transactivation of growth factor receptors, changes in cell matrix, and induction of multiple autocrine growth factors and their receptors.

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