Novel cardiovascular actions of the activins

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Abstract

Proliferation and directed migration of vascular cells are key components in vascular diseases such as atherosclerosis and restenosis following percutaneous transluminal coronary angioplasty. However, the precise cellular and molecular mechanisms involved in the control of vascular cell proliferation or migration at the tissue level remain largely undefined. Molecules contributing to these processes are elaborated by distinct cell types and act in both autocrine and paracrine modes. They include two broad classes, polypeptide growth factors and vasoactive G-protein-coupled receptor (GPCR) agonists. Examples of the former, such as platelet-derived growth factor, bind to and activate cell surface receptor tyrosine kinases, initiating intracellular biochemical signaling pathways associated with cell proliferation or migration. In contrast, recent evidence suggests that vasoactive GPCR agonists (e.g. angiotensin II, endothelin-1, a-thrombin) elicit cell growth indirectly by inducing the production of autocrine or paracrine factors in vascular cells. Recent studies have identified activin A as a novel component of conditioned medium obtained from GPCR agonist-stimulated vascular smooth muscle cells (SMCs). Although activin A alone only weakly stimulated rat aortic SMC DNA synthesis, it demonstrated a potent co-mitogenic effect in combination with either epidermal growth factor (EGF) or heparin binding EGF-like growth factor in these cells, increasing DNA synthesis by up to 5- and 4-fold respectively. Furthermore, in a rat carotid-injury model, activin A mRNA was upregulated within 6 h after injury, followed by increases in immunoreactive protein detected in the expanding neointima 7 to 14 days later. Taken together, these results indicate that activin A is a common vascular SMC-derived growth factor induced by vasoactive agonists that may, either alone or in combination with other factors, contribute to fibroproliferative vascular diseases.

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Introduction

Excessive proliferation and migration of several vascular cell types (e.g. vascular smooth muscle cells (SMCs), endothelial cells, myofibroblasts) are important components in vascular remodeling and associated pathologies, including angiogenesis, atherosclerosis, and restenosis following percutaneous transluminal coronary angioplasty (PTCA) (Schwartz et al. 1990, Ross 1993, Schwartz 1997). Accordingly, in normal vessels, cell growth and migration are closely regulated processes, although the precise molecular mechanisms involved in each are not well understood.

In the mature artery, complex interactions between the different resident cells, as well as circulating cells, influence the growth and metabolic rates of individual vascular cell types. Thus a balance of paracrine growth factors, vasoactive factors, circulating hormones, etc. controls tissue homeostasis. For example, endothelial cells lining intimal arterial surfaces produce nitric oxide and other factors that act to reduce vascular SMC proliferation (Gibbons & Dzau 1994). Consequently, endothelial cell damage, initiated either by direct injury or the accumulation of inflammatory cells, may alter the expression of these factors resulting in enhanced proliferation and/or migration of vascular SMCs involved in neointimal formation. These events contribute to the process of atherosclerosis, resulting in the formation of inflammatory fibroproliferative lesions that ultimately lead to myocardial and/or cerebral ischemia. Hence, a fundamental challenge in vascular pathobiology is the identification of the critical endogenous factors involved in vascular responses to injury.
Several G-protein-coupled receptor (GPCR) agonists including angiotensin II (AII) and α-thrombin have been implicated in vascular pathologies. These proteins are potent mitogens for vascular SMCs in vitro (Scott-Burden et al. 1991, McNamara et al. 1993, Weber et al. 1994) and also induce extracellular matrix formation (Scott-Burden et al. 1990, Carney et al. 1992). AII acts by direct activation of the angiotensin type 1 receptor (Sung et al. 1994), whereas α-thrombin binds to its receptor and, by enzymatic cleavage generates a new amino-terminus 'tethered ligand' which activates the receptor (reviewed in Coughlin 1994). Early intracellular signaling events in rat aortic smooth muscle (RASM) cells in response to AII and α-thrombin include increased levels of intracellular Ca\(^{2+}\), phosphoinositide turnover and formation of diacylglycerol (Berk et al. 1991, Matsusaka & Ichikawa 1997). AII and α-thrombin also stimulate signaling events associated with cellular proliferation including protein tyrosine phosphorylation, activation of the Raf-1/mitogen-activated protein kinase pathway, and nuclear proto-oncogene expression (for reviews see Van Obberghen-Schilling & Pouysségur 1993, Duff et al. 1995).

Many of the mitogenic signaling events activated by AII and α-thrombin are shared with growth factor agonists of receptor tyrosine kinases. However, the induction of DNA synthesis in response to AII or α-thrombin in vascular SMCs is delayed when compared with mitogens such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (FGF) or fetal bovine serum (Weber et al. 1994, Molloy et al. 1996). This delay may be due to a requirement for the production of endogenous growth factors in vascular SMCs that mediate the GPCR agonist-mediated responses. In this regard, AII and α-thrombin have been shown to stimulate the expression of growth factors including PDGF-A chain (Naftilan et al. 1989, Stouffer et al. 1993), basic FGF (Itoh et al. 1993, Weiss & Maduri 1993) and insulin-like growth factor (IGF)-I (Delafontaine et al. 1996) which may contribute to the mitogenic effects of these GPCR agonists. Taken together, these data support the hypothesis that various peptidic, GPCR agonists each stimulate vascular SMC proliferation by a similar autocrine mechanism (Fig. 1). In this model, delayed mitogenesis is preceded by the increased expression of a common subset of new gene products in vascular SMCs. Work from our laboratory, described below, has identified activin A as one of these common gene products upregulated by vasoactive GPCR agonists.

![Figure 1](image-url) Autocrine model of delayed mitogenesis stimulated by G-protein-coupled receptor agonists in vascular SMCs. Binding of vasoactive factors to the appropriate receptor results in rapid intracellular signal transduction leading to the expression of endogenous mitogenic factors (see text). The secretion of one or more mitogenic factors results in the formation of a productive autocrine loop, leading to cellular proliferation. Therapeutic agent(s) targeted against a key autocrine mitogen (growth factor (GF) antagonist) may functionally antagonize several GPCR agonists. ET-1, endothelin-1.

Identification of activin A in conditioned media samples from GPCR agonist-stimulated vascular SMCs

Our laboratory had previously demonstrated that RASM cells treated with the GPCR agonists α-thrombin or AII secreted potent mitogenic factors into the cell culture media (Weber et al. 1994, Molloy et al. 1996). Since previous studies had implicated growth factors such as basic FGF, PDGF, or IGF-I in this mechanism, we attempted to identify the mitogenic components in GPCR agonist-stimulated RASM cell conditioned media.
using a panel of specific growth factor-neutralizing antibodies. However, none of the antibodies significantly inhibited the mitogenic activity present in the RASM cell conditioned media samples. During these experiments we observed one notable exception. An individual lot of purified polyclonal antibodies (AD921221), obtained from a rabbit immunized with chromatographically-purified human placental basic FGF, was found markedly to inhibit the RASM cell-secreted mitogenic activity (Pawlowski et al. 1997). Since we previously had excluded basic FGF as a contributing mitogen, immunoblot analysis was performed on concentrated samples of conditioned media obtained from thrombin- or AII-stimulated RASM cells to determine whether these ‘neutralizing’ antibodies cross-reacted with another SMC-derived mitogen. SDS-PAGE and immunoblot analysis identified a protein of 14 kDa under reducing conditions (~26 kDa under non-reducing conditions) which was not recognized by other anti-basic FGF antibodies. This protein subsequently was purified and identified as activin A (inhibin \( \beta \) homodimers; for a review see Vale et al. 1990) by Edman sequencing and mass spectroscopy analysis. The reason for the cross reactivity of the anti-basic FGF antibodies with activin A is still unclear; however, these results demonstrated that activin A expression was strongly upregulated by GPCR agonists, but only weakly induced by PDGF or EGF (Fig. 2) or heparin binding EGF-like growth factor (HB-EGF) (Pawlowski et al. 1997). Furthermore, consistent with other studies, we found that EGF or HB-EGF alone were only weakly mitogenic towards vascular SMCs. Taken together, these results suggest that activin A facilitates the proliferative responses of vascular SMCs to a subset of endogenous growth factors, i.e. the EGF growth factor family.

Expression of activin A in vascular injury

To determine whether activin A expression was present in vascular injury, we measured activin A mRNA and protein levels following vascular injury using the rat carotid balloon-injury model (Pawlowski et al. 1997). We observed significantly increased levels of activin A mRNA by 6 h following injury, compared with uninjured vessels. However, the induction of activin A mRNA expression was transient since no increases were seen 3 or 6 days following injury. In contrast, we observed sustained and markedly upregulated expression of activin A protein

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**Mitogenic activity of activin A towards vascular SMCs**

Recombinant activin A was used to confirm its mitogenic activity towards vascular SMCs. In these experiments, we observed that activin A (20 ng/ml) stimulated only a modest 50% increase in \([^{3}H]\)thymidine incorporation compared with control cells (Pawlowski et al. 1997). These results indicated that activin A alone could not account for the pronounced mitogenic activity found in thrombin- or AII-stimulated vascular SMC conditioned media samples. Therefore, we explored the possibility that activin A might enhance the activity of other growth factors. In these experiments, we found that although activin A did not complement the mitogenic activity of basic FGF or PDGF, it strongly potentiated vascular SMC DNA synthesis stimulated by epidermal growth factor (EGF) (Fig. 2) or heparin binding EGF-like growth factor (HB-EGF) (Pawlowski et al. 1997). Furthermore, consistent with other studies, we found that EGF or HB-EGF alone were only weakly mitogenic towards vascular SMCs. Taken together, these results suggest that activin A enhances the proliferative responses of vascular SMCs to a subset of endogenous growth factors, i.e. the EGF growth factor family.
following carotid injury (Fig. 3). In these experiments, antibodies directed against either SMC $\alpha$-actin or activin $\beta_A$ chain were used to identify vascular SMCs and activin A protein respectively in rat carotid arterial specimens. In uninjured control arteries, $\alpha$-actin-positive medial SMCs expressed low levels of activin A which was also faintly identified in adventitial layers (Fig. 3A). Following injury, activin A expression was strongly induced. For example, at 2 and 4 days after injury, increased staining of activin A was observed in both the media and adventitial layers, as well as the slowly accumulating intimal vascular SMCs (data not shown). Seven days after injury, increased amounts of activin A were observed in the adventitia as well as in the SMCs of the media and neointima. By 14 days, activin A staining was most pronounced in neointimal SMCs, while its staining in the adventitia and media had diminished (Fig. 3C). By comparison, SMC $\alpha$-actin characteristically was detected in both medial and neointimal cells (Fig. 3D). Double immunostaining with anti-activin A and anti-$\alpha$-actin antibodies revealed many cells that contained both antigens, confirming that neointimal and medial SMCs identified by $\alpha$-actin staining also expressed activin A (data not shown).

Activin A expression following vascular injury: pathophysiological implications

Activin exists as disulfide-linked homodimers or heterodimers of the inhibin $\beta_A$ and/or $\beta_B$ polypeptide chains respectively (Vale et al. 1990). These proteins were originally identified by their ability to stimulate the secretion of follicle-stimulating hormone from anterior pituitary cells. However, activin A has been reported to affect many other cell types; for example, it acts as an erythroid differentiation factor (Yu et al. 1987), as well as a potent inducer of mesoderm in Xenopus laevis (van den Eijnden-van Raaij et al. 1990, Smith et al. 1990). Activin A has also been shown to induce DNA synthesis in porcine thyroid cells (Kotajima et al. 1995), MC3T3-E1 osteoblasts (Hashimoto et al. 1992) and preleptotene spermatocytes (Hakovirta et al. 1993).

As outlined above, activin A may function in the cardiovascular system by mediating the proliferative response of vascular SMCs stimulated with certain vaso-active GPCR agonists. Thus, although activin’s mitogenic properties on vascular SMCs and fibroblasts have been described (Kojima et al. 1993, Sakurai et al. 1994), we observed that it was only weakly mitogenic when added
alone to vascular SMCs, but significantly potentiated the levels of DNA synthesis stimulated by EGF or HB-EGF. Although the exact mechanism for this effect awaits further investigation, it could be due to an induction of EGF receptor expression or modulation of EGF–receptor binding affinity as previously reported for transforming growth factor-β (TGF-β) and EGF (Assoian et al. 1984, Massague 1985). Furthermore, since EGF (Rossi et al. 1992, Sambhi et al. 1992) and related factors including HB-EGF (Temizer et al. 1992, Miyagawa et al. 1995) and epiregulin (Taylor et al. 1999) have been implicated in vascular SMC growth control, our results support a modulatory role for activin A in this process.

Additional support for a role of activin in the cardiovascular system derives from the observation that intimal activin A protein content was found to be markedly upregulated following arterial injury in vivo. These data reinforce previous studies that have identified activin A expression in atherosclerotic lesions from hyperlipidemic rabbits (Inoue et al. 1994) and in the medial layer of human coronary artery samples (Kozaki et al. 1995). More recently, de Vries and colleagues have reported that monocytes/macrophages, as well as human vascular SMCs, express activin upon stimulation with atherogenic stimuli (C J M de Vries, personal communication). They also showed that activin A induces the expression of specific proteins associated with vascular SMC differentiation, and postulate that activins may have a role in atherosclerotic plaque stabilization as well as vascular injury repair. Activin A has also been shown to increase monocyte/macrophage migration (Petraglia et al. 1991) and vascular SMC (M C Reidy & C E Turner, personal communication) migration. Taken together, these results imply that activin A may contribute to the progression of fibroproliferative vascular diseases, including atherosclerosis and post-PTCA restenosis (Fig. 4). Thus, activin A secreted from ‘injured’ vascular SMCs might act upon adventitial fibroblasts, SMCs, and monocytes/macrophages to promote arterial remodeling.

Activin A is a member of the TGF-β superfamily comprised of other factors that have previously been implicated in vascular diseases (Border & Ruoslahti 1992). For example, Wolf et al. (1994) have demonstrated that neutralizing antibodies directed against recombinant TGF-β1 could suppress neointimal formation in the rat carotid-injury model. Additionally, other TGF-β isoforms are well-characterized stimuli for extracellular matrix protein production, in connective tissue cells (Roberts et al. 1986, Roberts 1995). The functions of activin A in vascular injury, therefore, may include the direct and indirect stimulation of tissue remodeling. In support of this concept, a recent study reported that the formation of embryonic cardiac cushion tissue, which ultimately contributes to formation of heart valves and septa, is dependent on the expression of activin A mRNA as measured by Northern blotting and in situ hybridization (Moore et al. 1998). Thus, localized expression of activin A in the heart suggests a possible role in the endothelial–mesenchymal transitions related to vascular remodeling.

The expression of activin A has also been correlated with fibrosis and tissue repair in other organ systems. In this regard, increased expression of activin A has been reported in rat liver fibrosis/cirrhosis (Sugiyama et al. 1998) and in pulmonary fibrotic lesions (Matsuse et al. 1996). Other studies have identified activin A expression...
associated with skin wounding (Hübner et al. 1996). In a related report, Munz et al. (1998) demonstrated that transgenic expression of activin A in mouse epidermis led to skin thickening, fibrosis, and accelerated wound healing in response to epidermal injury. These results support experiments that correlated delayed wound healing with reduced activin A expression following cyclosporine A administration in a rat model in vivo (Petri et al. 1998). Taken together, these findings indicate that activin A may be an important mediator of both normal and pathological wound healing in a variety of tissues.

Conclusion

The adverse vascular remodeling that occurs in diseases such as atherosclerosis and restenosis involves the migration and proliferation of multiple cell types in the vessel wall. Despite the fact that many endogenous molecules have been reported to stimulate these processes, accumulating evidence suggests that only a subset of factors (e.g., PDGF, GPCR agonists) significantly contribute to the progression of fibroproliferative vascular diseases. Activin A has recently been identified as a secreted protein expressed by vascular SMCs in response to GPCR agonists. The adverse vascular remodeling that occurs in diseases such as atherosclerosis and restenosis involves the migration and proliferation of multiple cell types in the vessel wall. Despite the fact that many endogenous molecules have been reported to stimulate these processes, accumulating evidence suggests that only a subset of factors (e.g., PDGF, GPCR agonists) significantly contribute to the progression of fibroproliferative vascular diseases. Activin A has recently been identified as a secreted protein expressed by vascular SMCs in response to GPCR agonists.

References


