Review

Multifactorial basis for coronary collateralization: a complex adaptive response to ischemia

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Abstract

Angiogenesis and vasculogenesis are adaptive responses of the coronary collateral circulation to myocardial ischemia. This review focuses on the concerted action of growth factors, growth factor receptors, extracellular matrix, and inflammatory cellular responses to regulate angiogenesis and vasculogenesis in response to myocardial ischemia and alterations in shear stress. Therapeutic angiogenesis represents a novel approach to increase myocardial perfusion in patients with coronary artery disease and provides an opportunity to further clarify the mechanisms that regulate collateral development. Impairment of angiogenic adaptive responses to ischemia during disease states is an important subject for future investigation. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Angiogenesis; Coronary collateral; Growth factors; Myocardial ischemia; Vasculogenesis

1. Introduction

Chronic imbalances of myocardial oxygen supply and demand produced by a coronary artery stenosis or occlusion have been shown to increase growth of the coronary collateral circulation. Human, canine, pig, and pony hearts respond to either a slow, progressive coronary artery stenosis [1] or repetitive brief coronary artery occlusions [1–3] with a growth adaptation of the collateral circulation. Chronic ischemia-induced enhancement of collateral perfusion most likely involves de novo angiogenesis [4–6] and expansion of preexisting collaterals [7] via the addition of new vascular components (endothelium, smooth muscle, and fibroblasts). Mechanical factors including increases in blood flow and vascular shear stress contribute to collateral development and serve as remodeling influences [8–11]. Collateralization leads to enhanced oxygen delivery to the area at risk and may prevent myocardial infarction if the compensatory growth process proceeds with sufficient rapidity. Modulation of this adaptive response to ischemic challenge and its investigation have been directed at a continuum of events from potential triggers of collateral development with subsequent growth factor release to the implications of therapeutic angiogenesis during various disease states.

2. Growth adaptation of the coronary collateral circulation

2.1. Angiogenesis

The growth of new capillary vessels, termed ‘sprouting’ angiogenesis, is a highly regulated, complex sequence of events that has been established in other tissues [12] and is assumed to be similar during coronary vascular growth. Initially, enzymatic degradation and dissolution of extracellular matrix architecture occurs as a result of elaboration of proteases (including collagenase and plasminogen activator) by endothelial cells [13,14]. This process allows
endothelial cell migration into the perivascular space [15]. The angiogenic stimulus must be chemotactic for the migrating endothelial cells [16]. Endothelial cells that lag behind the leading edge of migration must also proliferate, suggesting that angiogenesis requires a mitogenic component [17]. Endothelial proliferation also results from loss of contact inhibition and disruption of cell–cell membrane interactions [18]. Coalescence of intracellular vacuoles in adjacent endothelial cells may lead to the formation of a new vascular lumen [19]. This process is accompanied by pericyte migration along the endothelial sprout and new basement membrane structure is subsequently laid down by the combined actions of endothelium and pericytes.

2.2. Vasculogenesis

Coronary collateral development also requires proliferation and remodeling of vascular endothelium and smooth muscle cells in preexisting collateral vessels, a process described by Schaper [1,20] as ‘recapitulated’ vasculogenesis. Vasculogenesis is initiated by passive dilation of existing channels, rupture of the elastic lamina, and extravasation of platelets and white blood cells, including monocytes. Invasion of the vascular wall by monocytes is frequently observed in developing collaterals, and release of angiogenic factors from these and other inflammatory cells appear to play an important role in vasculogenesis [4,21–24].

3. Triggering coronary collateral development

The inciting event responsible for coronary collateral development remains controversial despite intense investigation. In 1971, Schaper et al. [25] demonstrated a relationship between myocardial ischemia, DNA synthesis, and endothelial and smooth muscle cell mitosis in canine coronary collateral vessels. The authors [25] originally proposed that physical forces resulting from altered coronary arterial flow during ischemia induced collateral vessel growth. A subsequent investigation from the same laboratory demonstrated [3H]thymidine incorporation into adjacent arterioles, veins, and capillaries, suggesting that physical forces could not be the only stimulus for collateral growth because tangential wall stress in veins and capillaries is significantly less than in arterioles [26].

The importance of myocardial ischemia as the trigger of angiogenesis has been suggested by early findings demonstrating that the duration of repetitive coronary artery occlusions are crucial for collateral development to occur. Very brief episodes of myocardial ischemia (15 s) were an insufficient stimulus whereas longer periods (2–5 min) of coronary artery occlusion produced marked coronary collateral development [27–29]. While these findings do not exclude differential degrees of shear stress as a contributing factor to collateral development, production of multiple microvascular occlusions by experimental embolization of a distal coronary perfusion territory promoted coronary collateral growth without altering pressure gradients or shear stress between large epicardial coronary arteries [30]. In a canine ameroid constrictor model, during which coronary artery occlusion is produced over several weeks by gradual swelling of hygroscopic material (ameroid) implanted around a coronary artery, collateral blood flow also increased between vascular beds that were not immediately adjacent to the ameroid-occluded artery [31]. Thus, pressure gradients or shear stress are not necessarily responsible for epicardial coronary collateral development. Finally, the expression of cytokines [32], whose actions are vital to collateral development, are enhanced by hypoxia or ischemia [33,34], providing strong evidence of a link between myocardial ischemia and induction of collateral development.

Vascular mechanical forces or shear stress have also been suggested to initiate collateral development. However, these forces are probably most important during remodeling of the collateral circulation as growth progresses and the ischemic signal wanes. Increases in shear stress that accompany increased flow through stenotic coronary arteries or occur during periods of rapid collateral development cause activation of endothelial cells and initiate an inflammatory cellular reaction [1]. Monocytes, macrophages and platelets attracted to activated endothelium observed under these conditions appear to be important sources of mitogens contributing to vascular remodeling [1,4,21]. The endothelium itself is also responsive to increases in shear stress, and this stimulus causes upregulation of platelet-derived growth factor [35] and nitric oxide synthase [36], two potential modulators of collateral development.

4. Growth factors and related receptors

Schaper and co-workers [25,37] speculated two decades ago that diffusible vascular growth factors may play an important role in the induction and regulation of coronary collateral development in vivo. Subsequently, the identification of growth factor(s) that initiate or regulate angiogenesis or vasculogenesis has been the subject of comprehensive investigation. Table 1 summarizes the experimental evidence supporting a role for several potential modulators of collateral development in the heart.

4.1. Fibroblast growth factors

The angiogenic potential of fibroblast growth factors (FGFs) were the first to be evaluated. Acidic FGF (FGF-1) and basic FGF (FGF-2) are heparin-binding polypeptides [38] that are widely distributed in a variety of tissues [39], serve as potent cellular mitogens [40], and stimulate endothelial cell migration [41,42]. Although FGFs stimu-
Table 1
Potential modulators of angiogenesis and vasculogenesis in the heart

<table>
<thead>
<tr>
<th>Modulator</th>
<th>Reference</th>
<th>Model</th>
<th>Species</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF</td>
<td>[46]</td>
<td>+</td>
<td>Porcine myocardium</td>
<td>FGF-1 detected in myocytes beyond an ameroid constrictor</td>
</tr>
<tr>
<td></td>
<td>[47]</td>
<td>+</td>
<td>Canine myocardium</td>
<td>Increases in collateral blood flow correlate with FGF-2-induced mitogenesis</td>
</tr>
<tr>
<td></td>
<td>[48]</td>
<td>+</td>
<td>Human heart</td>
<td>FGF-2 increases in pericardial fluid in patients with unstable angina</td>
</tr>
<tr>
<td>VEGF</td>
<td>[52]</td>
<td>+</td>
<td>In situ and isolated rat hearts</td>
<td>Coronary artery occlusion or global ischemia induce VEGF expression</td>
</tr>
<tr>
<td></td>
<td>[61]</td>
<td>+</td>
<td>Porcine myocardium</td>
<td>Coronary artery occlusion induces VEGF expression</td>
</tr>
<tr>
<td></td>
<td>[60]</td>
<td>+</td>
<td>Human myocardium</td>
<td>VEGF expression increases in infarcted myocardium</td>
</tr>
<tr>
<td>IGF</td>
<td>[82,83,85]</td>
<td>+</td>
<td>Porcine myocardium</td>
<td>IGF and IGF binding protein expression is altered by microembolization or coronary artery occlusion</td>
</tr>
<tr>
<td>Growth factor receptors</td>
<td>[95]</td>
<td>+</td>
<td>Rat myocardium</td>
<td>VEGFR-1 and -2 expression increases in infarcted myocardium</td>
</tr>
<tr>
<td></td>
<td>[96]</td>
<td>+</td>
<td>Porcine myocardium and coronary microvessels</td>
<td>VEGFR-1, VEGFR-2 and FGF-2 receptor expression increases in collateral-dependent myocardium</td>
</tr>
<tr>
<td></td>
<td>[100]</td>
<td>+</td>
<td>Human endothelial cells and rat cardiomyocytes</td>
<td>Myocyte hypoxia increases endothelial cell VEGFR-2 expression</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>[52]</td>
<td>+</td>
<td>Isolated rat heart and rat cardiomyocytes</td>
<td>Hypoxia increases VEGF expression</td>
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<td></td>
<td>[61]</td>
<td>+</td>
<td>Rat cardiomyocytes</td>
<td>Hypoxia increases VEGF expression</td>
</tr>
<tr>
<td></td>
<td>[63]</td>
<td>+</td>
<td>Rat cardiomyocytes</td>
<td>Hypoxia and transition metals increase VEGF expression</td>
</tr>
<tr>
<td></td>
<td>[67]</td>
<td>+</td>
<td>Canine coronary vascular smooth muscle cells</td>
<td>Hypoxia increases VEGF expression</td>
</tr>
<tr>
<td>Proteases</td>
<td>[102]</td>
<td>+</td>
<td>Porcine myocardium</td>
<td>uPA expression increases after coronary artery occlusion</td>
</tr>
<tr>
<td></td>
<td>[103]</td>
<td>+</td>
<td>Canine myocardium and coronary collateral vessels</td>
<td>MMP and PA expression are temporally regulated in coronary collaterals</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>[139]</td>
<td>+</td>
<td>Bovine coronary venular endothelial cells</td>
<td>VEGF stimulates cell proliferation through production of NO</td>
</tr>
<tr>
<td></td>
<td>[143]</td>
<td>+</td>
<td>Bovine coronary venular endothelial cells</td>
<td>NO induces cell proliferation and increases in uPA by inducing FGF-2</td>
</tr>
</tbody>
</table>

Late several processes required during angiogenesis [43], they may also serve as trophic factors in myocardium [20,44]. FGF-1 and FGF-2 have been localized to the nucleus and perinuclear regions [44] of normal cardiac myocytes [38,44,45]. This evidence suggests that FGFs act as autocrine factors [41] and regulate normal cellular processes [42]. However, FGFs may also be important during the adaptive response to myocardial ischemia. FGF-1 was localized to collateralized ischemic myocardium adjacent to focal areas of necrosis after ameroid-induced coronary artery occlusion in swine [46]. Temporal increases in mitogenic activity of FGF-2 in the ischemic zone after coronary artery occlusion in dogs also paralleled increases in collateral blood flow. FGF-2 activity acutely increased 1 week but returned to normal levels 8 weeks after coronary artery occlusion concomitant with an increase in collateral perfusion [47], findings that support a relationship between FGF activity and collateral development.

A potential role for FGFs during coronary collateral development in humans has been suggested by substantial increases in FGF-2 concentrations in the pericardial fluid obtained from patients with unstable angina [48] as compared to those without evidence of ischemic heart disease [48–50]. Increases in plasma concentrations of FGF-2 were temporally related to the onset of acute myocardial infarction in patients with coronary artery disease [49]. Plasma FGF-2 increased in patients 10 days and returned to normal values 30 days after acute myocardial infarction [50]. This association suggests that coronary collateral development may be stimulated by increases in FGFs. FGF may be released following cell death or from
binding sites present in the extracellular matrix [51]. Release of FGF following cell lysis may account for changes in FGF-2 expression that were detected after prolonged, but not transient myocardial ischemia in rats [52].

4.2. Vascular endothelial growth factors

The vascular endothelial growth factors (VEGF) are secreted, soluble heparin-binding vascular endothelial cell mitogens that stimulate angiogenesis in vitro and in vivo [53,54]. Four distinct isoforms of VEGF (VEGF-A: containing 121, 165, 189, and 206 amino acids) exist that are products of alternative mRNA splicing [55,56]. Additional genes in the VEGF family have also been identified encoding the proteins VEGF-B and VEGF-C [57–59], however, the significance of these other VEGF family members during coronary collateral development are unknown. VEGF contains signaling peptide sequences that allow its active secretion [53], an important property that distinguishes this growth factor from FGFs. VEGF mRNA is constitutively expressed in normal myocardium [60]. VEGF mRNA transcription is also increased in arteriolar smooth muscle cells adjacent to areas of myocardial infarction, and has been localized to infiltrating macrophages [60]. Brief periods (2–10 min) of myocardial ischemia are sufficient to induce 3- to 5-fold increases in VEGF mRNA expression in pigs [61]. Similarly, maximal expression of VEGF mRNA may be induced within 30 min after a transient ischemic insult and may persist for as long as 3 h in isolated, perfused rat hearts [52].

4.3. Regulation of VEGF by hypoxia

A primary role for VEGF, and not FGF [62], during coronary collateral development is suggested by observations that this mitogen is upregulated by hypoxia [52,61–67]. VEGF mRNA expression in vascular smooth muscle cells isolated from the left anterior descending coronary artery of dogs is increased within 24 h of exposure to hypoxia and is reversible within 8 h after return to normoxic conditions (Fig. 1) [67]. Moreover, conditioned media from hypoxic smooth muscle cells causes marked proliferation of endothelial cells and the response is 2-fold greater than that observed during normoxia. Evidence from tumor cell lines indicates that regulation of the VEGF gene by hypoxia is similar to another oxygen-sensitive gene, erythropoietin [68–72]. Like erythropoietin [70], the expression of VEGF is regulated at the transcriptional and post-transcriptional levels. VEGF mRNA transcription is increased 3-fold shortly after introduction of hypoxic conditions and a cis-acting element of the VEGF gene confers this responsiveness to hypoxia in C6 glioma cells [73]. Transcriptional activation of VEGF by hypoxia is mediated by a hypoxia-inducible factor in a rat tumor cell line [34]. In addition to regulation at the transcriptional level, hypoxia substantially increases the half-life of VEGF mRNA [34,64,73]. Under normal conditions, VEGF mRNA is relatively unstable but during hypoxia, mRNA stability is increased [74]. This change in transcript stability is protein synthesis-dependent [66] via interactions with factor(s) produced or stabilized during hypoxia. One such factor, identified as HuR, has recently been shown to specifically enhance VEGF mRNA stability during hypoxia in cultured cells [75]. VEGF mRNA is also stabilized during ischemia-induced hypoglycemia [64], findings that suggest that VEGF is an important stress-response protein. Finally, VEGF expression may be regulated by an oxygen-sensing mechanism similar to erythropoietin. By substituting for iron in the protoporphyrin ring transition metals decrease oxygen binding to heme-containing proteins and increase expression of both VEGF [70] and erythropoietin [71]. These in vitro data provide an important conceptual framework underscoring the potential role of tissue hypoxia to modulate VEGF expression during coronary collateral development.

4.4. Contextual regulation of VEGF

While hypoxia may be a primary regulator of VEGF, the expression of VEGF is also determined by the presence of other growth factors. This interaction between VEGF and other growth factors may be especially important during vascular adaptation to myocardial ischemia and indicates redundancy in the regulation of collateral development. Such redundancy is a hallmark of crucial biological processes. The precise action of a given cytokine on an endothelial cell target is dependent on the concentrations of other angiogenic mediators in the pericellular environment, a concept termed ‘contextual’ angiogenesis [76]. For example, FGF [33,62], transforming growth factor β (TGF-β) [62], and platelet-derived growth factor BB (PDGF-BB) [77] increase the expression of VEGF, pre-
growth factors amplify the hypoxia-induced signal for VEGF. The expression of VEGF during hypoxia is synergistically increased by FGF-2 [33], TGF-β [33], and PDGF-BB [77] in vascular smooth muscle cells. Synergism between VEGF and other growth factors has been demonstrated in an in vitro model of angiogenesis. VEGF stimulates endothelial cells to invade underlying collagen gel matrix and form tube-like structures [78]. The actions of VEGF are markedly enhanced by FGF-2 in this model [78,79]. VEGF and FGF-2 are also temporally regulated during coronary collateral development in a canine model of intermittent myocardial ischemia [80]. Interstitial fluid sampled from the collateral-dependent region is mitogenic for endothelial and vascular smooth muscle cells in vitro, and this response occurs concomitantly with increases in coronary collateral blood flow in vivo. Anti-VEGF antibody decreased the mitogenic response of interstitial fluid within 7 days after initiation of repetitive coronary artery occlusions, whereas antibody to FGF-2 neutralized the mitogenicity later (days 12–14) during collateral development [80]. These findings indicate that the release of growth factors into interstitium may be temporally [81] and sequentially [80] regulated. It also emphasizes that coronary collateralization is probably not related to a single growth factor but may require many factors acting in concert. This concept has important ramifications for therapeutic maneuvers based on administration of a single growth factor.

4.5. Insulin-like growth factors

Insulin-like growth factors (IGF) are constitutively expressed in cardiac myocytes [82,83] where they may produce insulin-like metabolic effects [84]. Expression of IGF-1 mRNA is increased nearly 2-fold 72 h after experimental microembolization in pigs, suggesting that IGF-1 is inducible by an ischemic stimulus [82]. IGF-1 has been localized to invading monocytes in regions of focal necrosis and the temporal association between IGF expression and the development of capillary sprouting in the ischemic region suggests that IGF-1 plays a role in coronary collateral development in vivo [82]. Multiple genes in the IGF system, including IGF-1 and several IGF binding proteins, are simultaneously expressed in ischemic myocardium during inflammation-linked angiogenesis [85]. VEGF expression is also enhanced by IGF-1 in human osteoblast-like cells [86]. These findings suggest that IGF-1 may alter the expression of other growth factors, enhancing their action during coronary collateral development.

4.6. Growth factor receptors

Angiogenesis and vasculogenesis require the concerted action of both growth factors and their receptors. Alterations in receptor number or affinity may be as important as the availability of their respective growth factors. Three high-affinity, VEGF tyrosine kinase receptors have been identified that are predominantly expressed by endothelial cells. VEGFR-1 (Flt-1) and VEGFR-2 (KDR or the murine homologue fetal liver kinase-Flk-1) are required for normal embryological development of the vascular system [87,88], and the more recently identified VEGFR-3 [89–91] appears to be important during lymphatic development [90]. VEGFR-2 has been shown to be crucial during endothelial cell differentiation and early vasculogenesis [92], and disruption of the VEGF-2 gene results in embryonic death [92]. VEGFR-1 appears to regulate the formation of normal vascular channels [93], but stimulation of this receptor alone does not cause endothelial cell proliferation or mediate chemotaxis [94].

Enhanced expression of VEGF receptors may play an important role during collateral development following myocardial infarction. Messenger RNA encoding VEGFR-1 and VEGFR-2 is substantially augmented and transcript expression occurs in a precise temporal and spatial fashion in rats after myocardial infarction [95], VEGF receptor mRNA is strikingly increased in vessels bordering the infarcted region and receptor expression is also elevated in areas remote from the infarction 24 h after coronary artery ligation. VEGF receptor expression in remote regions returns to normal levels 7 days after infarction. In contrast, both VEGFR-1 and VEGFR-2 expression remain markedly enhanced in newly formed small vessels within the healing infarct and VEGFR-1 is also increased in large vessels at the border of infarcted myocardium. In another model of coronary collateral development, the expression of VEGFR-1, VEGFR-2, and the FGF-2 receptor is increased in collateral-dependent, but not normal, swine myocardium 7–9 weeks after implantation of an ameroid constrictor (Fig. 2) [96]. VEGFR-1 is also expressed by monocytes [97] and the presence of monocytes temporally correlates with collateral development [98], findings that support an important interaction between growth factors and inflammatory cells.

4.7. Regulation of growth factor receptors by hypoxia

The connection between inadequate tissue oxygen delivery and collateral development is further strengthened by observations that hypoxic stimuli induce expression of VEGF receptors [99–101]. This association was first identified in chronically hypoxic rat lung. VEGF mRNA and protein are elevated in pulmonary parenchyma after 28–32 days of exposure to hypoxia concomitant with a parallel, more pronounced increase in VEGFR-1 and VEGFR-2 mRNA expression. Gene expression is upregulated in isolated perfused lungs after only 2 h of ventilation with a hypoxic gas mixture, and VEGF and VEGF receptor
5. Role of extracellular matrix during collateral development

Coronary collateral development requires the coordinated synthesis and degradation of extracellular matrix to facilitate invasion of migrating endothelial cells and remodeling of newly formed collateral vessels. Several proteases are important in this process including: plasmin [activated by urokinase and tissue plasminogen activators (uPA and tPA, respectively)] and matrix metalloproteinases (MMP) that degrade collagen and elastin. Urokinase plasminogen activator gene expression and protein activity are increased in ischemic myocardium during gradual coronary artery occlusion with an ameroid constrictor in pigs [102]. Plasminogen activator inhibitor (PAI) expression is simultaneously enhanced, indicating that extracellular matrix turnover is tightly regulated during coronary collateral development. MMPs are active during migration of endothelial and vascular smooth muscle cells and play an important role in the proteolytic cascade required during the development and remodeling of coronary collateral vessels. The activities of MMP and tPA are increased in canine coronary collaterals 2–4 months after ameroid constriction.
occlusion and mRNA transcripts for MMP-1 are elevated 5–8-fold in collateral vessels [103]. In contrast, concentrations of the endogenous tissue inhibitor of MMP are reduced during vascular adaptation to chronic myocardial ischemia.

Recent evidence suggests that growth factors and proteases are coordinately modulated during angiogenesis. VEGF stimulates MMP expression in human aortic smooth muscle cells. MMP expression occurs in response to phosphorylation of the VEGFR-1 receptor and these actions accelerate smooth muscle cell migration through a synthetic extracellular matrix barrier [104]. Degradation of extracellular matrix by proteases also cause the release of heparin-bound growth factors, including FGF and VEGF [105,106]. Interestingly, growth factors may be involved in a positive feedback loop with plasminogen activators during endothelial cell migration. FGF-2 increases uPA activity [43], and the expression of uPA and its receptor is localized to the leading edge of migrating endothelial cells in culture [107]. VEGF-induced angiogenesis in a collagen gel and PA expression are also dependent on endogenous FGF-2 in vitro, whereas inhibition of FGF-2 in VEGF-treated cells causes an overall antiproteolytic effect [108]. Thus, coronary collateral development is likely to involve a similar, highly coordinated cascade of events integrating cellular responses to and modulation of growth factor release, plasminogen-activator expression, chemotaxis, and extracellular matrix remodeling.

6. Therapeutic angiogenesis

Intense investigation has been directed towards enhancing the development of coronary collaterals by exogenous administration of growth factors and gene therapy. Investigation of therapeutic angiogenesis provides further insights into the process of coronary collateral development. FGFs were the first growth factors administered in an attempt to enhance development of the coronary collateral circulation. Initial studies using FGF-1 were unsuccessful [109–111], whereas, administration of intracoronary FGF-2 shortly after acute coronary artery occlusion salvaged infarcted myocardium [112]. Angiographic and histological evidence of new arteries, arterioles, and capillaries extending from the surrounding normal zone to the infarcted myocardium were observed in FGF-2-treated dogs and were accompanied by significant reductions in infarct size. Similarly, intracoronary FGF-2 enhanced neovascularization of infarcted myocardium in pigs, although regional wall motion remained unchanged [113]. The temporal relationship between FGF administration and collateral development was extensively studied by Unger and colleagues [114–116]. Chronic administration of FGF-2 to dogs after ameroid constrictor placement enhances blood flow, promotes cellular proliferation, and increases vascular density in collateral-dependent myocardium [115]. The maximum benefit derived from exogenous administration of FGF-2 is achieved between 10 and 17 days after ameroid placement, an interval coincident with the presence of myocardial ischemia [116]. FGF-2 provided no additional benefit when administered for more than 5 weeks after ameroid constrictor implantation, however its salutary effect did not dissipate when treatment was withdrawn after 5 weeks. In contrast to these findings, FGF-2 treatment provided no benefit when compared with saline-treated controls 6 months after ameroid constrictor implantation in dogs [114]. FGF-2 also had no effect when administered to dogs with mature coronary collaterals. Because a beneficial effect of FGF-2 appears to be confined to a narrow time interval during closure of the ameroid constrictor, Shou et al. [114] hypothesize that ‘pharmacologically driven’ collateral development requires ischemia or the presence of a transcollateral pressure gradient for growth factors to be therapeutically effective.

Growth factor-induced development of coronary collaterals during therapeutic angiogenesis appears to depend on the route of administration. Myocardial infarct size is reduced [117], and coronary collateral blood flow, ejection fraction, and regional wall thickening are increased after ameroid constrictor placement and perivascular application of FGF-2-loaded beads in pigs [118]. Perivascular delivery of FGF-1, genetically modified to enhance its stability, increased resting coronary collateral blood flow and enhanced coronary collateral reserve [119]. Similarly, intrapericardial administration of FGF-2 increased the number of arterioles present in infarcted canine myocardium [120], and enhanced angiogenesis in a rabbit model of left ventricular hypertrophy [121]. In contrast to intrapericardial, perivascular or intracoronary delivery, less than 1% of FGF-2 is recovered from myocardium after intravenous administration and FGF-2 fails to enhance coronary collateral development in dogs when administered via an intravenous route [122]. Myocardial recovery rates are 10- and 100-fold higher after intracoronary or intrapericardial administration, respectively, and FGF-2 has been localized to extracellular matrix and vascular endothelium under these experimental conditions [122]. Thus, important pharmacokinetic effects contribute to differences in therapeutic efficacy during administration of FGF.

Intracoronary VEGF also enhances collateral blood flow, increases the density of intramyocardial vessels in the collateral-dependent zone [123], decreases myocardial infarct size, and improves left ventricular ejection fraction [124] in animals after ameroid constrictor implantation. Similar to the findings with FGF, differences in VEGF pharmacokinetics may also contribute to marked differences in therapeutic efficacy. Intracoronary [123,124] and extraluminal administration of VEGF [125] effectively enhanced coronary collateral development. In contrast, left atrial administration failed to increase collateral blood flow [126]. Novel alternative approaches using gene transfer techniques may enhance the effectiveness of therapeutic
angiogenesis. The gene for the lesser known growth factor FGF-5 was administered by intracoronary injection using an adenovirus vector and improved coronary collateral blood flow at rest and during ventricular pacing in pigs after ameroid constrictor placement [127]. Evidence of capillary angiogenesis and improvements in regional contractile function further demonstrated the efficacy of this gene transfer technique.

The application of these exciting new techniques to humans with coronary artery disease is currently underway. Recently, Schumacher et al. [128] performed a seminal study during which FGF-1 was injected directly into the ischemic myocardium of patients undergoing surgical revascularization of the left anterior descending coronary artery perfusion territory with an internal mammary artery graft. Forty patients were randomly assigned to receive either active or heat-denatured FGF-1. Follow-up angiography was performed 12 weeks after surgery. Angiogenesis was demonstrated by the presence of an enhanced capillary network in a region surrounding the site of FGF-1 injection. Retrograde filling of stenosed vessels through newly formed vascular networks was also visible in patients treated with active FGF-1. Subsequently, a clinical trial of seven patients with coronary artery disease undergoing direct myocardial revascularization received an injection of FGF-2, contained within a heparin-alginate slow-release device, into myocardium that could not be surgically revascularized [129]. Three months after surgery, three patients demonstrated enhanced perfusion to FGF-2 treated myocardium that was not revascularized, however, three other patients had new small, fixed perfusion deficits consistent with infarction. Thus, while administration of FGF-2 enhanced angiogenesis in some patients, others failed to respond. A more complete understanding of how vascular adaptation to myocardial ischemia is modulated in vivo and how this process may be altered by different disease states is crucial if therapeutic angiogenesis is to be truly beneficial.

7. Growth factors and nitric oxide

Growth factors may increase coronary collateral blood flow not only through angiogenesis but also via interactions with nitric oxide. Intracoronary VEGF decreases mean arterial pressure and systemic vascular resistance in vivo, hemodynamic alterations that are blocked by inhibition of nitric oxide synthase [130,131]. VEGF relaxes coronary microvessels [131] and isolated coronary arteries [132] through calcium-dependent synthesis or nitric oxide release [96,132]. VEGF upregulates nitric oxide synthase mRNA expression, increases nitric oxide synthase protein concentrations, and enhances nitric oxide production in human endothelial cells in a dose-dependent fashion [133]. Growth factors also restore normal vascular responsiveness to microvessels harvested from ischemically injured tissue. Endothelium-dependent and β-adrenergic vascular responses are impaired in chronically ischemic collateral-dependent microvessels, however, treatment with VEGF [125,134] or FGF-2 [96,135] results in preservation of these responses. This preservation of vascular reactivity is dependent on activation of tyrosine kinase and nitric oxide synthase [96,132]. Furthermore, enhanced collateral-dependent microvessel responses are associated with increased expression of the tyrosine kinase receptors VEGFR-1, VEGFR-2, and FGF-1 [96]. Thus, VEGF and FGF cause endothelial release of nitric oxide mediated by activation of upregulated tyrosine kinases, and these actions may contribute to increases in coronary collateral blood flow in vivo [96].

While growth factors are unlikely to signal exclusively via nitric oxide, interactions with nitric oxide may directly modulate angiogenesis [136]. The activity of nitric oxide synthase is important during reconstitution of the circulation beyond a ligated femoral artery in a murine model of peripheral vascular disease. Distal limb perfusion and capillary density are severely impaired in mice with targeted disruption of the gene encoding endothelial nitric oxide synthase as compared with mice possessing a wild-type gene (Fig. 3) [137]. Administration of VEGF did not restore normal collateral development in nitric oxide synthase-deficient mice, findings that suggest that nitric oxide synthase may be a downstream effector of VEGF [137,138]. Nitric oxide also mediates the mitogenic effect of VEGF in endothelial cells isolated from coronary post-capillary venules [139]. VEGF-induced cell proliferation and DNA synthesis occurred concomitant with increases in cyclic GMP concentration, and these changes were blocked by nitric oxide synthase inhibition.

Growth factors cause differential activation of nitric oxide signal transduction pathways during angiogenesis. Inhibition of nitric oxide synthase abolishes endothelial cell migration and in vitro angiogenesis induced by VEGF [136,138], but not FGF-2 [138,139]. Nitric oxide has also been shown to cause antiangiogenic effects in chick embryo chorioallantoic membrane [140] and inhibits smooth muscle cell proliferation [141]. Recent findings may clarify these apparent conflictual results. Nitric oxide terminated the proliferative action of FGF-2 and promoted endothelial cell differentiation into vascular tubes in a 3-dimensional fibrin gel. The angiogenic effects of FGF-2 were abolished by nitric oxide synthase inhibition [142], findings that support the contention that nitric oxide acts as a molecular ‘switch’ to initiate endothelial cell differentiation during later phases of angiogenesis and in response to exogenous FGF. Nitric oxide also modulates angiogenesis by upregulating uPA expression and subsequently induces FGF-2 [143]. These novel findings suggest that nitric oxide enhances growth factor release and contributes to endothelial cell differentiation despite direct effects to inhibit smooth muscle cell proliferation. A specific role for nitric
oxide during coronary collateral development requires further investigation.

8. Altered regulation of collateral development in disease states

The mechanisms by which disease states alter angiogenesis or vasculogenesis are unclear. For example, the mechanisms whereby some patients with coronary artery disease develop coronary collaterals while others do not, are enigmatic. Sellke et al. [144] have taken a first step toward resolving this issue by evaluating coronary microvascular responses to VEGF in atrial microvessels harvested from 29 patients with coronary artery disease. VEGF caused relaxation of vessels from patients without coronary artery disease, and this response was blocked by inhibition of nitric oxide synthase or tyrosine kinase. In contrast, vasodilator responses to VEGF were profoundly impaired in atrial vessels from patients with coronary artery disease (Fig. 4) [144]. Alterations in expression of growth factors or growth factor receptors were not responsible for the abnormal vascular responses to VEGF. The expression of VEGF, the tyrosine kinase receptors, VEGFR-1, VEGFR-2, and constitutive and inducible nitric oxide synthase were unchanged in patients with versus without coronary artery disease. Thus, coronary artery disease is associated with a reduced response to VEGF in vitro. While the authors did not specifically investigate collateral development, the results support the contention that impaired responses to endogenous mediators of angiogenesis are of importance.
genesis or vasculogenesis, may account for inadequate collateral development in certain patients. The findings also suggest that therapeutic angiogenesis may be limited in certain patients as previously demonstrated [129].

Diabetes mellitus may also alter the regulation of coronary collateral development. Capillary density in infarcted myocardium of patients with diabetes is substantially less than that observed in normoglycemic patients [145]. Recent experimental evidence indicates that diabetes impairs collateral development in an ischemic hindlimb model [146]. Endothelial cell migration and tubular microvessel formation may be impaired in diabetes [145] by decreases in uPA activity [147]. IGF [148,149] and its receptor [149] are also downregulated in neural tissue in diabetes, however, it is unknown whether abnormal regulation of growth factors or their receptors contributes to alteration of coronary collateral development or increases in cardiovascular mortality in diabetic patients. The presence of anti-angiogenic factors actively secreted during diabetes or other disease states and impairment of nitric oxide-mediated mechanisms that may attenuate collateral development requires further study. Investigation of how disease states alter the development of the coronary collateral circulation is urgently required because the results of such experiments may impact the outcome of clinical trials of therapeutic angiogenesis.

9. Conclusions

Increases in coronary collateral blood flow by growth adaptation of preexisting coronary collaterals or neovascularization may reduce myocardial ischemia, prevent infarction, and preserve contractile function. It is clear that coronary collateral development is a complex sequence of events that proceeds through the orchestrated release of growth factors, expression of growth factor receptors, the concerted action of several cell types responding to ischemic stimuli, and alterations in vascular shear stress. Important recent advances have been obtained by the direct application of growth factors or gene therapy targeted to ischemic myocardium and recent investigations implicate nitric oxide as a potential regulator of angiogenesis and vasculogenesis. Interactions between disease states and the control of angiogenesis, particularly during therapeutic angiogenesis, represent critical areas of future research.

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