Vascular Failure: A Hypothesis

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Although cardiac failure has been studied extensively, vascular failure is not a recognizable term. We suggest that it is reasonable to argue that failure of the vessel to control its mass, contractile capacity, and lumen will involve pathways similar to cardiac failure. Vascular failure, or perhaps more accurately arterial failure, has very different consequences. Failure to control mass and external diameter will result in hypertension or loss of lumen in atherosclerosis. We review what is known about this normal remodeling response and its failure, and propose directions for research.

Introduction

Any high school student working with a battery, a few wires, and resisters could tell us what to expect once the blood vessels evolved closing the circulation. If the wires were simply connected across the battery (*ie*, if a blood vessel simply connected the two sides of the heart), the heart would burn up. A successful circuit needs to have resistance to disperse the potential energy (pressure or voltage) driving the flow or current. A circuit as sophisticated as the human circulation needs even more complex elements (*eg*, capacitators to even out the flow, variable resisters to adapt the circuit to different local needs, switching elements, and so forth).

This physics comparison is well and good, but the wires of the circulation are themselves living tissues. Just as the heart has a complex biochemistry to allow it to remodel in response to different physical needs, and just as failure of normal cardiac remodeling leads to cardiac failure, the same must be true of blood vessels. Failure of normal vascular remodeling is as likely to be important as is cardiac failure.

What is "vascular" failure? This review focuses on remodeling of the arteries and the role it plays in atherosclerosis and hypertension. We also review recent work from our own laboratory, and propose that a specific biochemical pathway is a candidate for research in vascular remodeling.

The Physical Basis of Arterial Remodeling: h and r_{ext} as Critical Values

Figure 1 summarizes our knowledge of the laws controlling flow through arteries. Arterial blood flow, according to the Pouseille equation for laminar flow in any tube, depends on the fourth power of the radius of the lumen, "r." These equations are derived from both pure physics (Pouseille and LaPlace) and biologic experiments (Glagov and Wolinsky). Pouseille and LaPlace tell us that the ability of an artery to conduct flow will depend on the size of the lumen (actually the fourth power of the radius), and that the wall must provide an adequate tension to retain the driving pressure. It is this tension issue that evokes a lot of the biology. Wolinsky and Glagov [1] showed that the number of layers of the vessel wall and wall thickness are a function of lumen size and, of course, that blood flow depends on lumen size. Glagov *et al*. [2], in turn, showed that even as the intima grows in atherosclerosis, the lumen enlarges to maintain blood flow. This discovery was highly anticanonical because the common assumption was that the atherosclerotic plaque is intrusive on the lumen.

Arteries need to keep their lumens open; however, as the radius increases, so does wall tension, "T." As a result, arteries need to control wall mass, "h," and do so without restricting the internal radius of the artery. In practice, the external diameter of the artery, r_{ext}, increases to compensate for changes in wall mass [1–4]. Failure of the artery to maintain normal values for h and r_{ext} results in restenosis, narrowing in atherosclerosis, graft failure, and hypertension. These physical principles must be followed in normal arterial development, as well as in development of an arterial supply to new tissues.

Biochemical Adaptation of Arterial Walls

The most obvious issue the equations raise is the molecular basis for the control of the two variables emerging from the equations … lumen radius, r, and wall thickness, h. These equations have had a major impact on our understanding of the increased resistance characteristic of high blood pressure. Folkow [3], and later Mulvany *et al*. [5,6], proposed that most of the elevation in resistance was due to an increase in wall thickness rather than a change in lumen diameter at rest. The concept is called an amplifier

Laws of flow

Pouseille

$$F = k_p \frac{\Delta P r^4}{1\eta}$$

LaPlace

$$T = P \frac{r}{h}$$

Wolinsky

$$T = k_w$$

$$\therefore k_w = P \frac{r}{h} \text{ ; and... } \frac{h}{r} = \frac{P}{k_w}$$

h/r is called the **Wall-Lumen ratio**.

Folkow

$$F = k_p \frac{\Delta P(r_{ext} - h)^4}{1\eta}$$

Glagov

for a given normal vessel

$$F = k_g$$

$$\therefore k_g = k_p \frac{\Delta P(r_{ext} - h)^4}{1\eta}$$

$$k_g = k_p \frac{\Delta P(r_{dil} (1 - \frac{P}{k_w}))^4}{1\eta}$$

but, under pathologic conditions, Wolinsky's Law fails, so **h/r** is no longer a linear function of **P** and/or Glagov's law fails and flow is restricted.

Figure 1. Fundamental equations.

effect and suggests, as is in fact observed, that the lumen size of hypertensive vessels when they are relaxed is approximately normal, and that the decrease in lumen can be explained by the contractile stimulus acting on a thickened wall rather than an increased contractility.

The amplifier concept ought to have led to biochemical studies. Surprisingly, however, an extensive review of the literature has provided very little information about the protein composition or RNA expression patterns of different arteries, arterializing veins, or, for that matter, even arteries adapting to hypertension. For example, despite the importance of remodeling in hypertension, the literature on changes in expression in arteries responding to hypertension is limited to changes in one cytokine, transforming growth factor-β, and the purported correlation with matrix proteins, especially fibronectin [7–9].

The other major process requiring arterial remodeling is collateral formation. Whereas new vessels are formed in angiogenesis by sprouting capillaries, new branches originating at the post-capillary vessels (*ie*, creation of new arterial capacity to provide circulation) in response to ischemia or in response to a short occurring by enlargement and remodeling of existing small arteries [10,11]. Nothing is known about how h and r_{ext} are controlled during this process.

This lack of data in arterial remodeling is striking when compared with extensive studies in the heart where an extensive literature exists because of the relationship of cardiac hypertrophy to cardiac failure [12,13]. That literature describes hypertrophy associated with up-regulation of fetal genes like β-myosin heavy chain, α-skeletal, α-smooth muscle actin, and atrial natriuretic factor, and down-regulation of a-myosin heavy chain and SR Ca^{2+}-regulating proteins [14,15]. The identity of the signaling pathways controlling this change in expression phenotype has identified critical signaling pathways via the β-adrenergic receptor, Gαq and downstream effectors, mitogen-activated protein kinase pathways, and calcineurin. The contrast with our lack of knowledge of vascular remodeling is obvious.

Wall Thickness as a Cause of Hypertension

The analogy to cardiac remodeling is most obvious in two conditions: hypertension and collateralization. As in cardiac adaptation to exercise, hypertension and collateral formation require the vessel wall to remodel in order to normalize wall tension. In the case of hypertensive remodeling, there has been a long debate about whether changes in wall thickening, perhaps in response to elevated cardiac output, can lead to a persistent increase in wall mass and a fixed increase in peripheral resistance in hypertension [6,16]. Direct evidence that vascular mass itself may control blood pressure came several years ago, when this laboratory attempted to use transgenic growth hormone mice to test the hypothesis that primary changes in arterial mass could cause an elevation in blood pressure. Growth hormone has no hormonal effect on blood pressure. Although we did report an increase in h, blood pressure was not increased [17]. However, Bohlooly *et al*. [18] repeated the study using more accurate measurements of blood pressure. They demonstrated hypertension, implying that an increase in wall thickness may be an independent variable in the etiology of hypertension.

The growth hormone mouse studies support mathematical models of circulation. These models predict that some structural change in h is needed for the maintenance of an elevated pressure [19]. At the same time, genetic studies show that a variety of defects in volume regulation are able to raise pressure. Although the numbers of such variant genes are, as of yet, small relative to the incidence of hypertension, the suggestion is that hypertension in general could be a multigenic disease of volume regulation [20]. Why, however, should volume per se elevate blood pressure? The equations in Figure 1 and the amplifier data suggest that volume must increase h. Because it seems unlikely that this happens without a change in protein composition, the question of the composition of the hypertensive wall remains important.

Why Stents Work: The Phenomenon of Pathologic Narrowing

The rules described in Figure 1 apply not only to atherosclerosis, but also to the formation of a neointima after a vessel undergoes angioplasty. This concept became important in the past decade because of the assumption that neointima should narrow blood vessels. Neointima is the tissue formed following angioplasty and it was believed that the same tissue formed after angioplasty in an atherosclerotic vessel was responsible for loss of the gain induced by the procedure. This concept failed to be useful in predicting the outcomes of drug trials aimed at limiting intima hyperplasia. The experimental and clinical literature explains this failure. First, studies of cell replication failed to find evidence that cell replication was increased in tissue samples of restenosis obtained by atherectomy [21,22]. Second, angiographic studies suggested that the most common effect of angioplasty was to crack the vessel and that loss of gain was simply due to healing of the cracks [23]. Third, studies in experimental animals showed that even when neointima is formed, the usual result is a compensatory enlargement of the lumen, just as is seen with atherosclerosis itself [24]. Thus, "restenosis" may be no more or less than a physiologic response that restores the atherosclerotic vessel to its adapted state. Rather than being an exception to the rule, restenosis is an illustration of the rule that vessels try to preserve their lumen size. The clinical challenge then becomes overcoming the normal processes of vascular remodeling. The obvious, and now successful, answer is to overcome this physiology with a stent.

Of course, stent therapy sidesteps the issue of why some atherosclerotic lesions renew. The answer may come from two experimental exceptions to the rule that vessels remodel when new intima forms. The exceptions are inflammation and transplant atherosclerosis. When inflammatory cytokines are placed on the adventitia, vessels form a neointima and narrow [25]. Transplantation atherosclerosis may involve a similar mechanism [26]. The transplant intima may form in a very different manner than the intima in a balloon-injured vessel. Recent data suggest that the intimal cells may arise from the circulation [26]. Whatever the source of the intimal cells in transplantation, they seem to overcome the normal form of adaptive remodeling, resulting in loss of lumen. Interestingly, an inhibitor of Rho kinase, fasudil, has been shown to block this process [27]. At this point, we can only note that intima formed in transplantation, possibly including some atherosclerotic lesions, shows a failure of normal remodeling.

Arterial Versus Venous Phenotypes

Arterial remodeling is a specialized property of arteries. The obvious place to look for genes associated with arterial specific properties is to ask how arterial RNA differs from venous RNA. We performed just such an analysis of the differences in expression between arteries and veins [28••]. The most impressive of the newly found differential markers was RGS5, a member of a new family of proteins that had, as recently as 5 years ago, only begun to be explored in mammals. This observation is exciting for many reasons. RGS proteins participate in negative feedback of G protein-coupled receptor (GPCR) signaling. It is a reasonable hypothesis that arterial mass is controlled by similar mechanisms to the more extensively studied mechanisms controlling hypertrophy in the heart. There is already extensive evidence implicating Gαq (the smooth muscle hypertrophy-inducing Gα subunit) and the associated RGS4 (Gαq-negative regulator) as important mediators in control of cardiac mass [29,30].

The most relevant artery in hypertension is the small, afferent artery of the glomerulus [6]. Our in situ hybridization shows a prominent RGS5 signal in that artery.

We have found the absence of RGS5 from only one normal artery, the coronary artery. This may reflect the need of the coronary circulation to permit dilation/remodeling during hypertension, whereas other arteries must thicken their vessel walls to increase their ability to respond to elevated pressure. Morphometric studies have not been done in this vasculature; however, the coronary tree behaves contrary to peripheral vasculatures because cardiac blood flow is increased during exercise or hypertension.

In the absence of a relevant literature on control of vascular mass, our hypothesis is largely derived from cardiac biology. Both the Gαq and Gαs signaling pathways have been shown to induce major effects on heart physiology and on cardiac myocyte hypertrophy and apoptosis in animal models [29–31]. Gαq transgenic over-expression mediates changes in heart function, leading to hypertrophy, cardiomyopathy, contractile failure, and apoptosis [29–31]. Transgenic over-expression of a dominant negative form of Gαq, the C-terminal 305-359 amino acid portion (which inhibits β-adrenergic receptor signaling by 50% of normal in vitro), reduces cardiac hypertrophy versus control nontransgenic subjects [32]. In addition, double knockouts in mice of Gαq and Gα11 in cardiomyocytes lead to hypoplasia, with severe thinning of the myocardial layer, and lack of Ca^{2+} release after stimulation with either phenylephrine or angiotensin II [33]. Low-level over-expression of Gαq in cardiomyocytes induces heart weight and myocyte size whereas higher-level over-expression leads to cardiac decompensation and heart failure [29]. In an aortic coarctation model, Sakata *et al*. [34] have shown that mice transgenically over-expressing Gαq develop eccentric heart hypertrophy with decompensation, whereas nontransgenic control mice exhibit concentric hypertrophy and maintain ejection performance.

The experiment most relevant to this review came from work by Rogers *et al*. [35], which was intended to test whether the transgenic over-expression of an R4 subfamily member, RGS4, could inhibit heart hypertrophy induced by aortic banding (coarctation) through its ability to inhibit Gαq signaling by GTPase antibody protein activity. Over-expression of RGS4 in this hypertrophy model,

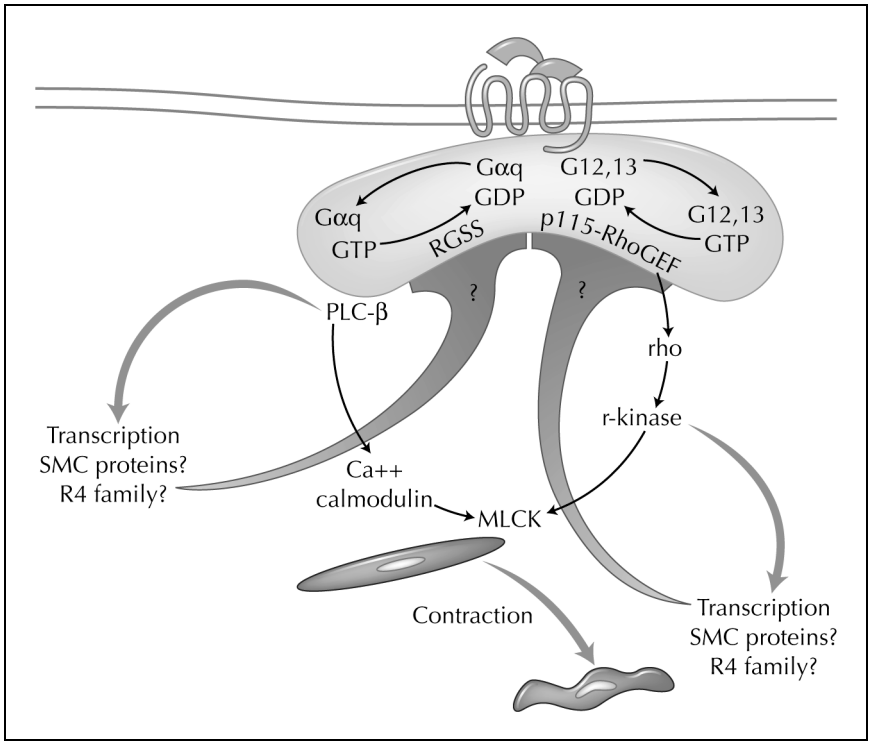

Figure 2. Pivotal role of RGS proteins in G protein-coupled receptor (GPCR) signaling. (MLCK—myosin light chain kinase; PLC—phospholipase C; SMC—smooth muscle cell.)

targeted to ventricular tissue using the α-myosin heavy chain promoter, prevented hypertrophic compensation and induced death within 3 days of coarctation, whereas nontransgenic control animals exhibited compensatory hypertrophy and survived the procedure. Furthermore, mice over-expressing both Gαq and RGS4 exhibit reduced contractile dysfunction and normalized levels of protein kinase C (PKC) compared with Gαq–over-expressing mice [36]; however, the over-expression of RGS4 did not fully compensate the observed abnormalities in left ventricular function, suggesting that additional factors, possibly other RGS proteins, are involved in normal heart homeostasis.

The fact that Rogers *et al.*'s [35,36] RGS animals were functionally normal until challenged supports the hypothesis that over-expressed RGS proteins may regulate remodeling while allowing normal postnatal development. By analogy, the presence of RGS5 in arteries may be indicative of a similar system for regulation of hypertrophy and growth in vessels.

Originally, it was believed that termination of the Gα signal was dependent on self-catalyzed hydrolysis of GTP to GDP, promoting dissociation of Gα subunits from their effectors and reassociation with βγ. However, the intrinsic Gα-GTPase activity is too slow to account for rapid physiologic responses [37,38]. Thus, the RGS family appears to play a pivotal role in regulation of activity of the GPCRs.

A Hypothesis

Role of RGS proteins in GPCR signaling

Figure 2 shows how we propose that RGS proteins occupy a critical place in determining contractile pathways for smooth muscle responding to GPCRs.

We suggest that expression levels of different RGS proteins, including R4 members and RGS-RhoGEFs, determine the ability of blood vessels to remodel, not only into arteries, but into arteries with the distinct properties required for different components of the afferent vascular circuit. The hypothesis also implies that RGS proteins should, in some way, control their own expression as part of a feedback system that turns off arterial remodeling and controls contractility once an artery is fully developed or adapted to changes in hemodynamic demand.

Based on this hypothesis, we might expect that over-expressed RGS5 would inactivate Gαq, driving the formation of a stable GαqGβGγ complex and, in effect, competing for RGS-RhoGEF, effectively acting as a dominant negative for both limbs of this signaling pathway. If our hypothesis is true, we would expect over-expressed RGS5 to block several components of vascular remodeling, including smooth muscle cell (SMC) contraction, movement, and hypertrophy.

Angiotensin II and smooth muscle cell hypertrophy

Figure 2 is based in part on a recent review of the effect of angiotensin II on smooth muscle contraction by Somlyo and Somlyo [39]. They describe two distinct pathways, one mediated by Gαq and the other by Gα13, that induce smooth muscle contraction and, presumably, trophic processes including cell growth, migration, and extracellular matrix deposition using the same pathways.

Stimulation of the angiotensin 1 (AT1) receptor by angiotension II induces a conformational change in the AT1 receptor that leads to GDP to GTP exchange on the Gαq subunit, causing its disassociation from Gβγ and subsequent

induction of an increase in intracellular Ca^{2+}. This occurs through activation of phospholipase C (PLC), which catalyzes hydrolysis of phosphatidylinositol-4,5-bisphosphate to inositol triphosphate (IP3) and diacylgycerol (DAG). IP3 induces Ca^{2+} release from sarcoplasmic reticulum stores and increases the concentration of Ca^{2+}/calmodulin, which binds and activates myosin light chain kinase, leading to phosphorylation of myosin light chain, causing the rapid induction of contraction seen in smooth muscle after angiotensin II treatment [40,41]. The DAG, on the other hand, activates PKC, which mediates the mitogenic effect of angiotensin II on SMCs and after SMC transcription.

The review also describes signaling via Rho-kinase, in this case via coupling of the receptor to Gα12 and 13. The Rho-kinase inhibitors Y27632 and fasudil inhibit hypertrophy induced in SMCs from angiotensin II stimulation [42]. Several GPCRs of vascular importance couple with Gα13 subunits, including receptors for endothelin 1, angiotensin II, thromboxane 2A, and thrombin. Gα13 binds to at least three RGS-RhoGEF proteins, the best characterized being p115-RhoGEF. When Gα13 binds to p115-RhoGEF, the RhoGEF domain is activated and Rho exchanges GDP for GTP and is then able to activate Rho-kinase, eventually inducing downstream contraction and migration. Rho is also required for a1-adrenergic receptor signaling, presumably through a similar competition with Gαq for activation via a ligated receptor.

Activation and inactivation of G proteins and identification of RGS proteins

G protein-coupled receptors account for the vasoactivity of all known vasoconstrictor molecules. Signal transmission depends on interaction with heterotrimeric complexes made up of αG, βG, and γG protein subunits. Gα subunits bind and hydrolyze guanine nucleotides. Binding of a variety of agonists to specific GPCRs at the cell surface activates G proteins by promoting guanine nucleotide exchange (GTP replaces GDP) on Gα subunits and subsequently dissociates the tightly bound Gβγ complex from Gα. Free Gα-GTP and Gβγ regulate the activity of target effector proteins, which, in turn, catalyze the production of intracellular second messengers and lead to biologic function.

Rho-kinase and vascular remodeling

As drawn, the hypothesis oversimplifies at least one important issue: the diversity of pathways controlling Rho. Particularly relevant to any effort to develop a biochemical explanation for a response to mechanical forces, Rho can be directly activated by as yet unknown mechanical transducers. There is an accumulation of evidence that such forces, including activation of β1 and β3 integrins, are also mediated through Rho-kinase [43–45]. Rho-kinase phosphorylates myosin light chain phosphatase, inhibiting its activity and thereby reducing the level of Ca^{2+} needed to induce contraction through the Ca^{2+}/calmodulin mechanism of myosin light chain kinase stimulation. Rho-kinase directly phosphorylates myosin light chain, further enhancing potentiating contractile response.

Recent studies also implicate Rho-kinase as critical, not only to control of vascular remodeling, but also to the transcription of smooth muscle differentiation genes, smooth muscle α actin, and smooth muscle myosin [15,46]. Moreover, RhoA is increased in hypertensive rat vessels, and Rho-kinase activity is required for remodeling of blood vessels in response to contractile stimuli [25,47]. It is intriguing to speculate that the promoters for these critical components of vascular mass are also ultimately controlled by RGS components because Rho itself is activated through Gα12/13 by a specialized RGS class, exemplified by p115-RhoGEF. Gene disruption of Gα13 leads to developmentally lethal alterations to blood vessel formation and endothelium morphology.

Because p115-RhoGEF and RGS5 must compete for receptors, we suggest that the control of mass by GPCRs depends on the interaction of RGS5 and related R4 subfamily members, p115-RhoGEF and related RGS-RhoGEFs, and Gαq and Gα12/13, with the serpentine receptors mediating vasotrophic signals. Recent studies support this hypothesis for α1-adrenergic signaling in myocardium.

Protein structure of RGS family

Our hypothesis suggests that over-expression of RGS5 will inhibit endogenous expression of this gene. Similarly, the pattern of RGS gene expression may determine the structural and biochemical features that differentiate one artery from another. The RGS gene family is characterized by the presence of an RGS domain, approximately 120 amino acids long consisting of 9 alpha helixes that interact directly with G protein alpha subunits. All four classes of G alpha proteins, αs, αi, αq, and α12, are now known to have their intrinsic GTPase activity accelerated by RGS proteins. More than one RGS protein is differentially expressed in the artery wall. There are greater than 20 known family members recently described as belonging to five subfamilies, of which RGS5 belongs in the R4 subfamily, along with RGS1, 2, 3, 4, 8, 13, 16, and 18. Most of the R4 subfamily are GTPase antibody proteins (GAP) for both Gαi and Gαq; however, RGS2 may preferentially inactivate Gαq. Mutational studies have shown that an intact RGS domain by itself is sufficient for functional GAP activity in vitro. Point mutations in the RGS domains of several RGS proteins, in positions shown by crystal structure to physically interact with the Gα subunit, also abolish GAP activity. Phosphorylation of at least one R4 protein in the RGS domain, RGS16, and palmitoylation in the RGS domain of two RGS proteins, reduces GAP activity.

The greatest diversity between RGS family members is contained in the C- and N-terminal accessory domains of the protein. Though over 17 different types of accessory domains have been identified, the R4 subfamily members have so far only been shown to contain three accessory domains [48,49]. The known form of RGS5 and at least one of the forms of each R4 member contain only one

accessory domain, an N-terminal amphipathic helix. The presence of the amphipathic domain in R4 subfamily members has been shown to target these proteins to the membrane. In some assays this domain is also required for GTPase activity, signaling regulation [50], and providing GPCR specificity [51]. The accessory domains of RGS proteins also determine binding specificity to other proteins, such as PDZ-RGS to Ephrin-B, SRB-RGS to estrogen receptor α, and RGS16 to MIR16. Other domains determine cytoplasmic, nuclear, and golgi localization.

Conclusions

Seeing h and r_{ext} as critical variables, the next question must be how these variables are controlled. Using cardiac remodeling as a model, we have suggested that GPCRs, and especially the regulation of G protein-coupled signaling RGS proteins, are likely candidates for at least one major pathway. However, even if this is true, many issues will need to be addressed.

1. What senses pressure and flow? The usual explanation for flow is that flow is sensed as sheer. However, there are other possibilities, such as the simple possibility of cell deformation or existence of a sensor for local diffusion or thermal gradients. In any case, the putative sheer receptor is not known. Even less is known about the pressure/tension sensor; we do not even have a really good idea about the transmission of sensor through the wall.
2. Are r_{ext} and h separable? Obviously atherosclerotic walls can thicken inappropriately, leading to loss of lumen. This suggests distinct biochemical pathways that control the external diameter of the vessel and wall thickness.
3. Are there specific molecular markers for remodeling vessels as there are for remodeling heart?
4. Is there a vascular equivalent of cardiac failure and if so, is this the reason atherosclerotic vessels eventually narrow rather than showing compensatory dilation?
5. Although there is extensive literature on the role of the endothelium in regulating vascular contractility, all vessels showing pathologic narrowing have intimas. What role does the intima play in control of the critical parameters h and r?

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