The Plastic Nature of the Vascular Wall: Reply to Lee, Sandow, and DeMay

This Letter to the Editor is in response to comments received from Drs. Lee, Sandow, and DeMey concerning our recently published review (29). In their Letter to the Editor, Lee et al. present a series of comments regarding the mechanisms we propose contribute to the process of arteriolar remodeling. Our proposal, and the model presented, depicts vascular smooth cells as having the intrinsic ability to alter their positional relationships within the vascular wall. Furthermore, we suggest that the vessel wall may have a plasticity not previously fully appreciated. Our model is based on direct visualization of the behavior of live vascular smooth muscle cells (VSMC) within the wall of functional resistance arteries. Conceptually, we acknowledge that a full understanding of this phenomenon will encompass aspects relating to regulation of cell-cell interactions, cell-extracellular matrix (ECM) interactions, and dynamic alterations of cytoskeletal structures.

1) The first point raised by Lee, Sandow, and DeMey takes the position that our isolated arteriole model system is problematic because of an absence of flow and because our pharmacological approach involved addition of vasomotor agonists abluminally into the vessel bath. Thus their primary argument is that the isolated arteriole, mounted in a pressure myograph and subsequently exposed to vasoconstrictor agonists, is not a physiologically relevant model. The pros and cons of the pressure myograph for the study of physiological phenomena in arterioles have been extensively reviewed and validated under numerous applications (11, 12, 18, 32). Therefore, we conclude there is no need to defend the basic isolated arteriole approach that continues to be state-of-the-art for many applications in which it is important to control or eliminate potentially confounding variables including intraluminal flow. This is not to say we do not appreciate the significance of such variables in the intact situation. Although we agree that the model (as any in vitro model and, indeed, many in vivo models) is not perfect, it has resulted in numerous important discoveries with clear in vivo relevance and pathological implications (36, 41). It is also clear that bath application of pharmacological agents can activate target receptors throughout the vascular wall compartment as presumably can result from synaptic spillover and raised circulating levels. However, the issue is not that this invalidates our remodeling observations but rather makes a case that we may be able to further refine our understanding of the receptor-based mechanism by detailed examination of receptor subtypes and modes of stimulation. All data are inescapably biased by experimental design, and we are comfortable with the test of our proposal that will come with time.

2) The second point raised by Lee, Sandow, and DeMey is a rather general statement referring to the links between chronic constriction and permanent structural changes. On the topic of arteriolar remodeling, the literature is overwhelming. In vitro data obtained using the pressure myograph consistently indicate that prolonged vasoconstriction leads to inward eutrophic remodeling (2, 3, 5, 6, 21, 27, 28). Other reports suggest this is also the case in vivo. Briefly, arterioles isolated from hypertensive individuals exhibit inward eutrophic remodeling (1, 16, 26, 30, 35). Treatment of these individuals with antihypertensive drugs that reduce cardiac output without affecting vascular tone successfully normalize arterial pressure but do not prevent the remodeling (38, 39). In comparison, reduction of arterial pressure with drugs that induce vasodilation eliminates the remodeling (14, 37). This suggests that stimuli other than the elevated intravascular pressure, likely related to the activation state of VSMC, are the driving stimuli for remodeling. These observations agree with in vitro data that indicate intraluminal pressure is not the primary stimulus responsible for inducing inward eutrophic remodeling (4). In vivo induction of hypertension with systemic vasoconstrictors or with nitric oxide synthase inhibitors results in inward eutrophic remodeling of the resistance arterioles (19, 45). Furthermore, genetic upregulation of the renin angiotensin system also results in hypertension and inward eutrophic remodeling of arterioles (20). Remarkably, in the latter model of hypertension, inhibition of integrin αvβ3 shifts the development of inward eutrophic remodeling toward medial hypertrophy. This observation is important because blockade of αvβ3 integrin inhibits VSMC migration during neointimal formation (13). In vivo, hypertension or the infusion or upregulation of vasoconstrictor agents is associated with significant changes in hemodynamics, endothelial function, and humoral factors that ultimately affect VSMC activation. Therefore, although multiple factors may participate in driving the remodeling process, data from in vivo as well as in vitro models consistently suggest that prolonged vasoconstriction leads to inward eutrophic remodeling of arterioles.

The question that concerns us is what are the mechanisms underlying the remodeling process? The fact that the remodeling is eutrophic denotes that there is no net change in the amount of wall material. For this to occur, it has been proposed that either the same wall material gets reorganized around a smaller luminal diameter or that there is a process of destruction and formation of new wall material that encroaches the arteriolar diameter (23, 24). We maintain that either of these two processes requires cellular repositioning. This is supported by in vivo data indicating that VSMC number and dimensions in eutrophic remodeled arterioles do not change (31). For this to occur, VSMC must reposition themselves around a smaller luminal diameter whether they are the same cells or newly formed cells to have the same longitudinal dimension around a smaller internal diameter. In vitro, we previously reported that this cellular repositioning increases the number of VSMC “layers” (28). After prolonged vasoconstriction, mid-diameter optical sections showed that remodeled arterioles had an increased number of VSMC “layers” compared with optical sections obtained before the induction of vasocon-
striction. If the cellular repositioning were to occur along the longitudinal axis of the vessel and laterally to other VSMC as suggested by Lee, Sandow, and DeMey, then not only the length of the arteriole would have to increase but the cross-sectional area of the wall would have to decrease, which was clearly not the case. Figure 2 in our review does not indicate that the cellular overlap occurs on the longitudinal axis of the vessel.

3) In their third point, Lee, Sandow, and DeMey raise the issue of endothelial cell damage. Their argument is that in our in vitro studies some of the functional and morphological changes we observed might be artifacts related to endothelial cell damage. A primary consequence of endothelial cell damage is the reduced bioavailability of vasodilator agents, in particular that of endothelium-derived nitric oxide (NO) (43). The net state of VSMC contraction depends on the balance that exists between vasodilators and vasoconstrictors. Since endothelial damage primarily reduces the availability of vasodilator compounds, it tilts the net state of VSMC toward contraction. Similarly, a lack of flow by relieving dilator production allows the effect of vasoconstriction to be more readily determined. Thus our contention is that an increase in vasoconstrictors, or a reduction in vasodilators, contributes to the inward remodeling process.

4) The fourth point raised by Lee, Sandow, and DeMey addresses the issues of VSMC slippage (ability of cells to move past each other). In 2004, we reported that optical sectioning obtained with multiphoton microscopy indicate that, during the initial phase of vasoconstriction, “a certain degree of cell slippage, that is cells sliding passed each other, occurs as the diameter of the vessel is reduced” (28). This conclusion was based primarily on the observation that, after the initial vasoconstriction, the number of individual VSMC cross sections (cell layers) observed at mid-diameter was increased. Lee, Sandow, and DeMey argue that this cannot be the same as the shifting of VSMC position previously reported (15) but do not explain why. Their premise is that VSMC are tethered to each other so strongly through tight and gap junctions and through the ECM that any movement is improbable. Our data argue otherwise. Indeed, VSMC are strongly attached to each other and the ECM, but these types of cellular attachments are being shown to be highly dynamic, fluidic, and elastic. It has been shown that the VSMC attachments to ECM rapidly rearrange in response to force (34, 44), that the sarcolemma is easily deformable (42), and that its lipid bilayer is indeed highly fluidic, allowing for the movement of lipids and proteins (17, 34). Moreover, a number of the molecules responsible for forming and maintaining intercellular connections have been shown to be short-lived, both in vitro and in vivo (for example, see Refs. 8 and 25), thus indicating that cellular interactions are highly dynamic. We, therefore, contend that the concept of the rigidly coupled VSMC within the wall of the blood vessel is too restrictive and cannot account for our observations. Indeed, Lee, Sandow, and DeMey mention that there are observations of cellular shifting reported in some studies but prefer to take the position that slipping and shifting are different.

5) Lee, Sandow, and DeMey then make a comment on “Intracellular matrix plasticity” and state that there is no medial collagen in cremaster arterioles. This remains debatable, and at this time there is insufficient nanostructural evidence available to conclude this point. Our position is that changes in the amount, cross-linking, and/or proportion of ECM proteins including but not limited to collagen, elastin, and fibronectin affect the structural and functional characteristics of remodeled arterioles. It is well documented that the wall of cerebral (7), cardiac (33, 40), renal (9), and skeletal muscle (10) arterioles contains collagen. Moreover, it has been shown that the collagen-to-elastin ratio changes in remodeled arterioles from hypertensive individuals and that these changes are believed to affect the viscoelastic characteristics of these vessels (22, 23).

6) Comment 6 revolves around the lack of proof for the proposed signaling pathway model. Our response is that this is a “proposed model.”

The general concept of vascular remodeling is in need of an improved understanding that includes extending our knowledge to the level of, and temporal behavior of, individual cells within the vascular wall. New approaches that allow insight at this cellular scale of organization will be essential so that the complex system level remodeling processes of the vascular wall can be integrated in a meaningful manner. Importantly, we believe that the concept of dynamic plasticity of the vessel wall provides an exciting hypothesis that will stimulate studies on control of the microcirculation.

References


