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Vascular Biology
Look What We Staggered Into

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The nuclear receptor superfamily, which includes receptors for steroid hormones, eicosanoids, and retinoic acid, is a group of ligand-dependent transcription factors.[1] Included in this family are "orphan" receptors for which regulatory ligands have yet to be defined (Figure). One such group is the retinoid-related orphan receptor (ROR) subfamily, which has three known members (RORα, RORβ, and RORγ). Previous work has suggested that RORs are involved in diverse processes including embryonic development as well as cell differentiation, cell proliferation, and cancer.[1]

Much less is known regarding potential roles for RORs in the cardiovascular system. RORα mRNA is expressed constitutively in human endothelium and vascular muscle in culture as well as within the wall of intact arteries.[2,3] The constitutive levels of RORα mRNA are reduced in human atherosclerotic plaques.[3] Several proinflammatory stimuli—interleukin-1β, TNFα, and lipopolysaccharide—all increase RORα mRNA expression in endothelium and vascular muscle. RORα can inhibit inflammatory responses in vascular muscle including TNFα-induced production of cytokines and expression of cyclooxygenase-2.[2] These effects seem to be mediated by increased expression of IκBα,[2] the primary inhibitor of NF-κB signaling. Activation of NF-κB has a significant effect on expression of many inflammatory-related genes. Thus, a major role for RORα may be to limit inflammation in blood vessels (Figure).

Pharmacological tools to study the functional importance of selective ROR members are limited. Fortunately, there are two genetic models available for the study of RORα. First, there is an animal with a natural mutation that makes it RORα deficient—the staggerer mouse (*sg/sg*).[1] Staggerer mice have neurological degeneration including cerebellar ataxia. In relation to vascular biology, it has already been established that *sg/sg* mice have lower levels of IκBα mRNA in blood vessels under normal conditions and develop greater atherosclerosis when fed a high-fat diet compared with wild-type controls.[2,4] These results are interesting in relation to the finding that RORα mRNA levels are reduced in human atherosclerotic lesions. Second, a RORα-deficient mouse has been produced using standard gene-targeting technology.

In the study by Besnard et al,[5] the role of RORα in blood vessels was examined using the *sg/sg* mouse. Several functional responses were examined using aorta, carotid artery, and small mesenteric arteries in vitro. In addition, arterial pressure was measured directly in anesthetized mice. The study has several major findings. First, arterial pressure was lower and the pressor response to intravenous phenylephrine was reduced in *sg/sg* mice compared with wild-type controls (C57BL/6J mice). Second, myogenic tone and flow-mediated vasodilation were less in mesenteric arteries (but not carotid arteries) in *sg/sg* mice. Third, contractile responses to serotonin and phenylephrine were selectively reduced in the mesenteric arteries from *sg/sg* mice. Fourth, depending on the blood vessel, relaxation in response to acetylcholine (the classic endothelium-dependent agonist), nitroprusside (a nitric oxide [NO] donor), and isoproterenol was impaired in the *sg/sg* mouse. Fifth, Western blotting suggested that levels of endothelial NO synthase (eNOS) were unchanged, but levels of several contractile proteins (SM-myosin, h-caldesmon, and calponin) were selectively reduced in mesenteric arteries from *sg/sg* mice.

These initial findings regarding vascular function in the staggerer mouse raise many questions. First, mechanisms that regulate myogenic tone, flow-mediated and endothelium-dependent relaxation, and receptor-mediated vasoconstriction are divergent. Hence, deficiency in which potential target genes for RORα could account for this spectrum of vascular changes? For example, NO produced by eNOS is a major mediator of endothelium-dependent relaxation in many studies in blood vessels from both experimental animals and humans.[6] The finding that relaxation of the mesenteric artery in response to acetylcholine was impaired in *sg/sg* mice raised the possibility that eNOS signaling is altered. However, eNOS protein levels were similar in control and *sg/sg* mice suggesting that differences in levels of expression of eNOS cannot account for this impairment. In addition, the finding that relaxation of this artery to nitroprusside was also impaired suggests that function of soluble guanylate cyclase and/or cGMP-dependent protein kinase I (the major signaling pathway for NO in vascular muscle)[7] may be abnormal. Conversely, vasorelaxation in response to isoproterenol, which was also impaired in small arteries from *sg/sg* mice, is likely mediated by cAMP and involves activation of potassium channels and calcium sparks in vascular muscle.[8]

Second, almost all the vascular changes in *sg/sg* mice were observed in the mesenteric artery raising the question of how representative is the mesenteric artery of blood vessels in general. A reasonable possibility is that the role of RORα

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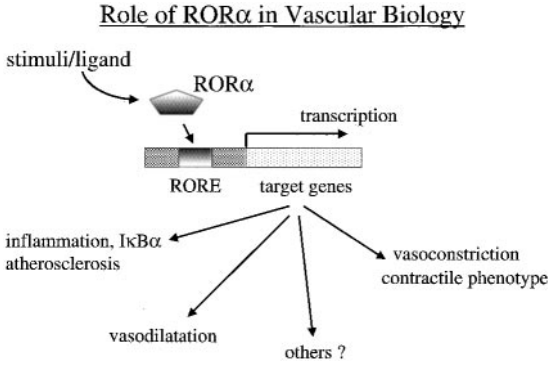

Schematic summary of known influences of RORα in vascular biology. The transcription factor RORα is activated by ligands that are presently unknown. Stimuli that regulate expression of RORα are only beginning to be defined (see text). It is also possible that RORα may be constitutively active in some cell types. RORα interacts and binds with ROR-response elements (RORE), which then influence transcriptional activity of RORα-sensitive target genes. Studies to date, including the present study by Besnard et al,[5] suggest that RORα target genes influence several prominent vascular phenotypes including vascular inflammation and development of atherosclerosis, expression of contractile proteins, and regulation of vascular tone (vasodilation and vasoconstriction).

varies in large conduit vessels compared with smaller arteries and microvessels. Although RORα deficiency did not alter function of aorta, RORα can affect vascular biology in large vessels because atherosclerosis in aorta is much greater in *sg/sg* mice fed a high-fat diet.[4] It is noteworthy that, by far, most studies of vascular function in genetically altered mice are performed using aorta.[6] If Besnard et al[5] had studied only that blood vessel, they would have obtained an essentially negative study in relation to altered vascular phenotypes in *sg/sg* mice.

Third, previous work with vascular cells in culture and studies of atherosclerosis suggest that RORα may be an important antiinflammatory molecule in blood vessels. In addition to contributing to the overall atherosclerotic process, inflammation and inflammatory stimuli are known to alter vascular function. Other antiinflammatory molecules including interleukin-10 and PPARγ[9] have protective effects on vascular function.[10–12] Interestingly, a target gene for RORα may be PPARγ,[13] another member of the nuclear receptor superfamily. Does RORα have similar protective effects on vascular function during inflammation? There are many pathophysiological conditions in which components of the inflammatory response are expressed within the vessel wall. In addition to atherosclerosis, these conditions include sepsis (endotoxin shock), diabetes, ischemia with reperfusion, subarachnoid hemorrhage, Alzheimer's disease, and aging. Thus, the role of RORα on vascular function may be potentially very broad.

Finally, it may be useful to consider what approaches, in addition to the use of *sg/sg* and RORα-deficient mice, might be used to better define the role of RORα in vascular biology. In addition to studies of vascular cells in culture (which have already been initiated),[2,3] other methods have potential. An adenovirus encoding RORα has been made[2] and might be used to overexpress locally the transcription factor in intact blood vessels. A variation of this approach might be to create a viral vector that expresses a dominant-negative form of RORα. Similarly, it may be possible to make transgenic mice that overexpress wild type or a mutant form of RORα. Because of the importance of ROR in embryonic development,[1] it might be necessary to limit the temporal or spatial expression of RORα within the vessel wall.

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