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Ischemia, Hyperemia, Exercise, and Nitric Oxide Complex Physiology and Complex Molecular Adaptations

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Since its discovery by Furchgott and Zawadzki in 1980,¹ endothelium-derived relaxing factor (EDRF) has been shown to play a central role in the cardiovascular system.² This endothelial product is chemically equivalent to nitric oxide (NO)^{3,4} or a biochemical congener thereof,⁵ and relaxes vascular smooth muscle in association with activating guanylyl cyclase and increasing cGMP. Operating through this basic paradigm, EDRF/NO is a critical determinant of several normal physiological responses of the vascular system that are essential for the maintenance of tissue perfusion.^{6,7}

In this issue and the forthcoming December issue of *Circulation*, the potential role of EDRF/NO in two related vascular responses is examined. Tagawa and

See p 2285

colleagues⁸ analyze the effect of EDRF/NO in reactive hyperemia of the human forearm vasculature, and Gilligan and coworkers⁹ evaluate the contribution of EDRF/NO to exercise-induced vasodilation in the human forearm. NO is synthesized from L-arginine through the five-electron oxidation of this basic amino acid by the enzyme NO synthase, one isoform of which is constitutively expressed in endothelial cells. Using (local) brachial artery infusions of an inhibitor of this enzyme, *N*^G-monomethyl-L-arginine (L-NMMA), these two groups of investigators examined the contribution of EDRF/NO synthesis to vascular responses in normal volunteers. Both studies demonstrated a 25% to 36% reduction in baseline blood flow during L-NMMA infusion and thus confirmed previous work suggesting an important contribution of EDRF/NO synthesis to resting arterial tone.^{6,10} Tagawa and colleagues⁸ demonstrated that after transient brachial artery occlusion, L-NMMA did not affect peak blood flow but significantly decreased the duration of hyperemia or flow debt repayment. Gilligan and coworkers⁹ observed that the absolute flow during forearm exercise was reduced modestly during L-NMMA infusion, although the relative increase in flow was in fact higher during infusions reflecting the reduction in baseline flow. Interpretation of their results is also complicated by the fact that L-NMMA increased systemic blood pressure and

slightly decreased heart rate. It is possible that L-NMMA would have had a more marked effect with greater degrees of exercise than were achieved with hand grip in this study, and such a finding would be consistent with previous studies in animals. Thus, these studies provide convincing evidence that EDRF/NO synthesis contributes to basal arterial tone, to the postischemic hyperemic response, and, perhaps, modestly to the forearm exercise response.

Reactive hyperemia is a protective adaptation that has evolved in mammals to ensure prompt restoration of blood flow when flow is interrupted abruptly and ischemia is induced. Vascular physiologists have considered the mechanisms responsible for this response for many years and have invoked a role for both direct myogenic responses and for local vasoactive substances, among which are included prostaglandins, adenosine, potassium, adenosine 5'-triphosphate, oxygen, and pH,¹¹ as well as hydrogen peroxide.¹² With the recognition that EDRF/NO is also an important endogenous vasodilator, investigators have recently begun to examine its role as well in reactive hyperemia. Most studies, all previously conducted in animals, support the view that EDRF/NO does contribute to the reactive hyperemic response and appears to do so in conjunction with other vasoactive agents previously considered essential for hyperemia. Importantly, the quantitative contribution that each of these agents or class of agents makes to the reactive hyperemic response appears to depend on the vascular bed studied, the experimental species used, and the mechanism by which ischemia or tissue hypoxia is produced.

Recent work from several groups supports the view that several of these locally active agents interact with EDRF/NO to promote reactive hyperemia. In the guinea pig coronary circulation, for example, Vials and Burnstock¹³ showed that while a major part of the vasodilatory action of adenosine is a consequence of the direct activation of A₂-purinoceptors on the vascular smooth muscle, activation of a subpopulation of this same receptor class on endothelial cells induces vasorelaxation through production of EDRF/NO. Baker and Sutton¹⁴ confirmed this view using rat cremaster muscle preparations, showing further that luminal endothelial purinoreceptors cause arteriolar dilation by an EDRF/NO-dependent mechanism, whereas abluminal (smooth muscle) purinoreceptors induce vasodilation by a mechanism independent of EDRF/NO. In the isolated guinea pig heart, Kostic and Schrader¹⁵ add another level of complexity to the interactions among locally

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active hyperemic vasodilators by showing that inhibition of NO synthase leads to an increase in adenosine release in the coronary circulation both in the basal state and during reactive hyperemia.

Like the study by Tagawa and colleagues,⁸ most of these studies used transient vascular occlusion to induce reactive hyperemia. In a unique departure from this standard experimental protocol, Park and colleagues¹⁶ induced transient hypoxic coronary vasodilation in isolated guinea pig hearts by exposing the coronary circulation to 100% N₂ for 1 minute. They showed that EDRF/NO and adenosine were both important for the increase in coronary blood flow induced by hypoxia in this system. However, by contrast with experimental systems in which transient occlusion of the vessel is used to induce reactive hyperemia, in this system, EDRF/NO was primarily responsible for the peak increase in blood flow observed; adenosine and vasodilatory prostaglandins appeared to play more of a role in the late phase of the reactive blood flow response. What distinguishes this experimental system from those involving vascular occlusion is, of course, the continued presence of blood flow throughout the hypoxic period. That EDRF/NO plays a greater role in this system than in an experimental system in which blood flow is arrested to induce ischemia and tissue hypoxia is indicative of the importance of flow in stimulating the release of EDRF/NO from the endothelial cell.

Early work by Rubanyi and colleagues¹⁷ and by Pohl and coworkers¹⁸ suggested that flow and the shear stress to which the endothelium is exposed under flow conditions induce the release of EDRF/NO from the endothelial cell. Flow and shear stress increase intracellular calcium concentration in the endothelial cell, and the increase in calcium, in turn, leads to activation of NO synthase, the endothelial form of which is calcium-calmodulin dependent. This view was later given support by our observations that physiologically relevant levels of shear stress induce the release of EDRF-like activity from cultured endothelial cells.¹⁹ We demonstrated that the physical stimulus of shear stress is transduced through the activation of a calcium-sensitive potassium channel in the endothelial cell to induce EDRF/NO release.²⁰ In addition to shear stress, mechanical deformation of the endothelium also appears to be important for EDRF/NO release,²¹ and in the reactive hyperemic experiments, cessation of pulsatile flow with its deforming potential through the forearm bed may limit the extent to which a brisk EDRF/NO response can occur upon restoration of flow.

Flow-mediated release of EDRF/NO is also believed to be important for exercise-induced vasodilation, as suggested by the work of Gilligan and coworkers.⁹ This response appears to be different for different divisions of the vasculature in a given bed. In hamster cremaster vasculature, Hester and colleagues²² showed that inhibition of EDRF/NO decreased both resting diameter and dilation of first-order arterioles in the setting of electrical muscle stimulation. EDRF/NO inhibition also impaired the functional dilation of second-order arterioles but had no effect on the resting diameter of second- or third-order arterioles or functional dilation of third-order arterioles. In dogs, Wang and colleagues²³ showed that acute treadmill exercise caused dilation of the circumflex coronary artery by 4.33% and increased

coronary blood flow by 32 mL/min; however, after administration of the NO synthase inhibitor nitro-L-arginine, the circumflex coronary artery constricted by -4.13% without a significant alteration in blood flow. This finding contrasts with the observations of Gilligan and coworkers⁹ and argues against a role for EDRF/NO in resistance vessel dilation under these conditions. However, caution must be exercised before generalizing observations made in a different species and a different vascular bed.

EDRF/NO responses to exercise have implications for the adaptive response to ischemia, as suggested by the recent work from Hintze's group (Wang et al).²³ Regular treadmill exercise in dogs for 7 days enhanced reactive dilatory responses after brief coronary artery occlusion.²³ More recently, this group of investigators has shown that the EDRF/NO adaptive response to exercise is associated with increased transcription of endothelial NO synthase.²⁴ The increase in transcription may itself be shear-stress dependent, given the recent observations that endothelial products that increase in response to shear have in the 5'-untranslated regions of their genes a response element (GAGACC) that is shear sensitive²⁵ and that nine of these shear-stress response elements are present in the 5'-untranslated region of bovine endothelial NO synthase.²⁶

The findings of these studies also have important implications for control of blood flow in a number of pathological states where EDRF-dependent dilation of conduit and resistance vessels is known to be impaired. Cox and colleagues²⁷ demonstrated impaired flow-mediated epicardial coronary dilation in patients with angiographic evidence of early atherosclerosis. Maximal flow responses during infusion of endothelium-dependent vasodilators are blunted in the coronary and peripheral circulation in patients with hypercholesterolemia,²⁸⁻³⁰ hypertension,^{31,32} diabetes mellitus,³³ and dilated cardiomyopathy.³⁴ Although flow debt repayment was not examined, peak hyperemic response after cuff occlusion does not appear to be impaired in the brachial circulation of patients with hypercholesterolemia,²⁸ diabetes mellitus,³³ and hypertension.³⁵ These findings are consistent with the observation by Tagawa and colleagues⁸ that EDRF/NO synthesis does not affect the peak hyperemic response after ischemia.

In contrast to the effects of these disease states on reactive hyperemia, the maximum flow response to increased oxygen demand is impaired in the setting of atherosclerosis. Nabel and colleagues³⁶ observed that patients with angiographic evidence of atherosclerosis had impaired epicardial dilation and a decrease in the coronary blood flow response to atrial pacing, findings that probably reflect loss of flow-mediated dilation. Preliminary work by Creager and colleagues (Lieberman et al³⁷) showed impaired dilation of the conduit brachial artery in a number of disease states, and Sorensen et al²⁹ observed impaired femoral arterial flow-mediated dilation in children with heterozygous familial hypercholesterolemia. However, the effects of these common vascular disease states on shear-mediated dilation of resistance vessels have not been well studied in patients. In light of the evidence that EDRF/NO synthesis plays a role in normal control of basal and stimulated tone of large and small vessels, impairment of EDRF action in these pathological con-

ditions could contribute to impaired tissue perfusion and ischemia.

Current data, including the article presented in this issue and the one to appear in the December issue of *Circulation*,^{8,9} strongly support the view that the endothelial cell can be viewed as a transducer of important vascular signals to which the vascular bed must respond in order to ensure adequate tissue perfusion at rest. Acting through the paracrine agent, EDRF/NO, the endothelium is responsible for moment-to-moment changes in vascular tone that form the basis for responses to ischemia and possibly to exercise. Ischemia and exercise may be viewed as two extreme situations in which the EDRF/NO system is called upon to provide adaptive responses promptly, in the former case to restore inadequate perfusion, in the latter case to provide enhanced perfusion in the setting of excess demand. EDRF/NO does not act alone in these settings; other vasoactive determinants, some of which are also derived from the endothelial cell, such as vasodilatory prostaglandins, promote these responses and interact synergistically with NO to optimize beneficial vascular effects.^{38,39} In these physiological scenarios, the central role of shear stress in acute EDRF-dependent responses and in chronic adaptive changes in the vasculature is also clearly supported by the recent data reviewed here.^{17,20,23,24} The complex physiology evinced by the vascular stresses of ischemia and exercise are well matched by the complex molecular responses that accompany them, all of which operate to maintain tissue perfusion. Therapy directed toward restoration of these mechanisms has the potential to improve tissue perfusion and limit ischemia in disease states associated with impaired EDRF action.

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