The discovery of prostacyclin and its endothelial origin established the idea of endothelium-derived vasoactive eicosanoids and led to the realization that endothelial cells were a source of autacoids that regulate vascular tone.1,2 In 1980, Furchgott and Zawadzki3 described endothelium-derived relaxing factor (EDRF) and presented evidence that EDRF was a lipoxigenase metabolite of arachidonic acid. Subsequent research indicated that EDRF was nitric oxide.4 Several laboratories have described an endothelium-derived vasodilating factor that is distinct from nitric oxide or prostacyclin.5–10 These laboratories reported that acetylcholine caused endothelium-dependent relaxation and hyperpolarization of vascular smooth muscle. The relaxations and hyperpolarizations were not altered by arginine analogs that inhibit nitric oxide synthase or inhibitors of cyclooxygenase or lipoxygenases. They were blocked by inhibitors of calcium-activated potassium channels such as tetraethylammonium or charybdotoxin but not by inhibitors of ATP-sensitive potassium channels such as glibenclamide. Subsequent studies indicated that this factor was released by other agonists including bradykinin and substance P. It was concluded that this vasodilating factor acts by opening calcium-activated potassium channels and hyperpolarizing the smooth muscle membrane. This factor has been termed endothelium-derived hyperpolarizing factor or EDHF.

The article by Thollon and coworkers11 in this issue of Circulation Research further emphasizes the importance of EDHF in normal coronary arteries and arteries with regenerated endothelium. Several important insights are provided into the action and nature of EDHF by this work. First, this study demonstrates the importance of measuring membrane potential in defining the contribution of EDHF to the action of agonists. Hyperpolarization of vascular smooth muscle unequivocally defines EDHF activity. Second, the study indicates the importance of the resting membrane potential of the smooth muscle on the magnitude of the response to EDHF. Removal of the endothelial lining of porcine coronary arteries results in depolarization of the underlying vascular smooth muscle cells, suggesting a tonic hyperpolarizing influence of the endothelium. When the endothelium is allowed to regenerate, the hyperpolarizations to serotonin are abolished while the hyperpolarizations to bradykinin are preserved. The arteries with the less negative membrane potentials exhibit the greatest hyperpolarizations to bradykinin. Third, there are fundamental differences in the vascular effects of serotonin and bradykinin. Serotonin induces vascular smooth muscle hyperpolarizations of 3 to 13 mV that are transient in duration. In contrast, bradykinin hyperpolarizes the coronary smooth muscle by 40 mV at the highest concentration of the peptide, and the hyperpolarizations are long lasting. The authors raise the possibility that different EDHFs mediate the responses to these two agonists. Bradykinin clearly acts to hyperpolarize coronary arterial smooth muscle through the release of a transferable endothelial factor that activates calcium-activated potassium channels.12–14 Serotonin has not been as extensively studied. This Editorial will examine the possible identity of EDHF(s), the need to apply chemical, biochemical, electrophysiological, and pharmacological approaches to defining EDHF and the importance of the experimental conditions in interpreting these studies.

Many studies define EDHF activity as relaxations to an agonist in the presence of inhibitors of nitric oxide synthase and prostaglandin synthase. However, it should be emphasized that endothelium-dependent hyperpolarization of smooth muscle is the hallmark of EDHF activity. Thollon and coworkers11 emphasize this point by demonstrating that agonist-induced changes in membrane potential may be dissociated from mechanical activity of the vessel. Serotonin produces small, transient hyperpolarizations but sustained relaxations.11,15 The possibility must always be considered that endothelial factors other than a hyperpolarizing factor may mediate relaxations that are resistant to inhibitors of nitric oxide and prostaglandin synthase.

Thollon and coworkers11 also emphasize the importance of the resting membrane potential on the magnitude of the response to agonists that release EDHF and the influence of the experimental conditions on these electrical events. The resting membrane potential of arteries pinned to a supporting matrix in the present study is markedly different from arteries under physiological transmural pressures. Arterial smooth muscle cells of pressurized coronary arteries exhibiting physiological transmural pressures are depolarized compared with arteries under nonpressurized conditions. Furthermore, pressurization of arteries activates second messenger systems.
that can alter the active state of arterial smooth muscle and endothelial smooth muscle interactions. The response to agonists that release EDHF should be enhanced in pressurized and depolarized arteries. Thus, the experimental conditions may minimize or emphasize the role of EDHF.

The identity of EDHF and the possibility of multiple EDHFs have received considerable attention. Recent studies from a number of laboratories have shown that in coronary, cerebral, and renal arteries EDHF is a cytochrome P450 metabolite of arachidonic acid, an epoxyeicosatrienoic acid or EET12–14,23–27 (Figure). Endothelial cells metabolize arachidonic acid by the cyclooxygenase, lipoxigenase, and cytochrome P450 metabolic pathways.28,29 The EETs are the only cytochrome P450 metabolites of arachidonic acid produced by the endothelial cell.29 The cytochrome P450 isozyme of the endothelial cell appears to be a CYP 2C epoxygenase.30 The EETs are released by vasoactive substances such as acetylcholine or bradykinin as well as arachidonic acid. They activate calcium-activated potassium channels and hyperpolarize and relax vascular smooth muscle. Arteries from other vascular beds such as the guinea pig carotid artery and rat mesenteric artery also exhibit EDHF activity. However, this activity is not reduced by inhibitors of cytochrome P450.31–33 The reason for this discrepancy may lie in the fact that EETs are not the only cytochrome P450 metabolites of arachidonic acid produced by the blood vessel. Smooth muscle cells do not synthesize EETs but rather metabolize arachidonic acid by cytochrome P450 to 20-hydroxyeicosatetraenoic acid (20-HETE)34–36 (Figure). This reaction is catalyzed by a CYP 4A isozyme, an \( \omega \)-hydroxylase. This metabolite is made by the smooth muscle of renal and cerebral vessels but not coronary arteries. 20-HETE has the opposite effects of the EETs on vascular tone, membrane potential, and potassium channel activity. It inhibits the opening of the calcium-activated potassium channel, depolarizes the cell, increases intracellular calcium, and vasoconstricts vascular smooth muscle.34,35,37 Unlike the EETs that act as a transferable paracrine factor,12–14 20-HETE acts as an intracellular second messenger in smooth muscle. It is released in response to stretch or increases in transmural pressure and is proposed to mediate myogenic tone.35,36

There are a number of drugs that inhibit cytochrome P450 including clotrimazole, miconazole, SKF525A, metyrapone, and 17-octadecylic acid (17-ODYA).38–40 These drugs are equally active in inhibiting the CYP 2C epoxygenase, and CYP 4A \( \omega \)-hydroxylase isozymes so inhibit both EET and 20-HETE synthesis in blood vessels. In the coronary artery, the endothelium produces EETs; however, 20-HETE is not made by the coronary smooth muscle.23,29 As a result, inhibitors of cytochrome P450 clearly inhibit the relaxations and hyperpolarizations to acetylcholine, bradykinin, and arachidonic acid, indicating that a cytochrome P450-derived factor mediates these smooth muscle effects.23,24,26,27 In contrast, with CYP 2C producing vasodilatory EETs in the endothelium and CYP 4A producing the vasoconstrictor 20-HETE in the smooth muscle, it is not surprising that inhibitors of cytochrome P450 give variable results in vessels that produce both of these eicosanoids. If the vessel produces both EETs and 20-HETE, the action of an inhibitor of cytochrome P450 will depend on which of the two cytochrome P450 pathways predominate. For example, if there is stretch on the vessel or if pressurized vessels are studied, the CYP 4A-derived 20-HETE pathway will predominate with closure of the calcium-activated potassium channel, depolarization, and vasoconstriction. An inhibitor of cytochrome P450 will decrease 20-HETE synthesis, open the potassium channel, hyperpolarize the smooth muscle, and relax the vessel.34,37 This effect of the cytochrome P450 inhibitor will mimic the activity of EDHF. Agonists that release EDHF will compete with 20-HETE for control of the calcium-activated potassium channel and membrane potential. Inhibitors of cytochrome P450 will inhibit both pathways, and the effect on membrane potential and vascular tone may be minimal. This finding may be interpreted to indicate that inhibitors of cytochrome P450 have direct effects on smooth muscle and on potassium channel activity and do not block the activity of EDHF. Under conditions of low 20-HETE synthesis, the effects of the endothelial EETs will predominate. Agonists that release EETs and EDHF will open calcium-activated potassium channels and hyperpolarize and relax the smooth muscle. Inhibitors of cytochrome P450 will block EET production, inhibit potassium channel activation, and prevent the hyperpolarization and relaxation.24,25,27 These findings with the inhibitors would indicate that EDHF is a cytochrome P450 metabolite.

Some inhibitors of cytochrome P450 have direct effects on potassium channels.41–43 Clotrimazole and ketoconazole inhibit calcium-activated potassium channels in vascular smooth muscle, colonic cells, and leukemia cells. The inhibition by these drugs occurred in whole-cell
Vascular Cytochrome P450 Eicosanoids and Tone

Although arachidonic acid metabolism has been studied in the bovine coronary artery, canine renal artery, rabbit aorta, rabbit carotid, and cat cerebral artery, this is not the case for arteries from other vascular beds. EDHF activity has been reported in the guinea pig and rabbit carotid artery, rabbit aorta, rat mesenteric artery, rat portal vein, and rat hepatic artery. The rabbit carotid artery and rabbit aorta have EDHF activity but do not produce metabolites of arachidonic acid by the cytochrome P450 pathway. Thus, some other compound(s), possibly nitric oxide or another metabolite of arachidonic acid, may mediate the effect. In the rat mesenteric artery, inconsistent effects have been reported with inhibitors of cytochrome P450. Under some conditions, the inhibitors of cytochrome P450 block acetylcholine-induced relaxations and hyperpolarizations, and induction of cytochrome P450 enhances these effects. In other studies, inhibitors of cytochrome P450 do not alter acetylcholine-induced relaxations or hyperpolarizations. None of these studies documents that the drugs inhibited the metabolism of arachidonic acid by cytochrome P450. The absence of biochemical studies of arachidonic acid metabolism in these vessels makes interpretation of the data impossible. It is not known whether these vessels metabolize arachidonic acid by cytochrome P450, whether they metabolize arachidonic acid to EETs and/or 20-HETE, or whether the drugs inhibit cytochrome P450 metabolism of arachidonic acid in the concentrations used. Potassium ion and anandamide are proposed as EDHFs in this vascular bed.

In summary, the studies of Thollon and coworkers emphasize the importance of measuring membrane potential of vascular smooth muscle in defining the endothelium-dependent response to an agonist as mediated by EDHF. The experimental conditions may determine the magnitude of the contribution of EDHF to an agonist response by setting the resting membrane potential. Depolarization of the arteries by pressurization or regeneration of the endothelium will enhance the EDHF response. Evidence from several sources suggests that there are multiple EDHFs and that the chemical mediator of the EDHF response may vary with the vascular bed.

In coronary, renal, and cerebral arteries, EETs appear to have the properties associated with the activity termed EDHF. In other vessels such as the rabbit carotid artery and aorta, arachidonic acid is not metabolized by cytochrome P450, so other factors must mediate the hyperpolarization and vasorelaxation. In the guinea pig carotid artery, rat mesenteric artery, hepatic artery, and portal vein, there is a lack of biochemical information on arachidonic acid metabolism to allow a determination of whether an arachidonic acid metabolite or another mediator is involved. Thus, it must be stressed that when studying the physiology and pharmacology of the vascular cytochrome P450 pathway(s), the arachidonic acid metabolites produced by the smooth muscle cells and endothelial cells must be known. Without a clear biochemical definition of the metabolites produced, a rational interpretation of the results with inhibitors or inducers is not possible.

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