

## Review Article

# The Coronary Microcirculation in Health and Disease

**Judy M. Muller-Delp**

*Department of Physiology and Functional Genomics, University of Florida College of Medicine, P.O. Box 100274, Gainesville, FL 32610-0274, USA*

Correspondence should be addressed to Judy M. Muller-Delp; [jdelp@ufl.edu](mailto:jdelp@ufl.edu)

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Coronary blood flow is closely regulated to meet the changing metabolic demands of the working myocardium. Resistance of the coronary vasculature is determined by metabolic, myogenic, endothelial, and neural mechanisms. The influence of these control mechanisms varies throughout the coronary circulation, as they have dominant sites of action in vessels of different caliber. Coronary vascular resistance depends upon the coordinated response to these influences. Within a segment of the coronary circulation, resistance may be determined, for example, by competitive interaction between neural vasoconstriction and metabolic vasodilation. Such a system in which control occurs through multiple mechanisms with varying effects allows for precise control of coronary blood flow. This system also provides protection against dysfunction of a single control mechanism. If one fails, other control mechanisms can compensate for that loss of function. Thus, adequate delivery of oxygen and nutrients can be maintained despite potential dysfunction and large fluctuations in metabolic demands of the myocardium. In disease states, these regulatory mechanisms may also fail, and endothelial dysfunction is commonly seen in the setting of cardiac disease. Optimal cardioprotective therapies must target the coronary microcirculation and cardiac myocytes in tandem. Similarly, reversal of cardiac dysfunction requires concomitant amelioration of coronary microvascular dysfunction.

## 1. Introduction

Coronary vascular resistance is determined by a variety of mechanisms including coronary vascular anatomy, extravascular cardiac compressive forces, and vascular smooth muscle contraction. The roles of coronary vascular anatomy and cardiac compressive forces in determining coronary blood flow have been the focus of numerous studies and various models describe the contribution of these factors to distribution of blood flow throughout the myocardium [1]; however, coronary vascular resistance is primarily determined by arteriolar tone. This review discusses (1) the factors influencing coronary vascular tone and their effects on coronary blood flow and (2) the impact of imbalance in factors that regulate coronary vascular tone on regulation of myocardial blood flow in disease states.

Vascular tone is governed by metabolic, myogenic, endothelial, and neural influences. These control mechanisms act in concert to vary coronary vascular resistance according to the demands of the working myocardium. Fine-tuning of vascular tone within the coronary circulation depends on

the balance of these controls. Furthermore, the influence of these control mechanisms varies depending upon location within the coronary vasculature [2–5]. In this paper, the role of these influences in determining vascular tone will be discussed and the segmental distribution within the vascular tree will be considered.

Because the importance of the microcirculation in determining total coronary resistance is well established [6], particular emphasis will be placed on mechanisms regulating arteriolar diameter. Only a small fraction of coronary resistance resides in large epicardial arteries; in fact, these large vessels are often termed “conduit arteries” for this reason. For many years, the coronary resistance vasculature was treated as a single unit consisting of all vessels distal to these large arteries (vessels decreasing in size from approximately 300  $\mu\text{m}$  to 20  $\mu\text{m}$ ). Vasoactive responses of “resistance vessels” were determined from pressure/flow relationships. For example, by perfusing the coronary bed at constant pressure and determining coronary blood flow before and after administration of a drug, an increase in the coronary blood flow in response to the drug would indicate that

resistance decreased due to dilation of resistance vessels. However, these techniques could not indicate the location of the vasodilation within the resistance vasculature.

Using stroboscopic illumination synchronized to cardiac motion, Nellis et al. [7] were able to visualize epicardial microvessels in the beating right ventricle and make micropuncture measurements of intravascular pressure throughout the coronary tree. These investigators observed that in the right ventricle, 70% of coronary vascular resistance was attributed to arteriolar and venular vessels smaller than  $140\ \mu\text{m}$  in diameter. Chilian et al. [6] adapted this technique to the left ventricle and found that more than 50% of total coronary resistance resides in arterioles less than  $150\ \mu\text{m}$  in diameter. Further, these techniques have demonstrated that within the microcirculation, responses to vasoactive stimuli are heterogeneous. Figure 1 illustrates the distribution of resistance along the coronary vascular tree and the predominant vasoactive influences within each segment of the coronary microcirculation. This schematic emphasizes the fact that the majority of coronary vascular resistance resides in small arteries and arterioles. It can also be seen that the major vasoactive influences differ in segments of the microcirculation. For example, neurohumoral and flow-dependent control mechanisms predominate in small arteries, whereas metabolic and myogenic controls are of primary importance in arterioles.

## 2. Metabolic Control

Metabolic activity of the heart is the most important physiological mechanism regulating coronary vascular resistance. This mechanism is in place to ensure adequate  $\text{O}_2$  delivery, as blood flow must increase in proportion to metabolic demand (e.g., increases in myocardial  $\text{O}_2$  consumption during exercise). This parallel relationship between  $\text{O}_2$  supply and demand is particularly important for the heart, as it differs from other organs in two important respects. First, the heart has very little capacity for anaerobic metabolism. In fact, the heart normally consumes lactate produced by other tissues. Myocardial lactate production is a pathophysiological sign of severe ischemia. Second, the heart consumes over 75% of the  $\text{O}_2$  delivered to it at rest, and thus no significant  $\text{O}_2$  reserve exists. Arterial blood typically has a  $\text{PO}_2$  of 100 mm Hg. Mixed venous blood, representing a sample from the entire body, normally has a  $\text{PO}_2$  of 40 mm Hg. In stark contrast, coronary venous blood has a  $\text{PO}_2$  of 18 mm Hg under resting conditions. During exercise, feed-forward sympathetic vasodilation mediated by  $\beta$ -adrenoreceptors accounts for about 25% of coronary metabolic vasodilation [10–12]; however, the remaining 75% of exercise-induced hyperemia is, at present, largely unexplained. For nearly a century, we have been working under the assumption that the working heart produces a vasodilatory metabolite. As stated by Markwalder and Starling [13], “The most potent agent in causing dilatation of the coronary vessels is non-volatile metabolites produced by the heart muscle. By this means a local mechanism is supplied by which the heart muscle will increase the

circulation through itself whenever increased demands are made on its functional capacity.” The exact nature of the metabolite and the effector mechanisms activated remain unsettled; however, particular attention has been focused on adenosine and  $\text{H}_2\text{O}_2$  as candidates for metabolic vasodilators in the coronary circulation. Further, ATP-sensitive ( $\text{K}_{\text{ATP}}$ ) and voltage-dependent ( $\text{K}_v$ )  $\text{K}^+$  channels have received considerable attention as effectors of coronary vascular dilation.

**2.1. Adenosine.** Adenosine is a metabolite of cardiac myocytes and a potent coronary vasodilator [14, 15]. The adenosine hypothesis proposes that adenosine is released from myocytes as myocardial  $\text{PO}_2$  falls [16]. Interpretations of data addressing this hypothesis have been controversial [17]. Adenosine production increases during periods of severe hypoxia and ischemia [16]; however, these conditions are extreme and are accompanied by dramatic changes in venous  $\text{PO}_2$  and  $\text{O}_2$  content. During exercise, the changes in coronary venous  $\text{PO}_2$  or  $\text{O}_2$  content, reflective of tissue  $\text{PO}_2$ , are minimal [18]. Thus, because the adenosine hypothesis proposes a negative-feedback scheme in which myocardial  $\text{PO}_2$  constitutes the regulated variable, it is difficult to understand how minimal deviations from the set point (as occur during exercise) could operate as an error signal to increase coronary blood flow. Other evidence for adenosine as a metabolic dilator comes from studies where adenosine signaling is disrupted; however, the stimuli employed are typically more severe, nonphysiological perturbations such as coronary occlusion to elicit reactive hyperemia or hypoxic vasodilation. Some of the tools that have been used include adenosine deaminase (ADA), which metabolizes adenosine to inosine, and 8-phenyltheophylline (8-PT), an adenosine receptor antagonist. ADA reduces both coronary reactive hyperemia [19] and hypoxic coronary vasodilation [20] by over 30%. Kanatsuka et al. [21] showed that 8-PT blunted the increase in coronary flow and dilation of coronary microvessels during reactive hyperemia. Bache et al. [22] agreed that coronary reactive hyperemia is substantially reduced by ADA or 8-PT. ADA attenuated coronary functional hyperemia in response to norepinephrine infusion [23], but interpretation is complicated by the fact that ADA also reduced myocardial oxygen consumption. In contrast, attempts to demonstrate a role for adenosine in control of coronary blood flow over the autoregulatory range of perfusion pressures have yielded negative results [24, 25]. Although myocardial adenosine levels may increase during exercise [26], Bache et al. [22] demonstrated that the adenosine inhibitors, ADA and 8-PT, had no effect on coronary blood flow during exercise. Similarly, Tune et al. [27] reported no significant contribution for adenosine during exercise-induced coronary hyperemia. In fact, adenosine levels in the myocardial interstitium did not reach vasoactive levels during exercise [27]. Thus, although adenosine may contribute substantially to the regulation of coronary blood flow during conditions of limited flow or extreme cardiac demand, it is less clear that adenosine plays a key role in minute-to-minute matching of coronary flow to cardiac demand.

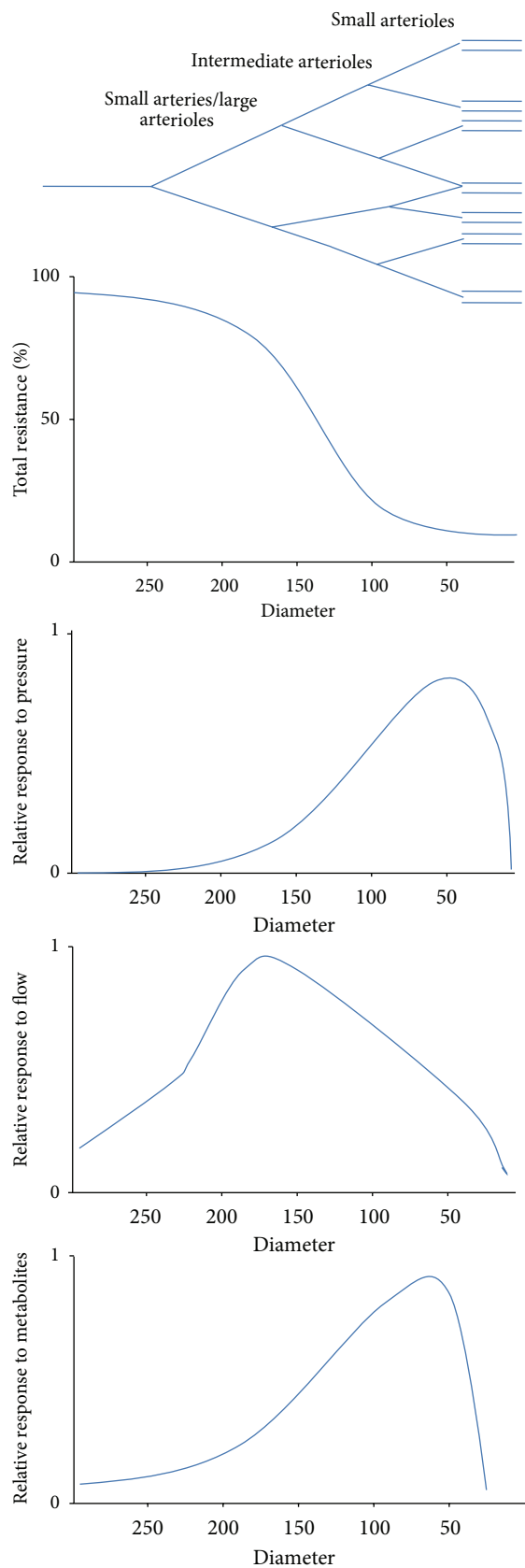


FIGURE 1: Schematic representation of the distribution of coronary vascular resistance and the mechanisms that contribute to control of resistance in successive segments of the coronary vascular tree. The majority of resistance resides in large, intermediate, and small arterioles. Distal small arterioles are most sensitive to changes in intraluminal pressure and contribute the most to coronary autoregulation. Intermediate arterioles are most responsive to cardiac metabolites, contributing substantially to metabolic regulation of coronary flow. Small resistance arteries and large arterioles demonstrate the greatest responsiveness to intraluminal flow.

When considering adenosine as a metabolic coronary vasodilator, it is important to determine whether measurements of interstitial adenosine concentrations are accurate and reliable. That is, during conditions of increased demand, does interstitial adenosine increase to vasoactive levels? Tissue levels of adenosine are not appropriate for estimating interstitial adenosine, as more than 90% of adenosine is bound to S-adenosylhomocysteine [28]. Adenosine measurements from tissue dialysis samples are typically higher than those returned by other assays [29] and may reflect tissue damage due to insertion of tubing into the myocardium. Applying mathematical models to account for adenosine uptake kinetics in the heart improves the ability to estimate interstitial adenosine [30]. At present, the best method for assessing interstitial adenosine is to sample coronary venous blood and pharmacologically inhibit further metabolism of adenosine prior to measurements.

The relative contribution of adenosine to an increase in coronary blood flow during varying conditions of myocardial demand (e.g., exercise, ischemia, and pacing) may be related to the sites of action of adenosine in the coronary microcirculation. Chilian et al. [31] used the nucleoside transport inhibitor, dipyridamole, to promote release of adenosine from the myocardium and found that the effects of endogenous adenosine production on coronary resistance were most profound in coronary arterial microvessels. During control conditions, approximately 25% of total coronary resistance occurred in the arterial compartment and 68% resided in the microvessel compartment. During dipyridamole infusion, arterial resistance increased to 42% of total resistance while microvascular resistance fell to 27%. These results indicate that adenosine exerts its greatest vasodilatory effects on arterial microvessels. These findings were confirmed by diameter measurements of coronary microvessels during adenosine infusion [32, 33]. Kanatsuka et al. [32] reported that dilation of arterial microvessels in response to intracoronary infusion of adenosine was inversely proportional to diameter; that is, greater dilation was seen in smaller vessels. Furthermore, the primary site of the dilation that occurred in response to adenosine differed from the site of dilation in response to changes in metabolic demand. Vasodilation that occurred in response to an increase in myocardial oxygen consumption, although heterogeneous, occurred in all coronary microvessels (40–380  $\mu\text{m}$ ) while dilation produced by adenosine and dipyridamole was most prominent in vessels less than 150  $\mu\text{m}$  in diameter. These small vessels that are most responsive to adenosine have also been shown to be the site of persistent vasodilatory reserve during severe hypotension [33]. Thus, adenosine may contribute most to changes in coronary resistance under conditions in which extreme metabolic vasodilation predominates over other regulatory factors. This may be the case during reactive hyperemia as opposed to conditions created by exercise or reductions of perfusion pressure, during which the decrease in the supply-to-demand ratio for oxygen is relatively mild.

Evidence of other metabolic regulators of coronary blood flow also exists. Oxygen [34–36] and carbon dioxide [37–39] have both been considered as possible metabolic regulators of coronary blood flow. In 1957, Berne et al. reported that

coronary blood flow did not change in response to moderate decreases in arterial oxygen content. Coronary blood flow only increased when coronary venous oxygen content dropped below a critical level. Changes in coronary vascular conductance that occur during alterations in myocardial metabolism and coronary perfusion pressure are correlated with changes in coronary venous  $\text{PO}_2$  [36, 40]. An increase in blood flow during hypercapnia has also been reported [38, 39]. Broten and Feigl [35] investigated the possibility that  $\text{O}_2$  and  $\text{CO}_2$  act synergistically in regulating coronary blood flow. Based on comparisons of the predicted coronary flow at a given venous  $\text{O}_2/\text{CO}_2$  content and actual vasodilation determined over the autoregulatory range, they suggested that 20 to 30% of the coronary dilation observed over the autoregulatory range was due to an  $\text{O}_2/\text{CO}_2$  sensitive mechanism.

While these data suggest that there is a mechanism that responds to changes in  $\text{O}_2$  and/or  $\text{CO}_2$  to produce vasodilation in the coronary circulation, a direct effect of either  $\text{O}_2$  or  $\text{CO}_2$  on the coronary vasculature *in vivo* has yet to be demonstrated. Myers et al. [41] have demonstrated that hypoxia causes dilation of isolated coronary microvessels through an endothelium-dependent mechanism. The vessels were isolated from the myocardium, indicating that the dilation was due to a direct effect of oxygen tension on the vascular endothelium and not a secondary effect of a metabolic vasodilator. However, the effect of hypoxia was not immediate (the dilation occurred over a 30-minute period) which does not support a role for oxygen in mediating rapid autoregulatory responses. Rather, the rapid vasoactive responses associated with changes in  $\text{O}_2$  are likely mediated by production of metabolites.

**2.2.  $\text{H}_2\text{O}_2$  and Redox-Sensitive Signaling.** Over the past decades, the identification of a primary metabolic feedback signal that regulates coronary blood flow has proved elusive. Subsequently, the notion of feed-forward regulation of coronary blood flow has emerged. In a feedback system, the error signal for metabolic dilation is the metabolite produced during periods when oxygen demand exceeds delivery. In such a feedback system, once blood flow increases the error signal is eliminated when the production of metabolites returns to baseline. In contrast, in a feed-forward signaling system, no error signal is generated. Rather, a metabolite is produced that is directly linked to oxygen utilization and, therefore, its level is regulated by oxygen utilization and not by a mismatch between demand and delivery. Vasodilatory signaling through reactive oxygen species, particularly hydrogen peroxide, has been proposed to function as feed-forward metabolic link that couples myocardial demand directly to coronary blood flow and oxygen delivery.

Reports of the coronary vasodilatory actions of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) began to emerge when  $\text{H}_2\text{O}_2$  was identified as an endothelium-derived hyperpolarizing factor in porcine [42] and human [43] coronary arterioles. More recent work [44] has demonstrated that mitochondrial production of superoxide ( $\text{O}_2^{\bullet-}$ ) during periods of increased myocardial demand (pacing) leads to increased release of  $\text{H}_2\text{O}_2$  from myocytes and subsequent vasodilation of coronary arterioles.

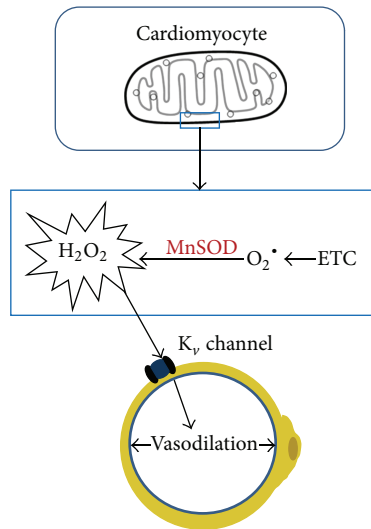


FIGURE 2: Feedforward metabolic vasodilation mediated by  $O_2^{\bullet-}$ -derived  $H_2O_2$ . Increased rate and strength of myocyte contraction increase mitochondrial metabolism and flux through the electron transport chain (ETC), driving greater production of  $O_2^{\bullet-}$ , which is converted to  $H_2O_2$  by manganese superoxide dismutase (MnSOD).  $H_2O_2$  diffuses to the vascular smooth muscle of coronary resistance vessels, activating  $K_v$  channels and resulting in membrane hyperpolarization and vasodilation.

Saitoh et al. [44] also performed *in vivo* experiments demonstrating that production of  $H_2O_2$  occurs in proportion to metabolism and that the vasodilatory action of metabolically produced  $H_2O_2$  functions to couple coronary blood flow to myocardial oxygen consumption. Subsequent work by Saitoh and colleagues [45] has indicated that the coronary vasodilatory effects of cardiac  $H_2O_2$  occur through oxidation of thiols and activation of mytogen-activated protein kinase. Yada and colleagues have performed extensive experiments that demonstrate the importance of  $H_2O_2$  in adjusting coronary vascular resistance during pacing and during ischemia followed by reperfusion [46, 47]. Although the cellular sources of  $H_2O_2$  that are produced *in vivo* have not been clearly identified, the work of Saitoh et al. [44] indicates that a portion of the vasoactive  $H_2O_2$  produced during pacing is released from myocytes. Collectively, these data support a broad role for  $H_2O_2$  in metabolic coronary flow recruitment. Although other redox-sensitive metabolic products may also contribute to regulation of coronary blood flow, Figure 2 illustrates that minute-to-minute regulation of coronary blood flow is likely to occur through production of a redox-generated metabolite that does not require generation of an error signal. Rather, increasing oxidative metabolism and flux through the electron transport chain results in greater generation of superoxide and superoxide-derived reactive oxygen species, including hydrogen peroxide. These reactive oxygen species stimulate vasodilation in a feed-forward manner, establishing a continuous signal that matches blood flow to myocardial demand.

Hydrogen peroxide may also link endothelial function to vascular resistance. Recent *ex vivo* evidence indicates

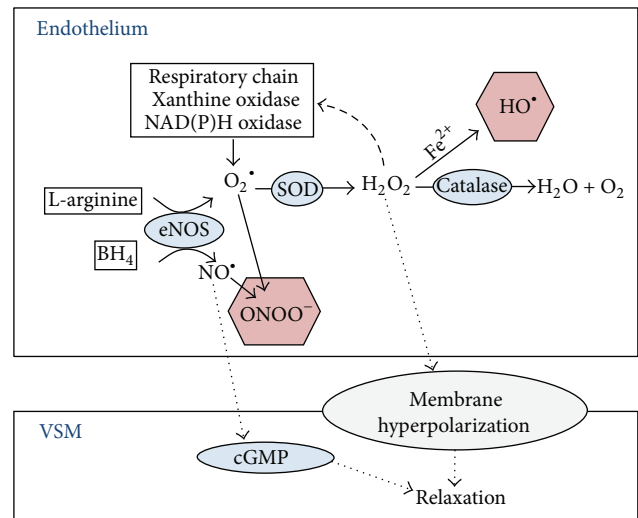


FIGURE 3: ROS generation and signaling in the coronary microvascular endothelium. In the presence of sufficient substrate (L-arginine) and cofactor (tetrahydrobiopterin,  $BH_4$ ) endothelial nitric oxide synthase (eNOS) reduces molecular  $O_2$  to nitric oxide ( $NO^{\bullet}$ ). When  $BH_4$  and/or L-arginine become limiting,  $O_2^{\bullet-}$  is produced by “uncoupled” eNOS. NADPH oxidases, xanthine oxidase, and the respiratory chain are also sources of  $O_2^{\bullet-}$ . Superoxide quickly reacts with  $NO^{\bullet}$  to produce peroxynitrite ( $ONOO^-$ ). Endogenous superoxide dismutase (SOD) dismutates  $O_2^{\bullet-}$  to form  $H_2O_2$ . Hydrogen peroxide ( $H_2O_2$ ) acts as a vasodilator by producing membrane hyperpolarization and relaxation of the vascular smooth muscle; however, in the presence of catalytic transition metals (i.e.,  $Fe^{2+}$ ), excess  $H_2O_2$  produced by treatment with exogenous SOD could produce the potent cytotoxic radical,  $^{\bullet}OH$ . Hydrogen peroxide can be eliminated from the cytosol through conversion to  $H_2O$  and  $O_2$  by catalase.  $H_2O_2$  can alter the activity of  $O_2^{\bullet-}$ -generating enzymes in a feedback manner.

that endothelial regulation of coronary vascular resistance occurs in part through redox signaling [48, 49]. Addition of the SOD-mimetic, Tempol, reduced endothelium-dependent, flow-induced vasodilation and addition of the iron chelating agent, deferoxamine, reversed this Tempol-mediated inhibition of endothelium-dependent dilation [48]. Figure 3 illustrates that treatment with exogenous SOD may drive overproduction of  $H_2O_2$  and promote formation of  $HO^{\bullet}$  in the endothelium. Together these data suggest that although  $H_2O_2$  may function as an important endothelium-dependent vasodilator, production of  $H_2O_2$  that exceeds the buffering capacity of the endothelium can impair endothelial function, and this is likely due to excess production of  $HO^{\bullet}$ .

**2.3.  $K_{ATP}$  Channels.** Increased activation of  $K^+$  channels hyperpolarizes smooth muscle and reduces contraction by reducing calcium entry through voltage-gated calcium channels. Thus, regulation of coronary arteriolar vasomotor tone is linked to  $K^+$  channel regulation of smooth muscle membrane potential. ATP-sensitive potassium channels have been shown to play an important role in autoregulation of coronary blood flow. These channels are inhibited by intracellular



ATP; thus, during hypoxia a decrease in intracellular ATP in arterial smooth muscle will activate these channels leading to hyperpolarization and relaxation [50]. The vasodilatory effect of ATP-sensitive potassium channel activators has been documented. In the coronary circulation of dogs [51], blockade of ATP-sensitive potassium channels by glibenclamide inhibits autoregulation. Coronary arteriolar vasodilation that occurred in response to reductions in perfusion pressure was abolished by glibenclamide. Glibenclamide also attenuated the vasodilation of coronary arterioles and small arteries during reactive hyperemia [21] and reduced coronary flow during the late phase of reactive hyperemia. Furthermore, hypoxic vasodilation in isolated guinea pig hearts was prevented by glibenclamide [52]. Metabolic vasodilation induced by  $\beta_1$ -adrenergic stimulation of the heart is also attenuated by glibenclamide treatment. Kuo and Chancellor [53] have shown in isolated coronary arterioles that adenosine potentiates flow-induced vasodilation through activation of ATP-sensitive potassium channels. Hyperosmolarity and hypoxia also activate ATP-sensitive potassium channels and may serve as stimuli for these channels, resulting in decreased microvascular resistance [54]. These data suggest that ATP-sensitive potassium channels are involved in both autoregulatory responses and metabolic vasodilation of the coronary circulation that occurs in response to an increase in oxygen demand. However, they must be interpreted cautiously in light of recent reports showing that glibenclamide has a partial blocking effect on calcium-activated potassium channels and L-type calcium channels [55].

**2.4.  $K_v$  Channels.** More recent studies indicate that voltage-gated potassium ( $K_v$ ) channels are critical in regulation of coronary arteriolar diameter. Immunohistochemical analysis of the human heart indicates the presence of  $K_v1.5$  channels in coronary artery smooth muscle [56] and multiple  $K_v1$  proteins are expressed in canine and rat coronary artery [57, 58].  $K_v$  channels contribute to regulation of basal and hyperemic coronary blood flow in the canine heart [57]. Blockade of  $K_v$  channels with 4-aminopyridine significantly reduces baseline diameter and inhibits adenosine-induced relaxation of isolated porcine coronary arterioles [59]. 4-aminopyridine also antagonizes endothelium-independent vasodilation of coronary arterioles by  $H_2O_2$  and abolishes  $H_2O_2$ -induced increases in coronary blood flow [60, 61]. Several studies have implicated  $K_v$  channels in mediation of endothelium-dependent vasodilation of coronary arteries and arterioles [62, 63]. NO and iloprost (a stable analogue of  $PGI_2$ ) activate  $K_v$  channels in coronary artery smooth muscle cells [64]. 4-AP inhibits EDHF-induced relaxation of guinea pig coronary arteries [65, 66]. These studies do not establish the relative contribution of  $K_v$  channels to the regulation of arterial tone in the coronary resistance vasculature *in vivo*; however, collectively these findings indicate that  $K_v$  channel activity is involved in both endothelium-dependent and -independent regulation of coronary vascular smooth muscle. These data also suggest that  $K_v$  channels are critical to vasodilatory responses of coronary arteries and arterioles.

### 3. Myogenic Control

Within the intact coronary circulation, the myogenic response may act as a regulatory mechanism maintaining blood flow within a steady range in the face of changing perfusion pressure. However, myogenic responses of the coronary circulation have been difficult to evaluate *in vivo* because it is necessary to separate the responses to alterations in perfusion pressure from the responses to changing flow or altered metabolic state. Eikens and Wilcken [67] demonstrated that a considerable reactive hyperemic response occurred in dog hearts even after coronary occlusions lasting only one to two cardiac cycles. Because the hyperemia occurred after such a brief occlusion they reasoned that it could not be the result of buildup of vasodilatory metabolites and must be due in part to dilation in response to the decrease in perfusion pressure during the occlusion, that is, a myogenic dilation. While the dilation may have been in part due to myogenic dilation this protocol also produced a large change in flow upon release of the occlusion and flow-induced dilation may have also contributed to the reactive hyperemic response. Additionally, data from a study by Schwartz et al. [68] indicate that a transient increase in cardiac metabolic demand may produce an immediate increase in blood flow. These investigators used an extra stimulus to potentiate systole (maximal ventricular  $dp/dt$  increased by 50%) without producing a discrete mechanical extrasystole. The potentiated systole, which was presumed to increase metabolic demand, was accompanied by an increase in coronary flow within the same cardiac cycle suggesting that changes in cardiac metabolic demand alter coronary vascular resistance on a beat-to-beat basis. However, these data do not refute the possibility that myogenic dilation contributes to coronary reactive hyperemia.

Myogenic responses are not present in all segments of the coronary circulation. Isolated resistance arteries (200–300  $\mu m$  in diameter) do not demonstrate active myogenic responses to changes in transmural pressure [69]. In contrast, using a technique in which coronary arterioles were isolated and perfused with a system that allowed pressure to be altered independently of changes in flow, Kuo et al. [70] demonstrated active myogenic responses in coronary arterioles between 80 and 100  $\mu m$  in diameter. These smaller coronary arterial vessels constrict in response to increases in intraluminal pressure and dilate in response to decreases in intraluminal pressure; however, transmural variation in the response was evident. The myogenic responsiveness of subepicardial arterioles was more pronounced than that of subendocardial arterioles. These investigators further demonstrated that the myogenic response in coronary arterioles is an intrinsic response of the smooth muscle which is independent of an intact endothelium [71]. They also showed myogenic responses of isolated coronary vessels interact with flow-induced vasodilation [72]. Human atrial arterioles also display variable myogenic activity, with the smaller arterioles exhibiting greater myogenic responsiveness [73]. Thus, as with other control mechanisms, myogenic responses appear to be more pronounced within a specific segment

of the coronary vascular tree, specifically in intermediate-sized arterioles. Although the contribution of the myogenic response to the regulation of coronary vascular resistance has not been definitively evaluated *in vivo*, these data suggest that myogenic activity contributes to control of coronary blood flow and especially to autoregulation.

#### 4. Endothelial Control

The endothelium modulates vascular tone through the release of vasoactive compounds that are capable of producing both vasoconstriction and vasodilation of the underlying smooth muscle. Endothelium-dependent release of factors such as nitric oxide, prostaglandins, endothelin, and endothelium-derived hyperpolarizing factor may occur in response to humoral substances, changes in intravascular flow (i.e., shear stress), and neural stimulation. Evidence suggests that coronary vascular tone is constantly being modulated by the endothelium under varying physiological and pathophysiological conditions. Using either pharmacological stimuli such as acetylcholine or changes in intraluminal flow to stimulate release of endothelium-dependent vasodilators, clinical studies have revealed the importance of nitric oxide and other vasodilatory compounds in control of vascular tone in both coronary arteries and arterioles [74].

**4.1. Nitric Oxide.** Both *in vivo* work and *in vitro* work indicate that endothelium-dependent release of nitric oxide, in particular, is important in control of coronary vascular resistance. Endothelium-dependent production of nitric oxide occurs via the enzyme nitric oxide synthase, which catalyzes the production of nitric oxide from L-arginine. Analogs of L-arginine act as competitive inhibitors of this enzyme and therefore, block production of nitric oxide. These inhibitors of nitric oxide synthase have been used extensively to investigate the role of nitric oxide production in modulation of coronary vascular resistance. *In vitro* studies have demonstrated that endothelium-dependent relaxation of large coronary arteries to acetylcholine [75], serotonin [35], and the  $\alpha_2$ -agonist, clonidine [76], are mediated through release of nitric oxide. In porcine coronary resistance arteries [77] and arterioles [72], antagonists of nitric oxide synthase produce constriction under baseline conditions, suggesting that there is tonic release of nitric oxide in small coronary vessels. In isolated porcine coronary microvessels, clonidine [78], serotonin [78], and substance P [72] produce endothelium-dependent relaxation through release of nitric oxide. In dogs, dilation of coronary resistance arteries to acetylcholine is also mediated through release of nitric oxide [75]. In the pig, where acetylcholine produces constriction of coronary microvessels, this response is modulated by release of nitric oxide from the endothelium [77].

*In vivo* studies indicate that nitric oxide may modulate coronary vascular resistance under varying physiological conditions. Chu et al. [79] reported that  $N^G$ -monomethyl-L-arginine (L-NMMA), an analog of L-arginine that acts as an antagonist of nitric oxide synthase, constricted epicardial arteries although it had no effect on baseline coronary blood

flow in conscious dogs. Consistent with these findings, Parent et al. [80] showed that infusion of  $N^w$ -nitro-L-arginine (L-NNA), another analog of L-arginine, did not alter baseline coronary blood flow in conscious dogs but significantly reduced the increase in coronary blood flow in response to acetylcholine, adenosine, and short coronary occlusions. More recent work from this group has also indicated that  $\beta$ -adrenergic vasodilation of the coronary circulation can be partially blocked by inhibition of nitric oxide production [81]. Jones et al. [82] studied vasoconstrictor responses to  $\alpha_1$ - and  $\alpha_2$ -adrenergic agonists in the coronary microcirculation and found that these responses were attenuated by endothelium-dependent release of nitric oxide in as much as inhibition of nitric oxide synthase potentiated constriction produced by  $\alpha$ -adrenergic activation. Several laboratories [83–87] have now shown that inhibition of nitric oxide production attenuates the reactive hyperemic response. Of these groups Yamabe et al. [83] and Gattullo et al. [84] reported that inhibition of nitric oxide synthesis did not alter basal coronary flow while Kostic and Schrader [87] found that nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase, reduced basal coronary blood flow by 16%. The reason for these differences is not certain; however, Kostic and Schrader [87] studied guinea pigs and the other two groups worked with dogs; the discrepancies may be related to species differences. Also, the arginine analogs used to inhibit nitric oxide synthesis production differed in these studies and this may have contributed to the opposing results.

Smith and Canty [86] showed that nitric oxide production does not contribute to basal coronary blood flow or to flow adjustments that occur over the autoregulatory range in conscious dogs. However, they did find that at perfusion pressures below the autoregulatory range (pressures at which myocardial ischemia occurs) synthesis of nitric oxide contributes to coronary vasodilation. They also found that nitric oxide contributed to the reactive hyperemic response. In contrast, Pohl et al. [88] found that both basal coronary blood flow and autoregulatory responses (pressures ranging from 45 to 120 mm Hg) were augmented in isolated rabbit hearts, following treatment with  $N^G$ -nitro-L-arginine. They also showed that nitric oxide contributes to reactive hyperemia. Duncker and Bache [89] studied the effect of nitric oxide inhibition on the coronary flow response to exercise in dogs under control conditions and in the presence of coronary stenosis. They found that under control conditions basal coronary blood flow and coronary blood flow responses to exercise were not reduced by treatment with an arginine analog but that during exercise with coronary stenosis present, increases in coronary blood flow were significantly inhibited by blockade of nitric oxide synthesis. In humans, Quyyumi et al. [90] reported that cardiac pacing produced less microvascular dilation after treatment with L-NMMA, suggesting that nitric oxide production contributes to coronary metabolic vasodilation. In contrast, Nishikawa and Ogawa [91] administered L-NMMA to patients at rest and during pacing but found that L-NMMA reduced coronary blood flow at rest but not during pacing. These investigators used a lower concentration of L-NMMA and did not perform

any other pharmacological interventions during their study, which may explain the differences between this study and the study of Quyyumi and colleagues [90].

Thus, conflicting results have been obtained concerning the contribution of endothelial nitric oxide to coronary blood flow under basal conditions and during experimentally induced elevations in heart rate. However, inhibition of nitric oxide synthesis alters the coronary flow response to pharmacological intervention with acetylcholine, adenosine, and  $\alpha$ - and  $\beta$ -adrenergic agonists. Data from a number of studies also support a role for endothelial nitric oxide in regulation of coronary blood flow during exercise, following coronary occlusion, and during hypoperfusion.

**4.2. Hyperpolarizing Factor(s).** In addition to the important effects of endothelial nitric oxide in control of coronary microvascular tone and blood flow, increasing evidence indicates that the endothelium produces factors capable of causing hyperpolarization of the underlying smooth muscle. Maintenance of vascular smooth muscle tone is directly linked to the membrane potential of the vascular smooth muscle. Any intervention which produces an adjustment in the membrane potential will alter vascular smooth muscle tone. For example, if the extracellular concentration of potassium is increased above 30 mM, this will produce membrane depolarization and opening of voltage-gated calcium channels. An influx of calcium through these channels will lead to an increase in the contractile state of the smooth muscle or increased vascular tone. In a similar manner, if an endothelium-derived hyperpolarizing factor (EDHF) acts on the underlying smooth muscle potassium channels to cause membrane hyperpolarization, this will lead to inactivation of voltage-gated calcium channels, a net decrease in the entry of calcium into the smooth muscle cells, and vasodilation.

Several studies have now shown that production of EDHF may be an important mechanism of control of vascular tone in the coronary circulation [92–94]. The epoxyeicosatrienoic acids (EETs), which are metabolites of cytochrome P 450, have been identified as likely candidates for endothelium-derived hyperpolarizing factors [95]. In the coronary circulation, these compounds have been shown to cause vasorelaxation both *in vitro* and *in vivo*. In an *in vivo* beating heart preparation, Widmann et al. [93] found that acetylcholine-induced vasodilation of small arterioles was completely inhibited when L-NNA was used to block NO formation and clotrimazole was used to block cytochrome P-450 metabolism. Neither L-NNA or clotrimazole alone produced complete blockade suggesting that small arteriolar dilation to acetylcholine is mediated through both NO and cytochrome P-450 metabolites [93]. Large arteriolar dilation to acetylcholine was not affected by clotrimazole but was blocked completely by L-NNA. These findings suggest that the production of EDHF may be especially prominent in smaller coronary vessels. Nishikawa and colleagues [94] performed an even more definitive study in the beating heart in which they showed that acetylcholine-induced vasodilation of arterioles in the beating heart was blocked completely by administration of indomethacin, L-NMMA, and 60 mM

potassium chloride. These investigators also demonstrated that miconazole, a more specific inhibitor of cytochrome P-450, when given in conjunction with L-NMMA and indomethacin, completely blocked the vasorelaxation to acetylcholine. In isolated arterioles, very low concentrations of EETs produced vigorous vasodilation [92]. Miura and Gutterman [96] have shown in isolated human coronary arterioles that 17-octadecyonic acid, an inhibitor of cytochrome P-450, significantly attenuated vasodilation to arachidonic acid, whereas L-NAME and indomethacin, a cyclooxygenase inhibitor, had no effect. Arachidonic acid also produced significant hyperpolarization of the vascular smooth muscle in the isolated vessels. Depolarization with 40 mM potassium chloride blocked the vasodilation to arachidonic acid. These results suggest that arachidonic acid metabolites produce prominent hyperpolarization and vasodilation in the human coronary microcirculation. The concentrations producing vasodilation were much lower than those which had been reported previously for large arteries, again suggesting a significant role of EDHF in modulating coronary vascular resistance in the small vessels of the coronary circulation.

H<sub>2</sub>O<sub>2</sub> has been identified as a major endothelium-derived hyperpolarizing factor in porcine [42] and human [43] coronary arterioles. Both bradykinin and substance P stimulate endothelial H<sub>2</sub>O<sub>2</sub> production [42] as detected by electron spin resonance. In addition, catalase inhibits coronary arteriolar dilation to bradykinin, substance P, and intraluminal flow [42, 43]. In addition to its role as an endothelium-derived vasodilator, H<sub>2</sub>O<sub>2</sub> may modulate the action of other hyperpolarizing agents such as the EETs [97]. In Type 2 diabetic mice, the contribution of H<sub>2</sub>O<sub>2</sub> to endothelium-dependent vasodilation increases in a compensatory fashion during the progression of endothelial dysfunction [98]. Similarly, in coronary arterioles from heart failure patients, H<sub>2</sub>O<sub>2</sub> is a prominent contributor to flow-dependent vasodilation [43]. In rats, the contribution of H<sub>2</sub>O<sub>2</sub> to flow-induced vasodilation of coronary arterioles declines with age [48]. Together, these data suggest that endothelium-derived H<sub>2</sub>O<sub>2</sub> functions as an important regulator of vascular smooth muscle tone in the coronary resistance vasculature. An increase in H<sub>2</sub>O<sub>2</sub> signaling can provide compensatory vasodilatory function, but a loss of H<sub>2</sub>O<sub>2</sub> signaling can also contribute to a loss of endothelial function in coronary arterioles.

**4.3. Humoral Influences.** Various humoral substances that are present in circulating blood or produced in the tissue may exert an effect on coronary vascular resistance through endothelium-dependent mechanisms. Many of these agonists produce heterogeneous effects within the coronary circulation that may be attributable to variations in endothelium-dependent modulation. Serotonin, a humoral agent produced by activated platelets, produces heterogeneous effects in the coronary circulation. Serotonin constricts large coronary arteries, yet it has been reported to increase total coronary blood flow suggesting that it has vasodilatory actions. In large arteries, serotonin produces constriction that is modulated by endothelium-dependent vasodilation, evidenced by the fact that removal of the endothelium results in augmentation



of vasoconstriction in response to serotonin [99]. Serotonin also constricts small coronary arteries (150–300  $\mu\text{m}$ ) [5]. In contrast, serotonin produces marked dilation of coronary arterioles less than 100  $\mu\text{m}$  in diameter, presumably due to a predominant effect on the endothelium in these smaller vessels.

The vasoactive effects of vasopressin also vary within the coronary circulation. In isolated epicardial arteries, vasopressin produces vasodilation. In the beating heart, diameter measurements of epicardial vessels ranging in size from approximately 50 to 300  $\mu\text{m}$  showed that vasopressin caused slight dilation of vessels greater than 100  $\mu\text{m}$  in diameter while it constricted vessels less than 90  $\mu\text{m}$  in diameter. These diameter changes were accompanied by an overall increase in vascular resistance and a decrease in coronary blood flow [5]. The diversity of these responses could be related to autoregulatory adjustments. The decrease in resistance in large arteries due to direct vasodilatory effects of vasopressin may produce an increase in downstream pressure promoting myogenic constriction of resistance vessels. Alternatively, the dilation of large vessels may be due to endothelium-dependent vasodilation that is absent in smaller vessels. Vasopressin relaxes isolated coronary arteries; this effect is significantly attenuated by removal of the endothelium [75], while isolated resistance arteries constrict in response to vasopressin. These data support the notion that the disparate effects of vasopressin in large and small coronary vessels are due to a difference in endothelium-mediated mechanisms.

Bradykinin induces potent endothelium-dependent dilation of coronary arterioles that is mediated through diverse, yet potentially interactive signaling pathways [97, 100–103]. Bradykinin has been demonstrated to produce relaxation of porcine coronary microarteries, through both *de novo* synthesis of nitric oxide and a non-nitric oxide mediator which activates  $\text{K}_{\text{Ca}}$  channels [100]. Exercise training improves bradykinin-induced, NO-mediated vasodilation of porcine coronary arterioles [104]. More recent evidence indicates that bradykinin-induced stimulation of  $\text{BK}_{\text{Ca}}$  channels is enhanced by exercise training in porcine coronary arterioles and  $\text{H}_2\text{O}_2$  is the likely mediator of channel activation and subsequent enhanced vasodilation [105]. Brief exposure to bradykinin preconditions the coronary microvasculature and improves the recovery of microvascular function following 60 minutes of ischemic arrest with cold crystalloid cardioplegia, with the preconditioning effect mediated through opening of  $\text{K}_{\text{Ca}}$  channels [101, 102]. Knockout of the kinin B2 receptor and the absence of B2R signaling result in capillary rarefaction and microvascular dysfunction in mice at twelve months of age [106]. Together, these data indicate that bradykinin signaling is critical to coronary microvascular endothelial function and maintenance and suggest that the bradykinin pathway is a target for both beneficial and pathological adaptations of coronary endothelial function.

Collectively, these data demonstrate the prominent role of the endothelium in modulating coronary vascular resistance. Endothelial release of nitric oxide modulates myogenic responses and  $\alpha$ -adrenergic vasoconstrictor responses. A number of humoral agents (e.g., acetylcholine, serotonin, substance P, and bradykinin) which produce profound

vasodilation of the coronary vasculature do so through endothelium-dependent mechanisms. Perhaps most importantly, vasodilation in response to changes in blood flow, a constantly changing physiological parameter, is dependent on the endothelium. Predominantly, the endothelium mediates a vasodilatory effect. Under some conditions, endothelium-dependent vasodilation may play a modulatory role by competing with vasoconstriction resulting from myogenic,  $\alpha$ -adrenergic, and humoral influences. Alternatively, endothelium-dependent vasodilation may actively contribute to the vasodilation which occurs during reactive hyperemia, exercise, and  $\beta$ -adrenergic activation.

## 5. Neural Control

Innervation of the coronary vasculature by both the parasympathetic and sympathetic nervous systems has been documented in both animals and humans [85, 107]. Both sympathetic and parasympathetic nerve fibers are located within the vascular wall of large arteries as well as resistance arteries and arterioles [107] with more dense innervation being present in resistance arteries and arterioles [108]. However, assessment of the direct influence of parasympathetic and sympathetic stimulation on coronary vascular resistance is complicated by changes in myocardial metabolism, which has a major role in determining coronary blood flow. A variety of techniques have been employed in studies to evaluate parasympathetic or sympathetic control of the coronary vasculature in the absence of autonomic influences on the myocardium and subsequent changes in heart rate, myocardial contractility, and perfusion pressure.

**5.1. Sympathetic Control.** Sympathetic control of the coronary circulation is exerted through both  $\alpha$ -adrenergic and  $\beta$ -adrenergic effects [109–115]; however, the direct effects of sympathetic stimulation of coronary vascular receptors are often masked by the metabolic vasodilation which occurs in response to sympathetic stimulation of the heart. Data obtained during conditions in which metabolic effects of sympathetic stimulation are controlled suggest that both  $\alpha$ -adrenergic and  $\beta$ -adrenergic regulation of coronary vascular resistance may be important during physiological and pathological conditions.

**5.1.1.  $\alpha$ -Adrenergic Regulation.** The primary effect of  $\alpha$ -adrenergic activation of coronary vessels appears to be a vasoconstriction of the vascular smooth muscle. However, a clear demonstration of coronary  $\alpha$ -receptor-mediated vasoconstriction has been difficult in the presence of competing metabolic influences secondary to augmented myocardial oxygen consumption. Coronary vasoconstriction in  $\beta$ -receptor-blocked hearts can be elicited by injection of phenylephrine and other  $\alpha$ -receptor agonists [108]. *In vivo* work suggests that although  $\alpha$ -adrenergic constriction may have little effect on resting coronary blood flow [116, 117], it plays an important role in determining coronary vascular resistance during conditions of increased myocardial oxygen demand created by sympathetic stimulation [118] and

exercise [113, 117, 119–121]. Mohrman and Feigl [118] measured coronary blood flow during conditions of increased myocardial metabolism created by sympathetic stimulation before and after  $\alpha$ -adrenergic blockade. They found that at the same level of myocardial oxygen consumption, blood flow was significantly higher in the presence of  $\alpha$ -adrenergic blockade. These results indicated that the adrenergic constriction competed with metabolic vasodilation, thus reducing oxygen delivery to the myocardium. Studies in exercising animals have shown that  $\alpha$ -adrenergic constriction restricts coronary blood flow during exercise [117, 119, 121–123]. During conditions such as hypotension [113, 124–126] and myocardial ischemia [113, 127, 128], in which myocardial oxygen delivery is impaired, adrenergic constriction competes with vasodilation produced by local metabolic mechanisms. Administration of  $\alpha$ -adrenergic blocking agents increases coronary blood flow during ischemia indicating persistent  $\alpha$ -adrenergic constriction which may, in fact, aggravate the ischemic condition [113, 127]. Similarly, coronary blood flow increases during hypotension following  $\alpha$ -adrenergic blockade [124–126]. The increase in blood flow that occurs after removal of  $\alpha$ -adrenergic constriction is accompanied by an increase in myocardial oxygen consumption suggesting that  $\alpha$ -adrenergic tone limits myocardial oxygenation as well as coronary flow. The physiological role of this adrenergic constriction remains to be determined; however, it is apparent that blockade of this response results in greater coronary blood flow and increased myocardial performance under conditions of physiological (i.e., exercise) and pathophysiological stress (i.e., ischemia produced by stenosis or hypotension).

Several studies have been performed in an attempt to determine whether the adrenergic constriction in the coronary circulation which persists during conditions of increased myocardial demand and limited oxygen delivery is mediated by  $\alpha_1$ - or  $\alpha_2$ -adrenergic receptors. Opposing results have been obtained. Gwartz et al. [117] determined that the adrenergic constriction that remains during exercise is mediated by  $\alpha_1$ -adrenergic receptors. They injected prazosin, a specific  $\alpha_1$ -antagonist, into the coronary circulation of dogs during submaximal exercise and found an increase in circumflex blood flow that was accompanied by an increase in left ventricular function (an increase in the rate of segmental shortening and in  $dP/dt$  max). These results have been confirmed by Dai et al. [121] who reported that coronary blood flow in running dogs was increased by blockade with prazosin. Combined blockade with both prazosin and idazoxan, an  $\alpha_2$ -antagonist, did not produce any further increase in coronary blood flow at the same level of exercise. In contrast to these results, Seitelberger and colleagues [127] reported that  $\alpha_2$ -adrenergic blockade attenuated myocardial ischemia in running dogs. The reason for the differences in these studies is not completely clear but may be related to the presence of ischemia produced by stenosis in the study of Seitelberger and coworkers. No ischemia was present in the studies by Gwartz et al. [117] and Dai et al. [121]; the measurements were made during conditions of submaximal exercise.

Varying results have also been reported concerning the contribution of  $\alpha_1$ - and  $\alpha_2$ -mediated constriction during conditions of coronary ischemia. Seitelberger et al. [127] determined that myocardial dysfunction produced by ischemia during exercise was attenuated by blockade of  $\alpha_2$ -adrenergic receptors with idazoxin. Heusch and Deussen [129] found that stimulation of cardiac sympathetic nerves produced an increase in resistance in coronary resistance distal to a severe stenosis. Blockade with the  $\alpha_2$ -antagonist, rauwolscine, but not with prazosin prevented the increase in resistance suggesting that the effect was mediated by  $\alpha_2$ -adrenergic constriction.

Some of the differences found in the studies described above may be related to the heterogeneous distribution of adrenergic receptors within the coronary vascular tree. These studies have considered the coronary resistance vasculature as a homogenous unit; however, even within the microcirculation a heterogeneous distribution of the effects of  $\alpha$ -adrenergic activation is present [3, 115]. Large epicardial coronary arteries constrict in response to  $\alpha_1$ -receptor agonists [128]; however, the primary sites of  $\alpha$ -adrenergic regulation in the coronary circulation appear to be in small arteries and large arterioles. In contrast to large epicardial arteries, there appears to be a functional distribution of both  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors in coronary resistance vessels [128, 130]. Activation of  $\alpha$ -receptors by exogenous norepinephrine infusion produces constriction of coronary arterioles greater than 100  $\mu\text{m}$  in diameter and a simultaneous dilation of arterioles less than 100  $\mu\text{m}$  in diameter [3, 115, 130]. These diverse effects are likely related to pressure changes created by the constriction of larger arterioles. If the upstream vessels (>100  $\mu\text{m}$ ) constrict this will reduce pressure in the smaller downstream arterioles. The decrease in pressure may then trigger a myogenic dilation in these smaller vessels. In contrast, if perfusion pressure is maintained below physiological levels (hypoperfusion), autoregulatory responses to pressure changes can be eliminated and both  $\alpha_1$ - and  $\alpha_2$ -adrenergic vasoconstriction can be unmasked in both large (>100  $\mu\text{m}$ ) and small coronary arterioles (<100  $\mu\text{m}$ ) [130].

$\alpha$ -Adrenergic receptors that mediate vasodilation are also present on the endothelium of coronary arterial vessels. The selective  $\alpha_2$ -agonist clonidine relaxes isolated epicardial coronary arteries precontracted with prostaglandin  $F_{2\alpha}$  and resistance arteries precontracted with acetylcholine [69, 76, 78]. Although intense activation of  $\alpha$ -adrenergic receptors has been consistently shown to produce vasoconstriction in the coronary circulation, some work has suggested that  $\alpha_2$ -adrenergic receptors may have dual effects in the coronary circulation. Threshold activation of  $\alpha_2$ -adrenergic receptors causes vasodilation of the coronary vasculature [131]. Maximal dilation of the coronary circulation in response to either exogenous or endogenous adenosine can be augmented by  $\alpha_2$ -adrenergic receptor stimulation with low doses of clonidine [132]. In the beating heart, the vasoconstrictor responses of small arteries and arterioles to  $\alpha_1$ - and  $\alpha_2$ -agonists were potentiated by the L-arginine analogs,  $N^G$ -nitro-L-arginine and  $N^G$ -nitro-L-arginine-methyl ester, suggesting that endothelium-dependent release of nitric oxide

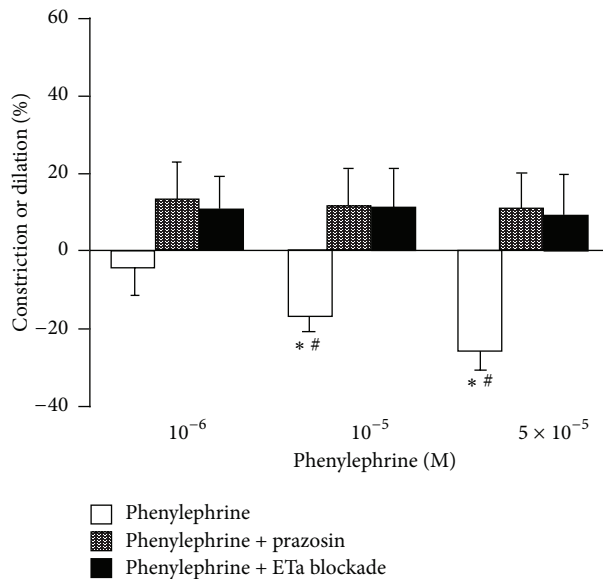


FIGURE 4: Vasoactive effects of supernatant-derived from myocytes in isolated coronary arterioles treated with 8-PSPT to block adenosine receptors. Myocyte-derived supernatant was obtained from myocytes treated with phenylephrine or simultaneously treated with phenylephrine and prazosin. Addition of the  $\alpha_1$ -adrenergic receptor antagonist, prazosin, to myocytes prevented the production of a vasoconstrictor compound in the supernatant and eliminated constriction of the isolated arterioles. Direct administration of prazosin to isolated arterioles was without effect, indicating that  $\alpha$ -adrenergic constriction is not due to direct effects on arteriolar smooth muscle. Administration of the ETa antagonist to the isolated arterioles abolished the constrictor response that was observed during exposure to myocyte-derived supernatant. \*  $P < 0.05$  versus other groups; #  $P < 0.05$  versus baseline. Adapted from Teifenbacher et al. [8], with permission.

occurs simultaneously with  $\alpha$ -adrenergic activation and may compete with the adrenergic constriction [82]. Under most conditions, this dilation to  $\alpha$ -adrenergic agonists appears to be masked by predominant constrictor effects. However, it is possible that under pathological conditions, such as in atherosclerosis in which endothelial function is impaired, the loss of adrenergically-mediated, endothelium-dependent vasodilation may contribute to hyperreactivity to adrenergic stimulation.

Paradoxically, it has been shown by Jones et al. [133] that isolated coronary arterioles do not respond to stimulation with  $\alpha$ -adrenergic agonists, despite experimental conditions similar to those in which venules demonstrate marked constriction. The disparity in experimental results found using *in vivo* and *in vitro* preparations of the coronary microcirculation may be due to indirect effects of  $\alpha$ -adrenergic stimulation in the heart. Endothelin antagonists block  $\alpha_1$ -adrenergic induced constriction of coronary arterioles in the intact beating heart [134], and cardiac myocytes stimulated with phenylephrine produce vasoactive substances (including endothelin) capable of constricting isolated coronary arterioles [8]. Figure 4 [8] shows that when coronary arterioles were exposed to supernatant derived from myocytes

treated with increasing concentrations of phenylephrine, a contractile response was elicited. This vasoconstriction was completely blocked by addition of the ETa antagonist, FR 139317. Autoradiographic studies indicate that the density of  $\alpha_1$ -adrenergic receptors on cardiac myocytes far exceeds that of coronary arterioles [135]; thus,  $\alpha_1$ -adrenoceptor-stimulated production of vasoactive substances by cardiac myocytes constitutes a likely mechanism for sympathetic constriction in the coronary microcirculation. Recently, Gorman et al. [136] demonstrated that although  $\alpha_1$ -adrenergic stimulation of endothelin production does not appear to contribute to adrenergic-mediated coronary constriction during exercise, endothelin receptor blockade countered the reduction in coronary blood flow produced by bolus injections of phenylephrine. Thus, although isolated arterioles appear refractory to stimulation with adrenergic agonists, experimental evidence indicates that adrenergic modulation of coronary microvascular tone occurs through indirect mechanisms that are not governed by myocardial demand, but which do occur through a myocyte-vessel interaction.

Considered together, these data indicate that adrenergic control of the coronary circulation is important during both physiological and pathophysiological conditions. The predominant effect of adrenergic stimulation of the coronary circulation is to increase vascular tone. Under various conditions that are associated with an increase in sympathetic stimulation, blockade of adrenergic receptors results in an increase in coronary blood flow. Within the coronary microcirculation, a direct adrenergic constriction can be demonstrated in both small arteries and in arterioles; however, under normal conditions in which the coronary vascular bed retains its ability to autoregulate, small arterioles escape from adrenergic constriction and show substantial vasodilation. For example, under control conditions in which autoregulatory mechanisms remain intact the  $\alpha_2$ -agonist, BHT-933, constricts large coronary microvessels but in a number of small arterioles autoregulatory dilation predominates over the vasoconstrictor effects of BHT-933 [130]. Significant constriction to  $\alpha_2$ -adrenergic stimulation is present in both large and small arterioles when autoregulation is prevented. Thus, although adrenergic receptors which mediate constriction are present throughout the coronary circulation, functional responses to adrenergic stimulation are heterogeneous. The heterogeneous responses to adrenergic stimulation result mainly from heterogeneous distribution of adrenergic receptors and modulation of adrenergic constriction by other controlling factors such as autoregulatory and endothelium-dependent responses.

**5.1.2.  $\beta$ -Adrenergic Regulation.**  $\beta$ -adrenergic receptors have also been identified in the coronary vasculature [137]. Activation of both  $\beta_1$ - and  $\beta_2$ -adrenergic receptors have been shown to elicit vasodilation in the coronary circulation. In general the vasodilation that occurs due to direct stimulation of coronary vascular  $\beta$ -receptors has not been readily distinguishable from the indirect metabolic vasodilation that occurs when heart rate and myocardial contractility are stimulated by cardiac  $\beta_1$ -receptors. The preponderance of data obtained



in the coronary circulation indicates that stimulation of  $\beta$ -receptors produces vasodilation of large epicardial arteries. Indirect evidence of  $\beta$ -adrenergic activation in resistance arteries comes from studies in which changes in coronary vascular resistance have been calculated from pressure/flow measurements in the whole heart under conditions in which myocardial metabolism has been controlled. Trivella et al. [110] demonstrated that isoproterenol induced vasodilation in the potassium-arrested heart during constant pressure perfusion. The dilation was blocked by the  $\beta_2$ -selective antagonist, L18,551, and by the  $\beta_1$ -selective agonists, practolol and L650,744. Radioligand binding studies indicate that as with  $\alpha$ -adrenergic receptors, the distribution of  $\beta$ -adrenergic receptors is heterogeneous within the coronary circulation. The ratio of  $\beta_1 : \beta_2$  receptors in large vessels is almost 2:1 [137], whereas a predominance of  $\beta_2$  receptors is found in resistance vessels [138]. Reverse transcription-polymerase chain reaction and immunohistochemistry indicate the presence of  $\beta_2$ -receptors in coronary arterioles, with a markedly greater level of both mRNA and protein expression of  $\beta_2$  receptors in subepicardial as compared to subendocardial arterioles [139]. Isolated porcine coronary arterioles relax in response to both norepinephrine and epinephrine [69, 140]. Recent data indicate that norepinephrine produces dilation of human coronary arterioles through activation of  $\beta_2$ -adrenoceptors on vascular smooth muscle [141]. Direct application of isoproterenol causes dilation of coronary microvessels in both *in vivo* and *in vitro* experimental preparations [139, 142, 143]. In isolated porcine coronary arterioles, isoproterenol-induced vasodilation was inhibited by the  $\beta_2$ -adrenoceptor blocker, ICI-118,551, but was insensitive to treatment with the  $\beta_1$  antagonist, atenolol [139].

Blockade of coronary  $\beta_2$ -adrenoceptors decreases coronary blood flow during exercise in both dogs and pigs [10, 11, 144]. A feedforward mechanism for  $\beta$ -adrenergic vasodilation has been demonstrated in the coronary microcirculation in response to intracoronary norepinephrine during exercise [10, 11, 112]. Gorman et al. [11] showed that in exercising dogs, treatment with an  $\alpha$ -adrenoceptor blocker increased coronary blood flow, whereas combined blockade of  $\alpha$ - and  $\beta$ -adrenoceptors resulted in a decrease in coronary blood flow. Gorman et al. [12] found that coronary venous oxygen tension was lower after specific blockade of  $\beta$ -adrenoceptors during exercise at an equivalent myocardial oxygen consumption [12], demonstrating that the myocardial oxygen supply-to-consumption ratio was reduced in the absence of  $\beta$ -adrenergic-mediated vasodilatory mechanisms.

**5.2. Parasympathetic Control.** Early studies of the effects of parasympathetic stimulation were inconclusive and attempts to demonstrate parasympathetic vasodilation produced negative results mainly because the negative chronotropic and inotropic effects of vagal stimulation were not controlled [108, 145]. However, Feigl [145] reported that vagal stimulation resulted in an increase in coronary blood flow in the dog heart. In that study, the effects of vagal stimulation on heart rate, contractility, and myocardial oxygen consumption were controlled and did not account for the increase in

blood flow. Additionally, atropine blocked the increase in coronary blood flow produced by vagal stimulation, indicating that the response was mediated through muscarinic receptors. Both *in vitro* and *in vivo* studies have shown that acetylcholine produces coronary vasodilation in dogs [146], rabbits, baboons, and goats [15]. However, the vasodilatory effect of acetylcholine is, in fact, species dependent. Species differences are likely related to differences in distribution of receptors on the endothelium and smooth muscle. In species that display coronary dilation to acetylcholine, muscarinic receptors on the endothelium mediate release of vasodilatory substances which cause relaxation of the underlying smooth muscle. In these species, removal of the endothelium from isolated coronary arteries converts the vasodilatory effect of acetylcholine to vasoconstriction [147]. In pigs and cattle, acetylcholine acts as a vasoconstrictor in coronary vessels presumably due to a lack of endothelial muscarinic receptors [147–150].

The role of the parasympathetic nervous system in controlling coronary blood flow under physiological conditions has not been established. The contribution of parasympathetic activity to control of resting coronary blood flow has not been considered in species where acetylcholine exerts a predominant vasodilatory effect. Presumably the contribution of parasympathetic vasodilation is small compared to the vasodilation exerted by metabolic mechanisms, especially since metabolic vasodilation would be greatest under circumstances in which a withdrawal of vagal tone would be expected. However, it is possible that under resting conditions there is a tonic vasodilatory effect exerted by the parasympathetic release of acetylcholine. In the pig, where acetylcholine produces constriction of large coronary arteries [77], resistance arteries [69], and arterioles [71, 77], Cowan and McKenzie [150] reported that neither cholinergic blockade with atropine nor vagal ligation altered resting coronary blood flow, indicating a lack of parasympathetic influence on basal coronary tone.

Vasodilation to acetylcholine is similar in isolated canine coronary arteries and arterioles. Isolated porcine coronary arterioles [71], resistance arteries [69], and epicardial arteries [77] all display similar sensitivity in their vasoconstrictor response to acetylcholine. Lamping et al. [151] found similar results in a beating heart preparation. In their study, both endogenous acetylcholine released in response to vagal stimulation and exogenous acetylcholine produced dilation of arterial vessels ranging from 50–400  $\mu\text{m}$  in diameter. The dilation produced by acetylcholine was similar in all vessel sizes. Using the same beating heart preparation, Komaru et al. [152] also reported that acetylcholine produced dilation in all sizes of coronary microvessels (50–250  $\mu\text{m}$  in diameter). However, they found that acetylcholine produced greater dilation in vessels less than 120  $\mu\text{m}$  in diameter. These investigators also found differences in the mechanisms by which acetylcholine produced vasodilation in large and small coronary arterioles; inhibition of nitric oxide formation eliminated the vasodilatory response to acetylcholine in large arterioles but only partially inhibited acetylcholine-induced vasodilation in small arterioles. The vasoconstriction of isolated porcine resistance arteries and large epicardial



arteries to acetylcholine also appears to be modulated by diverse endothelium-dependent mechanisms [77]. In large arteries, vasoconstriction in response to acetylcholine can be attenuated by blockade of the cyclooxygenase pathway. In resistance vessels, inhibition of nitric oxide synthase antagonizes the constriction produced by acetylcholine. In human coronary arterioles, responsiveness to acetylcholine has been reported to differ dramatically in atrial and ventricular vessels [153]. Atrial vessels constrict to acetylcholine whereas isolated ventricular arterioles display vasodilation. The results of this human study must be interpreted with caution, because the data were collected from a large sample of patients of diverse ages and various underlying cardiovascular diseases. Collectively, data from animal and human studies suggest that, like other control mechanisms, the vasoactive effects of cholinergic stimulation vary between segments of the coronary circulation.

## 6. Interactions Controlling Coronary Vascular Resistance

Control of coronary blood flow cannot be attributed to a single mechanism. Indeed, the close match between coronary blood flow and metabolic demand of the myocardium suggests that coronary blood flow is constantly changing and that such precision is due to integrated input from a variety of control mechanisms. Much work in the coronary circulation has shown that there is competition between metabolic vasodilation and adrenergic constriction [113, 117, 118, 123]. Thus, for example, metabolic vasodilation that occurs in response to sympathetic stimulation of the heart is tempered by adrenergic vasoconstriction. In the microcirculation, Chilian and Layne [33] found that small arterioles “escaped” from  $\alpha$ -adrenergic constriction. Autoregulatory dilation to the decrease in pressure created by constriction of upstream arterioles competed with and predominated over the  $\alpha$ -adrenergic constriction. As described previously, interaction between endothelium-dependent vasodilation and  $\alpha$ -adrenergic constriction has also been shown to occur in the coronary circulation. The endothelium-dependent vasodilation which appears to compete with  $\alpha$ -adrenergic constriction may serve a protective role in limiting vasoconstriction during conditions of intense sympathetic stimulation. Endothelium-dependent vasodilation may also counter the effects of circulating catecholamines, for example, during exercise. Exercise causes an increase in circulating catecholamines and also increases heart rate and thus increases metabolic demand of the myocardium. Endothelium-dependent vasodilation may help to ensure adequate blood flow despite the presence of adrenergic constriction.

Pohl et al. [88] reported that endothelium-dependent relaxation modulates autoregulatory responses of the coronary circulation. Using an isolated vessel preparation, Kuo et al. [72] demonstrated elegantly that flow-induced vasodilation interacts with myogenic responses in both a competitive and an additive fashion. When flow was introduced into

the lumen of a vessel demonstrating myogenic tone, flow-induced vasodilation counteracted pressure-induced constriction. Alternatively, flow-induced vasodilation potentiated myogenic dilation that occurred in response to a reduction in intraluminal pressure. Similarly, an increase in intraluminal pressure stimulated a vasoconstrictor response that opposed flow-induced dilation, whereas a step decrease in intraluminal pressure triggered myogenic vasodilation that augmented flow-induced dilation. Within a given segment of the vasculature, myogenic, flow-dependent, neural, and metabolic mechanisms could interact in either a competitive or additive manner. Close coupling of coronary blood flow to myocardial oxygen demand is accomplished through integration of vasoactive responses and achievement of a balance between vasoconstriction and vasodilation.

Alternatively, control factors that predominate at different levels of the vascular tree may act together to provide a coordinated increase in coronary blood flow under conditions of increased metabolic demand. Kuo et al. [154] speculated that an increase in metabolic demand may cause metabolic dilation of small downstream arterioles (these vessels show the greatest dilation to an increase in metabolic demand as indicated in Figure 1; [32]) which would then decrease upstream pressure and cause myogenic dilation of upstream arterioles. Dilation of these upstream arterioles would decrease arteriolar resistance and increase blood flow causing flow-induced dilation of feed arteries. Collectively, these changes would cause an increase in blood flow that would potentially be maintained until metabolic demand subsided. A decrease in metabolic demand would decrease the production of metabolic dilators and subsequently reduce the vasodilatory signal to small arterioles. A reduction in diameter in these downstream arterioles would cause upstream pressure to increase and promote myogenic constriction of upstream arterioles. This would increase resistance and decrease blood flow, thus decreasing flow-mediated vasodilation. Thus, a reversal of the process by which blood flow increased would occur and blood flow would return toward normal.

**6.1. Autoregulation.** Autoregulation refers to the intrinsic ability of an organ (the heart) to maintain relatively constant blood flow over a range of perfusion pressures. Thus, autoregulation occurs through mechanisms that act locally to control blood flow. Two major theories have been proposed to explain the mechanisms by which autoregulation of coronary blood flow occurs. These are the myogenic and metabolic theories of autoregulation. The myogenic theory suggests that blood flow remains constant within an organ despite large changes in perfusion pressure because blood vessels are able to constrict and dilate in response to changes in transmural pressure (i.e., changes in the pressure exerted at the vessel wall as a result of changes in perfusion pressure). Thus, an increase in perfusion pressure will cause an initial increase in flow and distention of blood vessels. The vessels will then constrict in response to elevated pressure. This increase in resistance will reduce flow, bringing it back within the initial range if myogenic gain is sufficiently high [155]. Similarly, a decrease in perfusion pressure, which would initially cause flow to decrease, will

trigger a myogenic vasodilation facilitating an increase in flow. This response acts to keep flow within a constant range although pressure has decreased. The metabolic theory of autoregulation proposes that coronary blood flow is regulated by vasoactive metabolites, the concentration of which is determined by myocardial metabolism. In keeping with this theory, an increase in perfusion pressure would transiently increase coronary blood flow causing increased washout of vasodilatory metabolites. The decrease in concentration of these vasodilatory metabolites would lead to constriction of blood vessels and an increase in resistance. The increased resistance would then reduce blood flow toward baseline. Conversely, a decrease in perfusion pressure would cause a reduction in flow leading to a buildup of metabolic vasodilators. An increase in the concentration of these metabolites would cause vasodilation and thus increase blood flow. Although both metabolic and myogenic mechanisms potentially play a role in regulation of coronary blood flow, the relative contribution of these mechanisms may differ within segments of the microcirculation. Additionally, both of these responses may be modulated by the endothelium.

Coronary arterioles smaller than 100–150  $\mu\text{m}$  in diameter dilate significantly in response to reduced coronary perfusion pressure [4, 33]. Kanatsuka et al. [4] showed that coronary arterioles dilated in response to both moderate and severe reductions in perfusion pressure. In contrast, arterial microvessels larger than 100  $\mu\text{m}$  did not change diameter in response to a moderate decrease in perfusion pressure and constricted when coronary perfusion pressure was reduced to 40 mm Hg. Chilian and Layne [33] reported that both small arteries and arterioles dilated when perfusion pressure was reduced to 40 mm Hg but the magnitude of the dilation was significantly greater in smaller microvessels (<150  $\mu\text{m}$  in diameter). Responses to complete coronary artery occlusion and dilation during reactive hyperemia following occlusion have also been shown to be heterogeneous within the coronary microcirculation. Coronary occlusion results in dilation of arterial microvessels less than 150  $\mu\text{m}$  in diameter while diameter remains unchanged in small arteries greater than 150  $\mu\text{m}$  in diameter [156]. Similarly, only arterioles dilated during early reactive hyperemia [21]. However, dilation of all arterial microvessels contributes to the later phase of reactive hyperemia. Collectively, these findings indicate that the microcirculation has an important role in autoregulation of coronary blood flow. The primary site of autoregulation in the coronary circulation is in arterioles less than 150  $\mu\text{m}$  in diameter. However, autoregulatory responses are not uniform within the microcirculation implying that diverse mechanisms may underlie autoregulatory responses within segments of the microcirculation. A significant myogenic contribution to autoregulation may be present in arterioles smaller than 100  $\mu\text{m}$  while metabolic mechanisms are a likely autoregulatory factor throughout the coronary microcirculation.

**6.2. Flow-Induced Vasodilation.** Increases in intraluminal flow elicit vasodilation of both large coronary arteries and coronary arterioles. In large coronary arteries from humans,

flow-dependent vasodilation has been shown to occur in proximal sections of epicardial arteries when distal vasodilation has been induced pharmacologically, thus increasing arterial flow [157–159]. In this situation, the proximal portion of the artery dilated although it was not exposed to the pharmacological vasodilator. Holtz et al. [160, 161] used temporary occlusion or adenosine to increase flow in dog hearts and then measured dilation of an epicardial artery when flow was either allowed to increase or was held constant by a distal stenosis. They showed that maintenance of constant flow eliminated the epicardial dilation during reactive hyperemia and adenosine infusion. Cyclooxygenase inhibition had no effect on the flow-induced vasodilation, indicating that the response was not mediated through release of vasodilatory prostaglandin (i.e., prostacyclin). Inoue et al. [162] also demonstrated that dilation of epicardial arteries during reactive hyperemia could be inhibited by a flow-limiting coronary stenosis. In addition these investigators found that the dilation of epicardial arteries during reactive hyperemia was attenuated by removal of the endothelium with a balloon catheter. In conscious dogs, inhibition of nitric oxide production reduced dilation of epicardial arteries in response to an increase in myocardial blood flow created by pacing at 200 beats per minute [163]. However, dilation of epicardial arteries in response to sustained increases in flow caused by adenosine infusion was not altered by treatment with L-NAME. These results suggest that flow-induced vasodilation of large epicardial arteries is endothelium-dependent and may be mediated, at least in part, by production of nitric oxide.

Kuo et al. [164] have demonstrated that coronary arterioles also exhibit flow-induced vasodilation. Using an *in vitro* preparation, responses to graded increases in intraluminal flow were evaluated in the absence of pressure changes. This was accomplished by cannulating isolated coronary microvessels with micropipettes that were attached to separate pressure reservoirs. Adjustment of the heights of these reservoirs in equal and opposite directions allowed for establishment of a pressure gradient across the vessel, thus generating flow through the vessel without changing mean intraluminal pressure. Graded increases in flow produced graded dilation; diameter increased as much as 30% at the highest flow rates. In contrast, flow-mediated increases in epicardial diameter have been reported to be in the range of 5–15%. Kuo et al. [164] further showed that, similar to large epicardial arteries, flow-induced vasodilation of coronary arterioles is abolished by removal of the endothelium. However, the mechanisms of endothelium-dependent vasodilation may differ in large and small coronary vessels. In large arteries the vasodilatory response to flow is not completely blocked by inhibition of nitric oxide suggesting that another mechanism is also involved. In arterioles, flow-induced vasodilation was abolished by inhibition of nitric oxide synthesis with an L-arginine analog, L-NMMA, the effects of which were reversed by addition of excess L-arginine [72]. Recent data suggest that in the intact coronary circulation, shear stress is regulated in small coronary arteries [165]. The regulation of shear stress in small arteries was abolished by inhibitors of nitric oxide synthase, suggesting

that the regulation occurs through production of nitric oxide. Although the role of flow-mediated vasodilation of the coronary microvasculature has not been definitely demonstrated *in vivo*, vasodilatory responses to flow and myogenic responses to pressure interact locally in isolated arterioles in both additive and competitive manner [72]. In arterioles that demonstrated myogenic dilation, diameter increased further upon exposure to intraluminal flow. Conversely, flow-induced vasodilation of isolated coronary arterioles opposed myogenic constriction to an increase in intraluminal pressure.

## 7. Disease States and the Coronary Circulation

**7.1. Metabolic Regulation.** Some of the most prevalent conditions affecting the coronary circulation are physical inactivity and obesity that lead to metabolic syndrome and, perhaps ultimately, Type 2 diabetes mellitus. Those afflicted have an increased risk of cardiovascular diseases such as coronary artery disease, cardiomyopathies, congestive heart failure, and substantially greater overall mortality rate [166, 167]. However, little is yet known about the coronary microvascular mechanisms that are altered and how they influence the progression of cardiovascular morbidity and mortality. Coronary flow reserve is reduced in obese patients [168, 169]. More invasive experiments in dogs and pigs with metabolic syndrome have shown that exercise-induced coronary vasodilation is impaired [170, 171]. Further, this is likely due to (a) increased vasoconstrictor mechanisms such as angiotensin II and alpha adrenoreceptor signaling [172, 173] and (b) reduced coronary vasodilator mechanisms such as smooth muscle  $K^+$  channel activity [174, 175]. These deficits may result from not only neurohumoral influences but also the adipose tissue that surrounds coronary vessels and alters reactivity, including endothelial function [176, 177].

**7.2. Endothelial Regulation.** Increasing evidence indicates that impairment of coronary blood flow regulation that occurs in a variety of pathological conditions is linked to endothelial dysfunction. Attenuation of endothelium-dependent nitric oxide production in the coronary circulation has been documented in several disease processes. Dysfunction of the endothelium is causally involved in the pathophysiology of atherosclerosis [154, 158, 159, 178], heart failure [142, 179], ischemia-reperfusion injury [180–182], and diabetes [183]. Endothelium-dependent vasodilation of atherosclerotic epicardial arteries to both pharmacological agents and to flow is impaired or absent [158, 159, 178]. Atherosclerosis impairs the vasomotor function of the coronary microcirculation [184], and endothelium-dependent vasodilation of isolated coronary arterioles is blunted despite the absence of overt atherosclerotic lesions in these vessels [178]. At high plasma concentrations, low density lipoproteins (LDL) constitute a major risk factor for atherosclerosis and are thought to play an important role in the development of atherosclerosis. In both large conduit arteries and coronary microvessels, oxidized LDL inhibits endothelium-dependent vasodilation [185, 186]. In microvessels, the vasodilatory

impairment produced by oxidized LDL is likely due to inhibition of nitric oxide synthesis because the effect was reversed by exogenous L-arginine and oxidized LDL did not alter endothelium-dependent vasodilation of microvessels to the nitric oxide donor, sodium nitroprusside [186]. Additionally, in both large arteries and arterioles from atherosclerotic animals, endothelium-independent vasodilation of the vascular smooth muscle to nitroglycerin and sodium nitroprusside is preserved indicating that the abnormality resides within the endothelium [158, 178].

Metabolic abnormalities are associated with cardiovascular risk factors that may greatly increase the risk for development of ischemic heart disease and heart failure. Abnormalities of coronary vasomotor function occur during the development of obesity and Type 2 diabetes; however, discrepancy exists concerning the nature and timing of the progression of vascular dysfunction that accompanies metabolic dysfunction and overt diabetes. The progression of diabetes is associated with a reduction of endothelium-dependent vasodilation to acetylcholine in coronary arterioles [187, 188]; however, endothelial function was preserved in obese rats until the development of Type 2 diabetes. In diabetic dogs, coronary blood flow responses to exercise are impaired [189]. In normal dogs, concentrations of leptin in the range found in obesity produce acute impairment of acetylcholine-induced coronary vasodilation [190]; however, prediabetic dogs without evidence of coronary atherosclerosis are protected from such leptin-mediated coronary endothelial dysfunction [191]. Impairment of coronary flow reserve, independent of occlusive artery disease, has been documented in diabetic [9, 192, 193] and prediabetic patients [194].

Episodes of ischemia also alter the reactivity of the coronary microcirculation in addition to producing damage to the myocardium itself. Damage continues even during restoration of blood flow (reperfusion) of the ischemic zone. Ischemia-reperfusion also produces selective damage to the endothelium of coronary vessels [180–182]. This effect is most prominent in small vessels. Endothelium-dependent relaxation responses to acetylcholine, ADP, and the calcium ionophore, A23187, were significantly reduced in isolated coronary arterioles following ischemia and reperfusion but were unaltered in large epicardial arteries [180]. Similarly, in a beating heart preparation, ischemia followed by reperfusion inhibited vasodilation of coronary arterioles to the endothelium-dependent agonists acetylcholine and serotonin but did not affect vasodilation to the direct smooth muscle vasodilator, papaverine [182]. These data suggest that production of nitric oxide by the endothelium is impaired in ischemia-reperfusion injury. The contributing mechanisms to this impairment of the endothelium are not entirely known; however, damage may be related to neutrophil adhesion to vascular endothelial cells [195], production of oxygen free radicals [196], or activation of platelet-activating factor [197]. Recent evidence suggests that part of the deficiency in endothelium-dependent relaxation may result from a decrease in availability of tetrahydrobiopterin, a co-factor necessary for metabolism of L-arginine to nitric oxide [198]. Impaired endothelium-dependent vasodilation following ischemia-reperfusion was restored by tetrahydrobiopterin



treatment [199]. As with a number of other diseases associated with dysfunction of the coronary circulation, an endothelium-mediated pathway appears critical in the pathophysiology of ischemia and reperfusion.

Endothelium-mediated increases in coronary blood flow to acetylcholine and arachidonic acid, which are indicative of a decrease in vasodilatory responses of resistance vessels, were decreased in dogs during heart failure produced by chronic rapid pacing [142]. In the same model of heart failure, endothelium-dependent vasodilatory responses of large coronary arteries were also depressed. As in other disease states that affect the coronary vasculature, vasodilatory function of the vascular smooth muscle was not altered by experimental heart failure. Selective impairment of endothelium-dependent function of coronary arteries has also been reported to occur in transplant patients who develop graft vasculopathy [200].

Thus, in all of these disease states the endothelium appears to be a target of the coronary vascular pathology, suggesting that a loss or decrease of endothelial modulation of coronary vascular resistance is a key factor in the coronary vascular pathology associated with various disease states. Collectively, these findings implicate the important role of the endothelium in balanced regulation of coronary vascular resistance.

**7.3. Vasoconstrictor Responses.** Balanced availability of both vasodilators and vasoconstrictors is needed to ensure adequate blood supply to the working myocardium. When the balance between endothelium-derived dilators and constrictors is shifted towards the latter, vascular function can be dramatically altered resulting in underperfusion of myocardial tissue, in particular during periods of heightened stress, such as exercise [201]. For example, vasoconstrictor responses to pharmacological stimuli are augmented in coronary arteries from atherosclerotic animals.  $\alpha$ -Adrenoceptor-mediated coronary vasoconstriction is augmented during exercise in diabetic dogs [202]. Endothelial endothelin production has been shown to increase in models of atherosclerosis and hyperlipidemia [203]. Responsiveness to endothelin also increases in coronary arterioles of aged female rats [204]. Contrarily, vasoconstrictor responses of coronary arterioles from male rats are reduced with advancing age [204, 205] possibly due to phenotypic changes in vascular smooth muscle and endothelin receptor expression. The contribution of altered vasoconstriction, whether increased or decreased, to coronary microvascular disease remains an ongoing and important question.

## 8. Coronary Microvascular Therapy

Recent clinical work has shown that impairment of coronary flow reserve is associated with increased cardiac mortality among both diabetic and nondiabetic patients in the absence of known large artery disease [193]; the inability to augment myocardial blood flow in response to stress, related in part to coronary microvascular dysfunction, identified subjects with significantly higher cardiac mortality. Demonstration

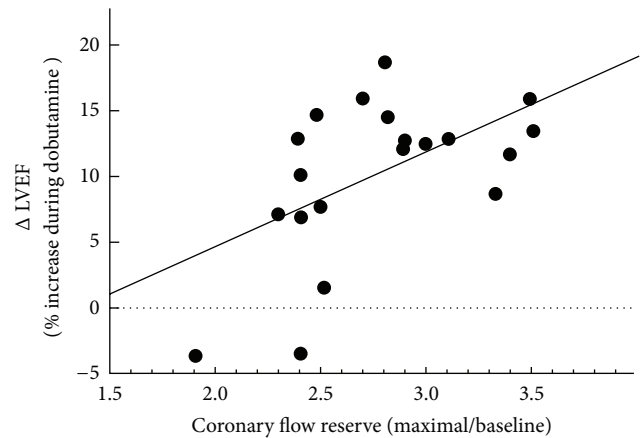


FIGURE 5: Correlation between coronary flow reserve and  $\Delta$ LVEF in diabetic patients ( $r = 0.68$ ,  $P = 0.0009$ ). Coronary flow reserve, the ratio of maximal hyperemic to baseline rest flow volumes;  $\Delta$ LVEF, the percentage change in left ventricular ejection fraction during dobutamine infusion. Adapted from Yonaha et al. [9], with permission.

of this association between critical cardiac dysfunction (sufficiently severe to result in cardiac death) and coronary microvascular dysfunction provokes the question, “Does the coronary microcirculation constitute a therapeutic target for treatment of myocardial disease and dysfunction?” Impairment of coronary flow reserve has also been documented in prediabetic patients [194] and diabetic patients [9, 192] and in patients with hypertension without atherosclerotic disease and evidence of diastolic dysfunction [206, 207]. Figure 5 illustrates that a significant correlation exists between coronary flow reserve and the ability to increase left ventricular ejection fraction during dobutamine infusion in diabetic patients. These data indicate the significant coupling between ventricular function and microvascular function in the progression of diabetic cardiac dysfunction [9]. Similarly, 80% of patients with dilated cardiomyopathy, in which systemic vascular disease and other cardiac disease were excluded, showed evidence of microvascular dysfunction by positron emission tomography [208]. The mechanisms that underlie development of microvascular dysfunction in the absence of occlusive large artery disease remain elusive, and it remains to be determined whether microvascular disease contributes to progression of fibrosis and myocyte dysfunction, or whether myocardial dysfunction and pathologic hypertrophy lead to aberrant microvascular signaling and function. Importantly, recent data support the notion that the coronary microcirculation and cardiac muscle must be treated in tandem, if optimal recovery and reestablishment of cardiac function are to be achieved. For example, in addition to the known effects of beta blockade therapy on LV function and exercise tolerance, chronic treatment with carvedilol improved coronary vasodilatory reserve in patients with idiopathic dilated cardiomyopathy [209]. Notably, studies of stem cell therapy also indicate that the greatest improvements in functional recovery following injection of cells occurs



when cells differentiate into cardiac, endothelial, and smooth muscle cells [210].

Recent developments indicate that the availability and delivery of progenitor and pluripotent stem cells may constitute important therapeutic means of improving coronary perfusion in diseased or injured myocardium [211]. Vascular progenitor cells exist in niches distributed throughout the human coronary circulation, and these cells are self-renewing and differentiate predominantly into endothelial and vascular smooth muscle cells [210]. In patients with the metabolic syndrome, a decreased number of circulating early endothelial progenitor cells correlates with reduced coronary collateral development and collateral blood flow [212]. Multipotent stem cells repair infarcted myocardium and promote collateral blood flow [213]. Similarly, delivery of mesenchymal stem cells immediately after myocardial infarction improved coronary blood flow and increased survival rates in pigs [214]. Progenitor cells and implementation of therapies that increase the activation and homing of these cells may represent novel therapeutic mechanisms for the restoration of coronary arteries and arterioles, thereby reducing injury of the myocardium following ischemic events.

## 9. Conclusion

Coronary blood flow must be closely regulated in order to meet the constantly changing metabolic demands of the myocardium. This requires rapid changes in coronary vascular tone over a wide range. Such precise yet extensive regulation can only be achieved by integration of a variety of control mechanisms. It has become clear that no single mechanism predominates in control of coronary vascular tone: neural, humoral, and local control mechanisms all participate. A lack of balance between these mechanisms is often apparent in disease states which are characterized by inadequate control of coronary blood flow. In addition to a balance between control mechanisms, close control of coronary blood flow is facilitated by heterogeneous distribution of these mechanisms within the coronary circulation. Coronary vascular tone is not determined by global responses of the entire coronary bed to vasoactive stimuli, but rather by coordination of diverse responses within segments of the coronary circulation. This provides for adequate and efficient control of coronary blood flow throughout the myocardium.

## 10. Summary

Resistance distributed throughout the vascular tree determines coronary blood flow. However, the majority of coronary vascular resistance resides in the coronary microvasculature. The responses of vessels less than 150  $\mu\text{m}$  in diameter to neural, humoral, and mechanical stimuli are, therefore, of particular importance in understanding control of coronary blood flow. Numerous studies have shown that large conduit arteries and resistance vessels often display disparate responses to similar vasoactive stimuli. Development of new techniques to study the coronary microcirculation has now demonstrated that even within the microcirculation,

responses to the same vasoactive stimulus may differ between large and small arterioles. Therefore, studies of the mechanisms that contribute to determination of coronary vascular resistance must not treat the resistance vasculature as a single unit but must consider the heterogeneity of these mechanisms within the microcirculation.

Neural control of coronary vascular resistance occurs through both sympathetic and parasympathetic mechanisms. Sympathetic control is mediated via  $\alpha$ -adrenergic and  $\beta$ -adrenergic receptors. The predominant response of the coronary microcirculation to  $\alpha$ -adrenergic activation is vasoconstriction. However, in small arterioles  $\alpha$ -adrenergic constriction may be overcome by autoregulatory dilation.  $\beta$ -adrenergic activation of the coronary vasculature produces vasodilation, assessed primarily by determination of pressure/flow relationships in *in vivo* studies. The response to parasympathetic activation of the coronary vasculature is species dependent. In many species, parasympathetic activation leads to vasodilation of both large arteries and microvessels through an endothelium-dependent mechanism. However, in some species, parasympathetic activation leads to vasoconstriction.

Myogenic responses, metabolic vasodilation, and flow-induced vasodilation all contribute to local control of coronary blood flow. Isolated coronary arterioles display both myogenic constriction and dilation to increases and decreases in transmural pressure, respectively. Isolated coronary arterioles also display vigorous dilation in response to increases in intraluminal flow, a response that is endothelium dependent. Additionally, responses to pressure and flow have been shown to interact in both a competitive and additive fashion *in vitro*. However, a clear demonstration of these responses has been difficult to ascertain *in vivo*. Metabolic vasodilation has been shown to be an important factor in local control of coronary blood flow. No single mediator of metabolic vasodilation has been identified; it is likely that there are several metabolic vasodilators that contribute to the observed increases in blood flow which occur in response to increases in myocardial metabolic demand.

In addition to mediating flow-induced vasodilation, the coronary endothelium acts as a modulator of vascular resistance under varying physiological conditions. Vasodilatory responses to a number of humoral agents are mediated by the endothelium. Endothelium-dependent release of nitric oxide may modulate myogenic and  $\alpha$ -adrenergic responses. Impairment of endothelium-dependent vasodilation has been reported in a variety of pathologies associated with dysfunction of the coronary circulation.

Coronary vascular resistance is determined by coordination of all these control mechanisms throughout the vascular tree. Clearly, no single control mechanism exerts a predominant effect and these control mechanisms are not uniformly distributed throughout the coronary microcirculation. Thus, it is important to consider segments of the coronary vasculature and the coordination of these segments into a vascular network when considering experimental results or contemplating therapeutic interventions. Effective

therapeutic interventions will consider the close coupling of the myocardium to blood flow and oxygen delivery, targeting both microvascular and myocyte dysfunctions simultaneously.

## Conflict of Interests

Z. Ren, L. Liu, R. Fu, and M. Miao declared that they have no proprietary, financial, professional, or other personal interest of any nature or kind in The Math Works, Inc., Trea, or J&K Chemical Ltd., cited in the paper. The stepwise behavioral responses: Behavioral adjustment of the Chinese rare minnow (*Gobiocypris rarus*) in the exposure of carbamate pesticides.

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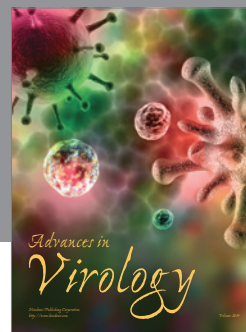
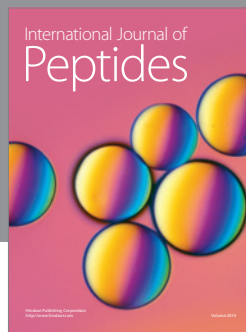
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