Research Advances Series

Understanding the Coronary Circulation Through Studies at the Microvascular Level

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Studies of the coronary circulation have divided vascular resistances into three large components: large vessels, small resistance vessels, and veins. Studies of the epicardial microcirculation in the beating heart using stroboscopic illumination have suggested that resistance is more precisely controlled in different segments of the circulation. Measurements of coronary pressure in different sized arteries and arterioles have indicated that under normal conditions, 45–50% of total coronary vascular resistance resides in vessels larger than 100 μm. This distribution of vascular resistance can be altered in a nonuniform manner by a variety of physiological (autoregulation, increased in myocardial oxygen consumption, sympathetic stimulation) and pharmacological stimuli (norepinephrine, papaverine, dipyridamole, serotonin, vasopressin, nitroglycerin, adenosine, and endothelin). Studies of exchange of macromolecules in the microcirculation using fluorescent-labeled dextrans have also identified the size of the small pore (35–50 Å) in coronary microvessels that can be altered by myocardial ischemia. Studies of the coronary microcirculation have demonstrated that the control of vascular resistance is extremely complex, and mechanisms responsible for these heterogeneous responses need further examination. (Circulation 1990;82:1–7)

Traditionally, coronary physiologists have envisioned two regulatory components of the coronary circulation: large conduit coronary vessels that could be visualized with angiographic techniques, and small coronary arterial resistance vessels that are too small to be imaged angiographically. These small arterial resistance vessels range in size from several hundred microns to about 10 μm in diameter. Older theories about regulation of coronary vascular resistance gave little importance to resistance in the venous segment of the coronary circulation except under extreme conditions. One of the tacit assumptions of this general theoretical formulation is that each of these three large components (conductance vessels, resistance vessels, and veins) functions in a homogeneous manner. Recent advances in research technology have made it possible to study the three major components of coronary circulation in greater detail than has been possible. The results of these investigations indicate that there is substantial heterogeneity of function within each of the three major components that regulate myocardial perfusion.

The purpose of this review is to describe advances in understanding the role of small coronary arterial resistance vessels and the regulation of myocardial perfusion. The interested reader is referred to recent reviews on the large conduit coronary vessels and the coronary veins.

Historical Aspects

The first direct visualization of coronary arterial microvessels in the beating heart was reported by Martini and Honig. Although their techniques were crude and laborious by present-day standards, their work stimulated interest in this area. The laboratory of Richard Bing was the birthplace of our current approaches to the direct examination of coronary microvessels in the beating heart. Tillmanns worked initially with Bing and subsequently used his regional immobilization method to study coronary microvessels in vivo. Nellis et al then developed an ingenious stroboscopic method of visualizing coro-
nary microvessels that required limited external restriction of movement of the myocardium in the area being examined. The Nellis technique has been significantly improved by Chilian and coworkers.9 Ashikawa and Kanatsuka et al.,10 working in Sendai, Japan, developed a floating objective approach that greatly facilitated studies of red cell velocity in coronary arterioles of the beating heart. Currently available techniques for studying the microcirculation in the beating heart only allow study of the most epicardial vessels in the heart, and it is quite possible that deeper subendocardial vessels may respond differently.

In addition to these advances in studying microvessels in vivo, Tesfamariam and Halpern11 developed an approach to study coronary microvessels in vitro. This technique has subsequently been extended in the laboratories of Harrison12 and Chilian.13 Coronary microvessels obtained from the subepicardium or subendocardium can be studied in vitro.13

As a consequence of these advances in technology, it is now possible to measure diameters in coronary vessels as small as 15–25 μm in the beating heart. It is also possible, with the servonull technique and a computer-controlled wobbler,8 to measure phasic pressures with high fidelity in vessels in the beating heart as small as 75–85 μm. Furthermore, red cell velocity10,14 and the flux of macromolecules (an index of permeability)15 can be assessed.

Investigations using these state-of-the-art techniques during the past few years have begun to accumulate results that are influencing the fundamental formulations about basic mechanisms that regulate myocardial perfusion. The results of conceptual importance will be briefly described below.

**Distribution of Coronary Vascular Resistance**

Direct measurements of phasic pressure in coronary arterioles as small as 85 μm in diameter have indicated that under control conditions, in the cat heart, about 45–50% of total coronary vascular resistance resides in vessels larger than 100 μm in diameter9,16 (Figure 1). Furthermore, the venous segment of the coronary circulation under control conditions accounts for 10% of total coronary vascular resistance. Thus, the “precapillary resistance vessels” less than 100 μm in diameter are responsible for less than half of total coronary vascular resistance under control conditions. Older theories suggested that arterioles (less than 100 μm in size) accounted for most of coronary resistance.17

Additional studies have shown that chronic hypertension18 or pharmacological coronary dilation with papaverine9 or dipyridamole16 can significantly shift the overall distribution of coronary vascular resistance. For example, with intense coronary dilation with dipyridamole, the coronary venous segment of the circulation is responsible for 31% of total coronary vascular resistance, whereas under control

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**Figure 1.** Distribution of microvascular pressure measured with servonull technique as a percentage of aortic pressure in different sized coronary arterioles and venules in the normal left ventricle in pentobarbital anesthetized cats during control conditions (●) and intravenous administration of dipyridamole (0.4 mg · kg⁻¹ · min⁻¹) (○). During infusion of dipyridamole, there is a redistribution of microvascular resistance such that larger arteries and veins account for a greater portion of resistance. (From Chilian et al.16)
conditions, it only accounts for 7% of total coronary vascular resistance.

The understanding that coronary vascular resistance is regulated by a broad size range of coronary microvessels and that the magnitude of regulation by specific segments can be dramatically shifted is an important concept that could not have been elucidated without direct studies at the microcirculatory level.

**Critical Closing Pressure in the Coronary Circulation**

In 1978, Bellamy reported data from Gregg’s laboratory that suggested that in awake dogs, coronary flow in conduit coronary vessels ceased when arterial perfusion pressure decreased below 45 mm Hg. Thus, Bellamy suggested that if flow stopped in the proximal left anterior descending coronary artery at a driving pressure of 45 mm Hg, it also stopped in other segments of the coronary circulation. This observation stirred the interest of many coronary physiologists who began to examine the possibility that “stop-flow” or “critical closing pressure” as opposed to coronary sinus pressure was the effective back pressure in the coronary circulation.

Soon, the importance of coronary capacitance and coronary collaterals on measured stop-flow pressure was recognized. Measurements of red cell velocity of coronary arterioles have resolved this issue. These studies have shown that antegrade movement of red cells in 20-μm coronary arterioles continues until coronary driving pressure is only a few millimeters of mercury higher than coronary sinus pressure and probably nearly equal to the pressure in very small coronary venous channels. Cessation of antegrade flow in large upstream vessels with continued forward flow in microvessels is due to capacitance in the coronary circulation. These microvascular studies also demonstrated that coronary arterioles remained open at extremely low coronary driving pressures. Thus, there was no direct evidence of critical closure of arterial vessels in the coronary circulation. In part, a positive stop-flow pressure may be a result of unaccounted for collateral flow from surrounding perfusion territories, the viscous properties of the blood, and elastic properties of the vascular wall. The back pressure to flow is most likely coronary sinus pressure.

**Coronary Microvascular Exchange**

Several laboratories have used indirect methods for examining microvascular exchange in the coronary microcirculation based on indicator-dilution techniques. Although these techniques can provide information regarding exchange of a variety of solutes in the heart, they are limited: the time needed to obtain measurements is long (several seconds), exchange processes in different regions or layers of the heart cannot be distinguished, and sample volume is large so that discrete changes cannot be assessed. To overcome these problems, McDonagh and coworkers have used direct measurements of capillary permeability in the arrested heart. They examined transvascular exchange of solutes in capillaries and venules after ischemia and reperfusion, with the advantage that discrete areas of myocardium could be examined and alterations in extravascular compressive forces and myocardial oxygen demands could be avoided. Limitations include the effects of cardioplegic solutions on microvascular permeability and alterations in the normal control mechanisms of microcirculatory processes in the arrested heart.

Because of the limitations inherent in previously used techniques to examine microvascular exchange, recent studies have used stroboscopic illumination of the left ventricle to measure discrete areas of the heart. Using an index of permeability based on leakage of fluorescein-labeled dextrans of various sizes, studies in the coronary microcirculation in vivo have shown that the small pore size in coronary microvessels is about 35–50 Å. This value is comparable to other regional circulations. In addition, the “apparent pore size” in the coronary circulation enlarges dramatically after 15 minutes of coronary occlusion followed by reperfusion. Whether this is a pharmacological increase in pore size or simply disruption of the endothelial lining needs to be resolved. Thus, profound changes in coronary permeability occur in advance of any evidence of myocardial necrosis in the setting of transient coronary occlusion.

**Microvascular Site of Autoregulation and Metabolically Induced Coronary Dilation**

Autoregulation and metabolically induced coronary dilation are the dominant mechanisms that modulate myocardial perfusion. The specific vascular segments that participate in these phenomena could not be elucidated without direct examination of coronary microvessels.

Measurements of microvascular diameter in beating ventricles indicate that when coronary perfusion pressure is decreased to 60 mm Hg and perfusion is maintained by autoregulation, the coronary microvessels responsible for this dilator response are less than 100 μm in diameter. Furthermore, in vessels less than 100 μm in diameter, the magnitude of dilation is inversely related to vessel size (Figure 2). Microvessels greater than 100 μm in diameter do not change diameter at all in the face of this autoregulatory stimulus. When coronary driving pressure is reduced to 40 mm Hg, coronary vessels less than 100 μm dilate, and the intensity of that dilation is inversely related to control diameter size (Figure 3). With this low perfusion pressure, which is beyond the autoregulatory range, coronary microvessels greater than 100 μm actually decrease in diameter. The reduction in diameter may not be entirely passive, because these vessels will dilate in response to intravenous norglycerin, intracoronary adenosine, or topical EDTA. These data suggest that submaximal dilation, which has been reported in ischemic myocardium, is related to inadequate dilator responses in specific size classes of coronary microvessels.
FIGURE 2. Effect of a mild decrease in coronary perfusion pressure (to 60 mm Hg) of the left anterior descending artery on diameter of coronary arteries and arterioles in the epicardium of the left ventricle in chloralose anesthetized dogs. Arterioles less than 100 μm dilated, whereas larger arteries and arterioles did not respond. (From Kanatsuka et al.44)

Studies of in vitro coronary microvessels obtained from the subendocardium and the subepicardium suggest that myogenic responses are less prominent in subendocardial microvessels. This observation may partly explain why autoregulation is less effective in the subendocardium than in the subepicardium of the left ventricle.41

Increases in metabolism during pacing increase the diameter of all size classes of microvessels (Figure 4). The magnitude of dilation is inversely related to the size of coronary microvessels.42

Two new concepts have come forth from microvascular studies of coronary autoregulation and metabolic dilation. First, both autoregulation and metabolic dilation result in strikingly heterogenous dilation of coronary microvessels. Second, the size classes of arterial microvessels responsible for the dilator response to autoregulation and metabolic dilation are not identical. In fact, with autoregulation, arterial microvessels less than 100 μm in diameter dilated, whereas larger microvessels constricted.

Neural Control

Studies of neural control of the coronary circulation at the microvascular level have been focused primarily on α-adrenergic and cholinergic mechanisms.

Selective activation of α-receptors in vivo either by electrical stimulation of sympathetic nerves or infusion of norepinephrine during β-blockade produces constriction of arterial microvessels greater than 150 μm in size and, surprisingly, dilation of vessels less than 100 μm in size (Figure 5). Studies in the pig in vitro suggest that coronary arterial microvessels smaller than 100 μm do not contain functional α-receptors.

Activation of cholinergic receptors in vivo by either vagal stimulation or acetylcholine infusion produces modest but relatively uniform dilation of coronary microvessels across all size classes.45 Very large intracoronary doses of acetylcholine produce intense dilation of coronary microvessels, and the dilator response is similar in all sizes of coronary microvessels.45 Studies in vitro indicate that in the dog, acetylcholine-induced coronary dilation of coronary microvessels is primarily endothelial dependent.12

Thus far, two new concepts about neural coronary regulation have come forth from microvascular studies. First, these studies suggest that α-adrenergic receptors and adrenergic regulation of coronary vascular resistance is heterogenous across different size classes of arterial microvessels. Because α-adrenergic stimulation simultaneously dilates and constricts different size classes of coronary microvessels, this may explain why α-adrenergic activation is a less potent constrictor in the coronary circulation than in other vascular beds.46 Second, these studies provide evidence that in the dog, acetylcholine and vagal stimulation dilate both large arteries and arterioles down to 50 μm in size.

Humoral Regulation

Effects of several humoral substances have been examined at the microcirculatory level. They include serotonin,47 vasopressin,47 the thromboxane mimetic U46619,46 norepinephrine,43 epinephrine,49 adenosine,42 and endothelin.50 With the exception of the thromboxane mimetic U46619, which produces relatively uniform constriction across all size classes of coronary arterial microvessels, all other humoral substances examined have strikingly heterogeneous effects on coronary microvessels. For example, adenosine only dilates microvessels less than 150 μm in diameter, whereas serotonin constricts microvessels greater than 100 μm in diameter and simultaneously dilates vessels less than 100 μm in diameter (Figure 6). In contrast, vasopressin intensely constricts...
Microvessels less than 100 μm in diameter and has either no effect or slightly dilates microvessels greater than 100 μm in diameter (Figure 7).

The observation that different size classes of coronary microvessels have strikingly heterogeneous responses to important vasoactive substances suggests that control mechanisms in microvessels above and below the 100–150-μm level must be strikingly different.

Pharmacological Agents

The effects of only two drugs—nitroglycerin and nifedipine—have been studied in detail in the coronary microcirculation. Infusion of nitroglycerin produces dose-related dilation of coronary microvessels greater than 200 μm in diameter and has little or no effect on smaller coronary microvessels. In contrast, S-nitrosoycteine, an active metabolite of nitroglycerin, dilates small microvessels very significantly both in vitro and in vivo. These data would suggest that microvessels lack the metabolic machinery to convert nitroglycerin to active nitrosoycteine metabolites. This may explain the finding that nitroglycerin selectively dilates large microvessels. Thus, these studies at the microvascular level suggest that drug metabolism may be heterogeneous in various size classes of coronary microvessels.

Infusion with the calcium blocker nifedipine produces impressive dilation of coronary microvessels. The dilator responses were homogeneous across all vessel sizes.

Why Do Coronary Arterial Microvessels Respond Heterogeneously to Vasoactive Stimuli?

Many mechanisms may account for heterogeneous responses of different size classes of coronary microvessels to vasoactive stimuli. They are: the variable length–tension relation of vascular smooth muscle in different size classes of microvessels, variable distribution of receptors, heterogeneous metabolic capacity of the vascular wall, differing levels of capacitance function, varying levels of myogenic tone, heterogeneous innervation, and variable extravascular compressive forces. In addition, constriction of upstream vascular segments will substantially decrease downstream pressure and thereby activate autoregulatory and myogenic mechanisms. These mechanisms are not mutually exclusive; it is likely that several may be acting simultaneously in the same vascular segment under a given set of conditions. Studies to elucidate the relative importance of these mechanisms are just beginning and no doubt will continue for decades. Full exploration of this area is vitally important to understanding control mechanisms that modulate coronary perfusion in normal and diseased states. Most of these experiments will require direct examination of the coronary vasculature at the microvascular level. This complex ability of the microcirculation to respond to the same stimulus in a nonuniform manner allows the coronary circulation to finely control vascular resistance. Segmental control of vascular resistance can precisely regulate perfusion and oxygen and nutrient delivery to the myocardium.
FIGURE 6. Effect of an intra-atrial infusion of serotonin (16 μg/kg/min) on coronary arteries and arterioles in the epicardium of the left ventricle of pentobarbital anesthetized cats. Arterioles less than 100 μm dilated to serotonin, whereas larger vessels constricted. (From Lamping et al.)

FIGURE 7. Effect of an intra-atrial infusion of vasopressin (0.5 units/min) on coronary arteries and arterioles in the epicardium of the left ventricle in pentobarbital anesthetized cats. Arterioles less than 100 μm constricted, whereas larger vessels dilated slightly or did not respond. (From Lamping et al.)

Summary

In less than a decade, studies of the coronary microcirculation have brought forth a substantial number of new concepts that importantly influence our understanding of basic mechanisms that regulate myocardial perfusion. New technology has opened a new vista for investigation, which promises to continue to enhance our knowledge about basic control systems that regulate myocardial perfusion. Research in this area should be encouraged, and coronary physiology laboratories should consider incorporating studies at the microvascular level in many of their basic investigations.

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