CLINICAL AND EXPERIMENTAL ASPECTS OF FUNCTIONAL AND FLOW RESERVE OF THE MYOCARDIUM

KLINISCHE EN EXPERIMENTELE ASPECTEN VAN FUNCTIONELE EN BLOEDSTROOMRESERVE VAN HET MYOCARDIUM

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CORONARY FLOW AND FUNCTIONAL RESERVE OF STUNNED MYOCARDIUM

Chapter 1

Introduction and aim of the thesis

INTRODUCTION

Coronary flow reserve

Basic research over the past 50 years has provided the clinician with important concepts for understanding human coronary physiology. One such tool is the determination of coronary blood flow reserve. Defined as the ratio of maximal to resting coronary flow, flow reserve may be helpful in assessing the need for revascularization in selected patients (1), as well as providing insight into pathophysiological states involving small arterial vessels (2-4). Many methodologies have been developed for measuring myocardial blood flow in patients, however most are limited by poor spatial resolution, the need for prolonged sampling periods or inherent inaccuracies with the measurements (5).

Two invasive techniques have recently shown promise in the determination of coronary flow reserve in patients. Ultrasonic Doppler catheters have become small and steerable and can accurately assess changes in coronary blood flow velocities following infusions of vasodilators such as papaverine and adenosine. The technique has gained widespread acceptance for use in the catheterization laboratory, particularly to evaluate the success of interventions such as percutaneous transluminal coronary angioplasty (6). Another invasive modality combines videodensitometry and digital subtraction angiography. This method uses a measurement of contrast density at two different vessel locations to determine the transit time and together with vascular volume can provide measurements of coronary blood flow (7). Both of these techniques are safe and show good results when validated against other accepted techniques. The major disadvantage however is that neither absolute blood flow nor transmural flow distributions can be measured.

Positron emission tomography (PET) is non-invasive, and thus does not theoretically alter baseline flows such as might occur with an intracoronary catheter. Positron emitting tracers such as H_2O^{15} are administered either intravenously or as inhaled $CO_2{}^{15}$ and are rapidly extracted by the myocardium. With the use of high resolution, rapid tomographic scanners, one can measure time activity curves in the left ventricular chamber and the myocardial regions of interest. Using tracer kinetic principles, total and regional myocardial blood flows can be accurately quantitated. The results correlate well with flow measurements obtained from radiolabeled microspheres in dogs, over a wide range of flows (8). Because of the short half-lifes of the tracers, sequential measurements can be made to evaluate multiple interventions.

Limitations of coronary flow reserve measurements: Abnormally low coronary flow reserve values may be important for the diagnosis and management of specific diseases. However, the maximal coronary flow response to infusions of vasodilators depend upon multiple factors, including the driving coronary perfusion pressure in diastole, coronary venous pressure, and transmural compressive forces (e.g. contractility). Thus coronary flow reserve measurements may be influenced by a number of hemodynamic variables independent of changes in coronary vasoreactivity. Likewise, when baseline coronary flow is altered, flow

reserve ratios may also vary, not necessarily reflecting changes in the coronary vasculature. Therefore, absolute coronary flow reserve measurements in the capacity of the vasculature to maximally vasodilate. Maximal coronary flow-pressure relationships more accurately characterize the coronary vasculature and are less influenced by changes in systemic hemodynamics (9). By obtaining multiple flow-pressure points over a wide range of perfusion pressure, one can accurately determine coronary pressure-flow lines during maximal vasodilation. Alterations in the capacity of the vasculature to vasodilate may be indicated by changes the slopes or flow-intercepts of the relationships. The tool allows for more accurate measures of altered flow reserve even in the presence of interventions which may cause modest changes in systemic hemodynamics factors such as heart rate, preload, or contractility.

Another means of assessing the response of vasodilators on the vasculature is the use of relative flow reserve (10). This is obtained from the ratio of maximal coronary flow during a known stenosis to the maximal coronary flow without a stenosis. Like maximal flow-pressure lines, relative flow reserve is less affected by changes in systemic hemodynamics. This concept has been applied to coronary flow reserve measurements with PET, where the vasodilator response in a region of myocardium supplied by a stenosed vessel is compared with a region supplied by normal coronary arteries. Thus PET measurements of flow reserve are more relative than absolute, and thereby offer a more theoretical approach to studying flow reserve in patients.

Regulation of coronary blood flow

Autoregulation: During a constant metabolic state, the myocardium like other vascular beds, exhibits the capacity to adjust its resistance in order to maintain coronary blood flow constant, despite increases or decreases in perfusion pressure (11). This phenomenon, termed autoregulation is depicted in figure 1. In response to abrupt changes in coronary pressure, coronary flow is initially altered, but rapidly returns to normal within seconds to minutes. The mechanism of this phenomenon is not completely understood, but probably involves a combination of myogenic and metabolic factors. In regards to the latter, such mediators as oxygen, carbon dioxide, and other biproducts of ischemia have all been shown to provide endogenous vasodilation for the maintainance of coronary blood flow (12).

During ischemia: In pathophysiological states such as coronary artery disease or ventricular hypertrophy, the balance between myocardial oxygen supply and demand may be disrupted. In such situations, coronary pressure may fall below a critical level where the capacity to autoregulate flow is exhausted and further reductions in perfusion pressure result in reductions in blood flow and oxygen delivery. This "break point" is illustrated in figure 1 as the onset of the descending limb of the autoregulatory curve. This can occur anywhere between 40 and 70 mm Hg, depending upon such factors as the animal model, the kind of anesthesia used, or systemic hemodynamics (13,14). A two fold increase in heart rate, for instance, increases the breakpoint of the autoregulatory curve from 40 mm Hg to 60 mm Hg in awake dogs (15). The capacity to autoregulate flow is also dependent on extravascular compressive forces, and thus the subendocardial regions of the myocardium are more

Correction: the second sentence on page 10 should read:
"Therefore, absolute coronary flow reserve is not a specific measurement of the capacity of the vasculature to maximally vasodilate."

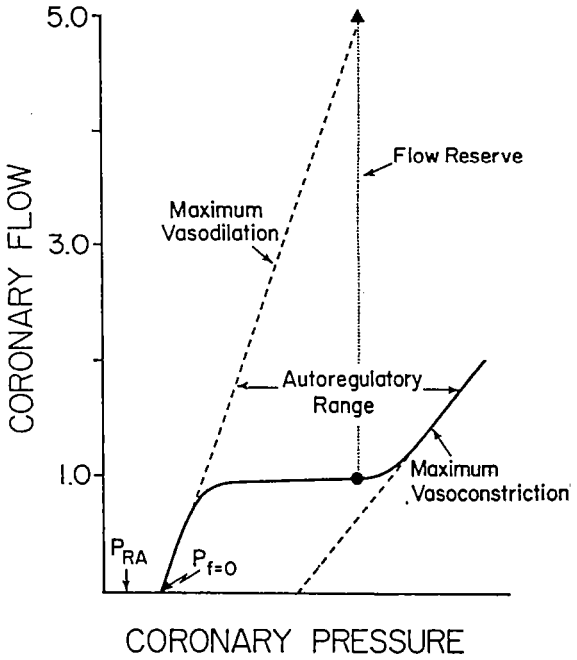

Figure 1. *Steady-state relationship between coronary flow and coronary arterial pressure in the left ventricle. The solid line depicts the normal relationship. At a constant level of myocardial metabolic demand, coronary flow is maintained constant over a wide range of coronary pressure, between the bounds of maximum coronary vasodilation and constriction (dashed lines). The solid circle represents the normal operating point under basal conditions; the solid triangle is the flow observed at the same pressure during maximum vasodilation. Flow reserve, the ratio of flow during vasodilation to that measured before vasodilation, is in this case 5.0.* P_{ra} *= right atrial pressure;* $P_{f=o}$ *= "back pressure" opposing coronary flow (from Klocke (45) with permission).*

vulnerable to ischemia than the subepicardium (16,17).

Below the autoregulatory "breakpoint", reductions in perfusion pressure, blood flow and transmural function have been shown to correlate well with one another.
In awake dogs, relative reductions in either segment length or wall thickening at constant levels of global oxygen demand are linearly related to graded reductions in coronary pressure (14). In anesthetized preparations, Verdouw and others have shown a similar relationship between function and flow once total coronary blood flow is reduced by 20% (18,19-21). This correlation between reductions in flow and transmural function during ischemia is even more significant when subendocardial blood flow is considered, thus emphasizing the importance of subendocardial flow to transmural function (16,17).

In addition to the coronary blood flow-function relationship, changes in flow and metabolism have been investigated during graded reductions in coronary perfusion pressure. In most animal species, coronary blood flow and oxygen consumption are closely coupled over a broad range of flow reductions (22). The maintainance of normal oxygen delivery to the myocardium during reduction in perfusion pressure are provided by the capacity to autoregulate blood flow. Once blood flow begins to fall during ischemia, some metabolic reserve does exist to enhance energy production. For example, myocardial oxygen extraction can increase during graded levels of ischemia (23). In addition, the amount of ATP production via fatty acid metabolism is drastically limited during ischemia but is partially compensated for by enhanced glycolysis. Thus, metabolic byproducts of anaerobic glycolysis, such as lactate and H^+ increase relative to the degree of flow reductions, although the correlation has only been shown to be weakly linear (24). In addition to enhanced glycolysis, other metabolic changes may occur relative to the degree of ischemia. In anesthetized dogs and swine, transmural changes in phosphocreatine and inorganic phosphate levels as measured by nuclear magnetic resonance are sensitive to changes in blood flow over a broad range of ischemic perfusion pressures during ischemia (25,26).

The above studies help characterize the relationships between coronary blood flow, perfusion pressure, transmural function and metabolism beyond the point when autoregulation of blood flow by the myocardium is exhausted. It is apparent that this capacity of the myocardium to autoregulate blood flow during reductions in perfusion pressure is the most important reserve for maintaining normal function and metabolism. Once this fails, some metabolic reserve during ischemia does exist however, this is limited.

Exogenous vasodilator reserve during myocardial ischemia: Traditionally, it has been assumed that the descending limb of the pressure-flow relationship during endogenous vasodilation overlaps that observed during pharmacological vasodilation (27). On the basis of recent studies however, this does not seem to be the case. In anesthetized swine, Pantely et al induced myocardial ischemia by lowering perfusion pressure to 45 mm Hg, and showed that coronary blood flow increased in all myocardial layers during an intracoronary infusion of adenosine (28). These results have been repeated by two other groups in anesthetized dogs (29,30), thus confirming the hypothesis that pharmacologically recruitable reserve exists despite moderate ischemia at a time when endogenous vasodilation is maximal. The reason for this discrepancy is speculative. Perhaps the myocardial vasculature loses the capacity to release potent endogenous endothelial vasodilators such as the "Endothelium Derived Relaxing Factor" (EDRF) leaving such vasoconstrictors as endothelin unopposed. It is also possible that the myocardial release of biochemical mediators of vasodilation are attenuated over time. Interestingly, none of these studies could show that the recruitment of this pharmacological vasodilator reserve had any beneficial effect on either function or metabolism. These results suggest that during myocardial ischemia, coronary blood flow may not be the only regulator of function and metabolism. Such factors as intracellular levels of calcium or lactate may also be important determinants of myocardial performance during ischemia.

Myocardial stunning

Over the past 15 years, basic cardiovascular research has focused on myocardial injury incurred following ischemia and reperfusion. In anesthetized dogs, Heyndrickx et al showed that 15 minutes of coronary occlusion followed by reperfusion induces transient reductions in function, despite the return in coronary blood flow and the absence of necrosis (31). This entity, referred to as "myocardial stunning" is clinically relevant in light of the current emphasis on early reperfusion of ischemic syndromes with thrombolytic agents, percutaneous transluminal coronary angioplasty and bypass surgery (32). Even though days to weeks may be required for the complete normalization of this post-ischemic function, infusion of inotropic agents such as epinephrine or the induction of post-extra systolic beats can recruit function (33,34). It is not understood why myocardial function remains depressed following ischemia and reperfusion, despite the presence of normal functional reserve. Once this is delineated, interventions that improve post-ischemic ventricular dysfunction in patients following reperfusion can be applied most effectively.

Coronary blood flow and stunning: Although sustained abnormalities in transmural blood flow are not the cause of stunning, it has become apparent that many abnormalities in the coronary vasculature may be associated with the functional abnormalities. In both awake and anesthetized dogs, subendocardial blood flow has been shown to be abnormally low following 15 minutes of complete occlusion and 3-4 hours of reperfusion (35,36). The mechanism of this abnormally low blood flow has not been well elucidated, but may involve capillary obstruction from edema formation (37) or microvascular plugging by leukocytes (38). The endothelial cell may also undergo changes that could alter reflow following stunning. For example, after one hour of occlusion and reperfusion in dogs, several morphological changes occur, including focal injury and partial detachment from the underlying cell matrix (39). In addition to these anatomical changes, the endothelial cells may lose the ability to release vasodilating factors such as EDRF, as shown by an attenuated response to known stimulators of EDRF release with ATP, bradykinin, serotonin and acetylcholine (40,41).

Even when basal coronary flow returns to normal following stunning, the capacity to maximally vasodilate may be altered. The degree of reactive hyperema following 30 seconds of coronary occlusion, for example, has been shown to be lower following myocardial stunning induced by 15 minutes of ischemia and reperfusion (42). This may not be a specific finding for changes in the coronary vasculature because of other factors which are altered following the stunning process. These include reductions in either myocardial oxygen demand during a brief period of occlusion or the concentrations of endogenous vasodilators such as ATP, both of which could explain the lower reactive hyperemia. Other studies however, have shown altered vasoreactivity to exogenous vasodilators. Following ischemia and reperfusion in anesthetized dogs, the coronary flow response to maximal doses of intravenous adenosine has been reported to be lower than prior to stunning (36). Contrary to these results, others have shown no alterations in coronary vascular reactivity during infusions of maximal doses of adenosine (43,44).

Several reasons may exist for the inconsistencies among these studies. First of all, the term myocardial stunning has been loosely applied to many models of ischemia and reperfusion, some of which involve prolonged ischemic times which also induce necrosis. It is not surprising that flow reserve in these infarcted areas is lower than in stunned but viable regions. Secondly, intravenous infusions of vasodilators may induce systemic effects on left ventricular pressure, heart rate and contractility and thus may not vasodilate to the same degree as compared with maximal doses of intracoronary infusions. Finally, because of the steep pressure-flow relationship during maximal vasodilation as shown in figure 1, small changes in perfusion pressure may induce large changes in coronary flow with the potential for error in comparisons between interventions. Thus, coronary vascular resistance is better characterized by maximal coronary flow-pressure relationships, defined by their slopes and flow-intercepts (45).

It is clear that the coronary vasculature may undergo a number of changes following ischemia and reperfusion that are important even though they may not be the primary causes of the contractile abnormalities. Further work needs to identify those factors involved with "vascular stunning" that are independent of known alterations in the vasculature such as "edema" or necrosis. Clinically, these questions are important regarding the efficiency of the vasculature to support increased myocardial demand following stunning. It is also interesting that enhanced blood flow following reperfusion may correct some of the functional abnormalities following reperfusion (46). Understanding these problems may give a better appreciation of the mechanisms behind stunning and in turn, provide more therapeutic applications.

Myocardial metabolism and stunning: In addition to the coronary vasculature, the metabolic state of the myocardium undergoes several changes following ischemia and reperfusion that may be associated with the functional abnormalities. The uptake of substrates utilized by the myocardium for energy production, for instance, are different, as measured by positron emission tomography (PET). In chronically instrumented dogs, 3 hours of coronary occlusion followed by reperfusion cause sustained abnormalities in the clearance of C-11 labeled palmitate (a marker of fatty acid metabolism). At the same time, uptake of the glucose analog F-18 deoxyglucose is enhanced implying that the reperfused myocardium preferentially utilizes carbohydrates rather than fatty acids for ATP production (47). This new imaging tool allows the researcher a means to quantitate changes in the uptake of myocardial substrates following reperfusion (48). It also affords the clinician a tool to identify the extent of post-ischemic myocardium in patients following exercise or reperfusion therapies (49). Although the technology has great potential, more basic research on the energy needs of the reperfused myocardium is needed before interpreting the results. For instance, the tracers used for PET only measure uptake of the substrates, and may inaccurately assess their overall utilization (50).

Following brief periods of ischemia and reperfusion in anesthetized dogs, ATP and other nucleotides have been shown to be persistently depressed (51). Because the time course of their recovery coincide with that of the functional abnormalities (52), it has been hypothesized that a reduced high-energy phosphate pool is the cause of stunning. Based on the results of further studies however, the relationship between post-ischemic function and

reductions in ATP is not causal. For example, providing substrate to increase myocardial ATP stores to normal values following reperfusion has not been shown to improve the contractile abnormalities (53). More importantly, the presence of normal contractile reserve suggests that a defect in the resynthesis of ATP is not the primary cause of stunning. The capacity for the mitochondria to phosphorylate appears to be intact as indicated by normal or elevated levels of phosphocreatine (54,51), thus leading some investigators to postulate that the lower ATP represents an energy drain following stunning. In support of this, myocardial oxygen consumption in regionally stunned myocardium may be unchanged, despite the reductions in regional function and thus oxygen demand (55). This "oxygen waste" theory suggests that the energy consumption of the myocardium is inefficiently high following reperfusion compared with the amount of measured work.

The relationship between oxygen consumption and post-ischemic ventricular function is of great interest, but has not yet been well characterized. Part of the problem is accurately measuring oxygen extraction, which has been shown to be heterogenous following reperfusion (55). Secondly, it is difficult to relate oxygen consumption and contractility following reperfusion because changes in coronary blood flow in regionally stunned myocardium may depend upon many determinants of oxygen demand other than thickening or shortening. Finally, different models of stunning report variable degrees of functional changes, thus confounding the interpretation of coronary blood flow, oxygen consumption and function following reperfusion.

Mechanism of myocardial stunning: The mechanism of myocardial stunning is not well understood but probably involves multiple processes that could potentially contribute to the mechanical dysfunction. A leading hypothesis suggests that free oxygen metabolites such as superoxide anion (O2-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH-) are generated during early reperfusion and cause damage to membranes, contractile structures or the sarcoplasmic reticulum (56). Potential sources of these free radicals include the activation of neutrophils (57) and the increased activity of the enzyme xanthine oxidase (58). In support of this oxyradical theory, several investigators have shown in anesthetized dogs that superoxide dismutase (an enzyme that converts O_2- to O_2 and H_2O_2) and catalase (an enzyme that reduces H_2O_2 to O_2 and H_2O) significantly attenuate the degree of myocardial stunning following ischemia and reperfusion (59,60). Further proof is provided by the fact that these agents reduce the level of free radicals produced following reperfusion as detected by spin trap and electron paramagnetic resonance spectroscopy (61).

An equally important component in the evolution of myocardial stunning involves intracellular calcium homeostasis or altered myofibrillar sensitivity to the anion. Several studies suggest that "calcium overload" occurs during reperfusion, thus inducing contractile abnormalities. For example, isolated hearts exposed to 15 minutes of ischemia and reperfusion demonstrate less myocardial stunning when the perfusate contains low calcium concentrations (54). In isolated ferret hearts, intracellular levels of calcium ("calcium transients") as measured by the fluorinated calcium indicator 5F-BAPTA and nucleur magnetic resonance spectroscopy (NMR) are elevated following ischemia and reperfusion at a time when contractile function is reduced (62). Although calcium levels may be elevated

only briefly during reperfusion, this transient excess may be enough to damage intracellular organelles responsible for contraction.

Another hypothesis links calcium abnormalities and stunning by proposing that the sensitivity of the myofilaments for calcium is reduced. In experiments with isolated reperfused ferret hearts using NMR, "calcium transients" are elevated but maximal calcium-activated pressure is decreased suggesting a lowered responsiveness to calcium by the contracile proteins (63). Contrary to these studies, in a canine model of post-ischemic dysfunction produced by multiple 5-minute occlusions and 10 minute reperfusions, isolated sarcoplasmic reticulum demonstrate a decrease in the ability to transport calcium, along with a reduction in the activity of calcium-magnesium-ATPase (64). This would suggest that the myofilament sensitivity is intact, but that the defect lies in the inability of the sarcoplasmic reticulum to deliver calcium to the contractile proteins. This theory is attractive in that it might explain why regionally stunned myocardium demonstrates functional reserve with inotropic agents. However, it does not explain some of the NMR observations that intracellular calcium transients are elevated during early reperfusion.

Aims of the thesis

In this thesis, we first address some clinical aspects of coronary flow reserve. In the anesthetized swine model, we then describe the relationships between myocardial blood flow, metabolism and function during both ischemia and following ischemia-reperfusion.

(1) In the first section, some of the principles of coronary blood flow reserve as applied to patient care will be presented. Such measurements may provide important information about abnormalities of the capacity of the coronary vasculature to maximally vasodilate, allowing specific diagnoses and therapies. A primary limitation in the clinical setting has been inaccurate, non-invasive tools to measure coronary blood flow. Positron emission tomography has enormous potential for the quantitative measurements of regional myocardial blood flow. We will show how the technique can be applied to selected patients with coronary artery disease both prior to and following intravenous infusions of dipyridimole. Coronary flow reserve will be measured in both normal and collateral dependent myocardial regions in patients, and the results will be compared with those obtained in normal volunteers.

We also investigate the effects of antiischemic agents used by patients on coronary flow reserve measurements. In anesthetized swine, absolute and relative coronary flow reserve measurements are presented as well as maximal mean coronary flow-pressure relationships prior to and following administration of two commonly used classes of drugs.

(2) Coronary artery disease progresses to the stage where the vascular supply can not meet the demands of the myocardium. At this point, autoregulation of blood flow fails and ischemia develops. With further increases in demand or decreases in coronary blood flow, ventricular function and aerobic metabolism are abruptly altered. In the second section, we define the relationship between myocardial blood flow, function and metabolism during graded reductions in perfusion pressure, in order to understand the adaptive processes of

the myocardium during acute ischemia. We also determine the degree of myocardial flow and metabolic reserve that is present at a time when autoregulation of blood flow is lost. This includes the assesment of pharmacological vasodilator reserve during increased work load and the importance of regional differences in vasomotor tone, during myocardial ischemia.

(3) With the clinical interest in thrombolysis, cardiovascular research has now focused on myocardial injury incurred following ischemia-reperfusion. It has now been established that the relationship between myocardial function, coronary blood flow and metabolism are transiently altered. In this final section, we characterize these relationships in models of myocardial stunning induced by less than 15 minutes of ischemia followed by 30-60 minutes of reperfusion, both under basal conditions and during inotropic and chronotropic stimulation.

Chapter 2

The assessment of myocardial blood flow and flow reserve in collateral dependent myocardium using positron emission tomography.

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Abstract

Using positron emission tomography and the inhalation of CO_2 (oxygen-15), we have measured coronary flow and flow reserve in myocardium perfused by normal and collateralized vessels in five patients and compared the results to five volunteers. All patients had chronic stable angina with coronary artery disease limited to a completely occluded vessel which received non-jeopardized, interarterial collaterals. Wall function was normal by ventriculogram and by 2D echocardiogram both at rest and following the dipyridamole infusion. During the basal state, flow in the control group was 0.89 ± 0.07 ml/g/min and was not statistically different from myocardium perfused by normal and completely collateralized vessels in the patient group (0.99 ± 0.10 and 0.86 ± 0.14 respectively). Following dipyridamole infusion, flow in the control group increased to 3.69 ± 1.00 and in the normally perfused regions in patients to 2.97 ± 0.94 ml/g/min (NS). In the collateral dependent regions, the increase was less than controls (1.66 ± 1.02; $p < .005$). Flow reserve in controls was 4.1 ± 0.9 compared with 3.1 ± 1.1 in normal regions (NS) and 1.9 ± 0.9 in collateralized regions ($p < .005$) in patients. Within each patient, the ratio of flow reserve in normal and collateralized regions was determined and correlated well with total exercise time ($r = 0.95$; $p = .01$) and peak double product ($r = 0.83$; $p = .08$) during symptom limited exercise tests.

In conclusion, coronary flow to collateral dependent noninfarcted myocardial regions is normal, but pharmacological reserve is limited. The altered relative flow reserve in these regions correlates well with exercise performance and explains in part, why these patients continue to have ischemia at high work load.

Introduction

The physiological significance of well developed coronary collaterals, in patients with coronary artery disease has been of great concern to clinicians. Unfortunately, inadequate means of myocardial blood flow measurements in humans has limited the understanding of their importance. When well developed in the distribution of an occluded artery, collaterals seem to provide enough perfusion to maintain myocardial viability. In support of this, such patients have been shown to have normal thallium perfusion scans (1-3) and regional wall motion measurements (4-9).

Their capacity to increase flow to myocardium supplied by diseased coronary arteries, either following stress or with vasodilators is also of great interest. In the chronic infarct model in dogs, collaterals have been shown to maintain both resting and exercise induced perfusion, although these results vary between individual animals (10-13). In humans, it is not clear whether collaterals recruit flow during high work load. For instance, studies performed in patients with advanced coronary artery disease and well developed collaterals have shown that flow reserve to collateral dependent myocardium is not great enough to prevent ischemia induced by either exercise (14-18) or pharmacological stress (19).

Positron emission tomography (PET) is evolving into an important technology for the quantitation of regional myocardial blood flow and flow reserve. Several groups have now shown that the results correlate well with flow measurements obtained from radio-labeled microspheres in dogs, over a wide range of flows (20-23). Using the inhalation of oxygen-15 labelled CO_2 as a tracer, we studied 5 normal volunteers and 5 patients with collateral dependent myocardium. Our aim was to first evaluate the efficiency with which collaterals perfuse non-infarcted myocardium at rest. Secondly, we wished to determine the pharmacologic flow reserve in collateral dependent myocardium, and how it compares with normally perfused myocardium. Thirdly, we hypothesized that the degree of altered flow reserve through these collaterals would predict the functional capacity of these patients, as measured by performance on graded exercise tolerance tests.

Methods

Patient selection

We chose a highly select group of patients with coronary artery disease to best determine the importance of collateral coronary flow. In review of angiograms performed at Hammersmith Hospital, we identified five men (54-64 years old), with disease limited to an occluded major epicardial vessel that completely opacified via interarterial collaterals. Coronary angiography was performed within 3 months of the PET study and ventricular function in the distribution of the occluded artery was normal by left ventriculography. In addition, no wall motion abnormalities were demonstrated with 2D echocardiography either at rest or following an infusion of intravenous dipyridamole (0.56 mg/kg over 4-5 minutes). Patients denied a prior history of myocardial infarction but all complained of chronic stable exertional angina.

Each patient underwent a symptom limited exercise tolerance test (Modified Bruce protocol) prior to the PET scan. The tests were stopped because of typical angina, and ischemia was confirmed by at least 1 mm ST depression at peak exercise. Table 1 lists the clinical and exercise data for each patient, showing total exercise time and double product (systolic blood pressure X heart rate) at peak exercise.

Table 1: *Clinical data in all 5 patients with normal and collateral dependent myocardium*

PT#	HISTORY	CORONARY ANGIOGRAPHY	EXERCISE TEST SBP X HR	Time (min)
1	63 yo male; Fx class II angina for 3 years	occluded RCA with LAD collaterals	24,000	10.7
2	64 yo male; Fx class II angina for 12 years	occluded LAD with RCA collaterals	26,790	10.1
3	61 yo male; Fx class II angina for 3 years	occluded LAD with RCA collaterals	18,000	6.0
4	54 yo male; "walk thru" angina for 2 years	occluded RCA with LAD collaterals	28,800	18.0
5	63 yo male; Fx class II angina for 12 years	occluded LAD with RCA and CX collaterals	25,960	12.3

Control group

5 healthy volunteers (25-39 years of age) served as a control group. All were men and none had any prior history of angina or heart disease. Myocardial blood flow was determined at rest and following the same dipyridamole dose as with the patients.

PET protocol

PET scanning was performed using an ECAT 931-08/12 tomograph (CTI Inc., Knoxville, Tennessee). The scanner provides for fifteen simultaneous slices covering an axial field of view of 10.8 cm, which is sufficient to comprise the entire heart and major vessels in a single scan. In clinical studies, images are reconstructed with a spatial resolution of 8.4 X 8.4 X 6.6 mm.

All scans were performed following an overnight fast and at least 72 hours after withdrawl of antianginal therapy. Informed consent was obtained and an intravenous heparin lock placed in an arm vein. Subjects were positioned on the bed and a 5 minute rectilinear transmission scan acquired using the gallium-68 ring source. This scan was used in such a way that the heart was completely viewed by the 15 slices. The skin was marked with a felt pen and a laser light source used to ensure constant positioning throughout the study period. A 20 minute transmission scan was obtained for attenuation correction of subsequent emission scans. Tracer amounts of radioactive gases were administered via a mouthpiece and a one-way exhaust valve which ensured adequate delivery of the tracers and proper removal of expired gas. Oxygen-15 ($t1/2$ = 2 min.) labelled carbon monoxide was given at a concentration of 3 MBq and at a flow rate of 1 ml/min. for 4 minutes. One minute after discontinuing the gas supply, a 6 minute emission scan was obtained and several venous blood samples were drawn through the intravenous line for measurement of $C^{15}O$ activity in a well counter. Samples were decay corrected and the scan was later used for determination of blood volume and the selection of arterial input regions of interest.

Fifteen minutes later, to allow for decay of the oxygen-15, regional myocardial blood flow was determined, using continuous inhalation of oxygen-15 labelled carbon dioxide over 3.5 minutes at 6 MBq. Under the catalytic influence of carbonic anhydrase, the gas is rapidly transferred to the water pool in the lung capillary bed. Inhalation of oxygen-15 CO_2 therefore, is a convenient way of administering oxygen-15 water. A total of 25 dynamic frames (ranging from 5 to 30 seconds) were collected over 7 minutes covering the inhalation and washout periods (23). Fifteen minutes later, intravenous dipyridamole (0.56 mg/kg) was infused over 4-5 minutes with continuous 12 lead EKG and and blood pressure monitoring. Within 1-2 minutes of completing the infusion, the protocol was repeated. At the conclusion of the scan, 75-100 mg of aminophilline was given intravenously to reverse any residual drug effects.

Data analysis

Over 60 myocardial and 10 arterial input regions of interest (ROIs) (3 mm diameter circles) were identified and stored for each subject. ROIs were selected from the left atrium and used as the input function only when recovery exceeded 95% of the values obtained from blood samples of oxygen-15 measured in a well counter. On a seperate extravascular density image obtained by subtracting the scaled $C^{15}O$ emission scan from the transmission scan, myocardial ROIs were localized on the center of the myocardium. An offline Sun computer network in conjunction with an Analyze soft ware program (Mayo Clinic) allowed reorientation of transverse sections into short and long axes. This facilitated the proper placement of ROIs onto myocardium in the distribution of either normal or collateralized

vessels. Time activity curves were then generated by projecting the myocardial and arterial input ROIs on the dynamic water images. The arterial time activity curves were averaged in order to generate a statistically sound input function. In the patient group, the myocardial time activity curves were divided and averaged into two regions: one perfused by normal and one perfused by collateral vessels. In volunteers, myocardial time activity curves were taken from all myocardial regions. Using the arterial input function, these myocardial curves were fitted to a two compartment model using standard non-linear interactive least-square fitting techniques for regional myocardial blood flow, volume of distribution of water and arterial blood volume (23). Figure 1 is a representative myocardial flow image in a normal volunteer with the blood volume subtracted.

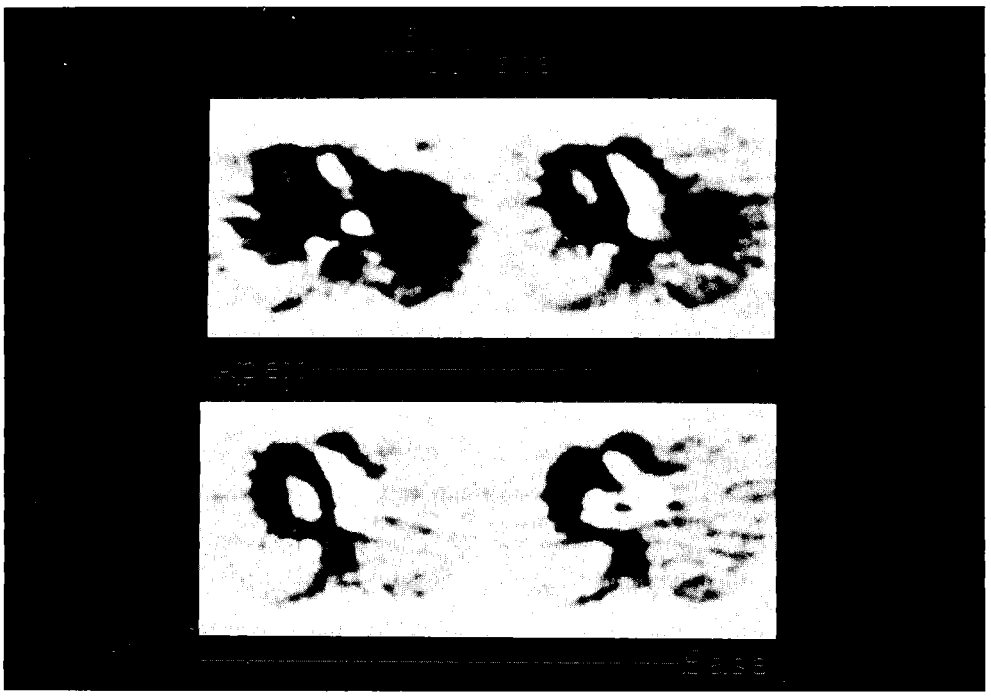

Figure 1: *Representative transaxial myocardial water image with blood volume subtraction in a normal subject after the administration of dipyridamole. The left of the image corresponds to the left of the subject. Note the homogeneous distribution of the tracer throughout the left ventricle.*

Statistics

In the control group, blood flows at rest and following dipyridamole were determined for four different myocardial regions (septum, inferior, anterior and lateral walls), as well as for the entire myocardium. In the patient group, flows were determined in two separate myocardial regions: normally perfused and collateral dependent myocardium. Flow reserve in each subject was calculated by the ratio of dipyridamole to rest flow. Results are expressed as means $\pm$ standard deviation. Comparisons are made between normally perfused and collateral dependent myocardium in the patients and the values obtained from the entire myocardium in the volunteers. Statistical significance is tested by unpaired t-test using the bonferroni correction. Myocardial flows are also tested by paired student's t-test in the two regions in the patients. Changes in heart rate and blood pressure following dipyridamole are also tested within each group by paired student's t-test.

In each patient, a ratio of collateralized to normal myocardial flow reserve was calculated. This was plotted against the patient's exercise time and double product. Regression lines were determined by simple curve fits.

Results

Hemodynamics

The effects of the dipyridamole infusion on resting hemodynamics were different between groups. In the normal volunteers, heart rate increased from 59 ± 8 at rest to 110 ± 14 beats per minute ($p<.01$) within 4 minutes of the infusion. Systolic blood pressure over the same period dropped only from 111 ± 12 mm Hg to 110 ± 14 (NS). In the patient group, heart rate increased to a lesser degree following dipyridamole (64 ± 4 to 79 ± 16 beats per minute (NS)) while systolic blood pressure also changed little from 116 ± 13 to 115 ± 19 mm Hg (NS). Two patients (#3 and #5) complained of chest pain during the infusion, and STT changes were noted on the EKG in patient #5. All subjects received intravenous aminophylline following the scanning protocol.

Myocardial blood flow in volunteers

In the control group, blood flows at rest and following dipyridamole were 0.89 ± 0.07 and 3.69 ± 1.00 ml/g/min respectively. Flow reserve was 4.1 ± 0.9. Figure 2 shows the results of myocardial blood flow in four different regions, determined separately. This emphasizes that in a group with presumed homogenous flow distribution, the model used for the flow calculation is not biased upon the location of the region of interest.

Myocardial blood flow in patients

Figure 3 is an example of the images obtained from patient #5. Table 2 gives the results of myocardial blood flow for all 5 patients, in normally perfused (N) and collateral dependent (C) regions. Flow reserve is listed for each region, as well as the relative flow reserve values (C/N).

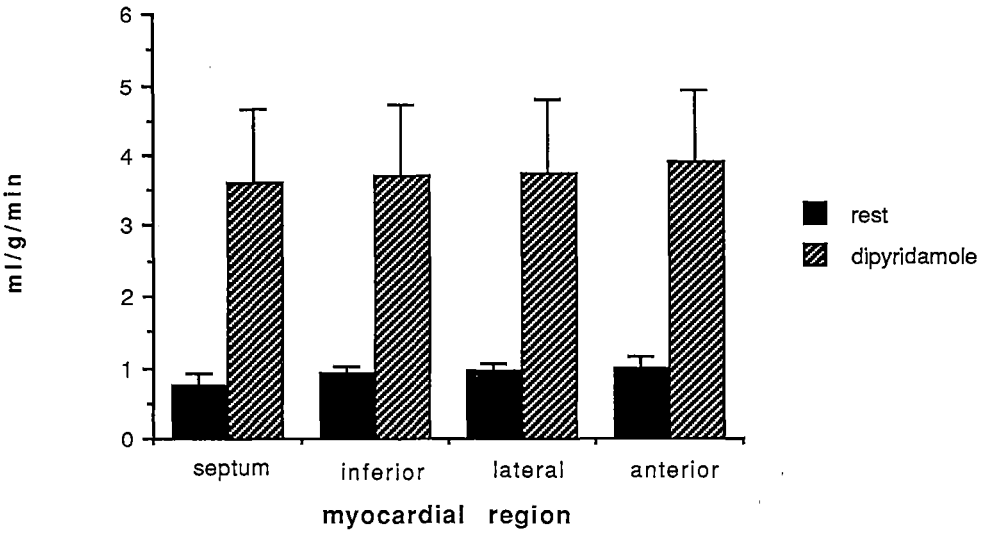

Figure 2: *Myocardial blood flow (ml/g/min) in four different regions in volunteers, during two flow states (means ± sd).*

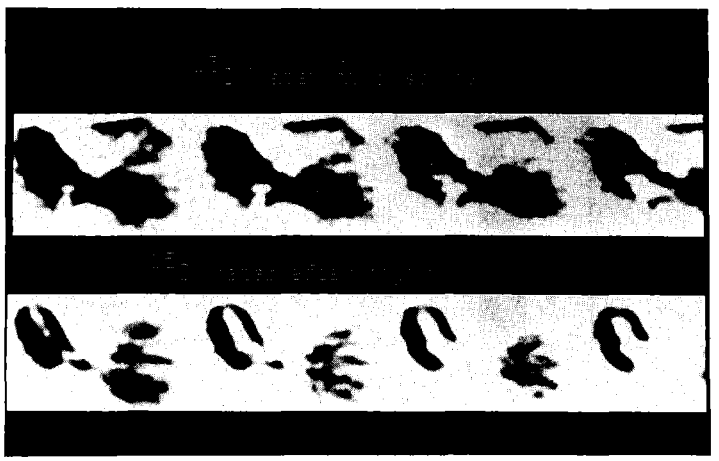

Figure 3 : *These water images were obtained from a patient with a complete occlusion of the left anterior descending coronary artery and collateral circulation from the right coronary artery. Note the perfusion defect in the anterior and anteroseptal segments during vasodilation.*

Table 2 : *Myocardial blood flow (MBF-ml/g/min) and flow reserve in normally perfused (N) and collateral dependent (C) myocardium*

Patient#	MYOCARDIAL BLOOD FLOW		CORONARY FLOW RESERVE				
	N	C	N	C	N	C	C/N
1	0.84	0.76	3.93	2.03	4.7	2.7	0.57
2	0.94	0.73	2.12	0.89	2.3	1.2	0.52
3	1.08	0.91	2.69	0.95	2.5	1.0	0.42
4	1.05	1.07	4.00	3.29	4.1	3.0	0.73
5	1.06	0.85	2.11	1.12	2.0	1.3	0.66
means ± SD	0.99 ± 0.10	0.86 ± 0.14	2.97* ± 0.94	1.66 ± 1.02	3.1 ± 1.1	1.9 ± 0.9	

* $p<0.05$ vs rest

Figure 4 compares myocardial blood flow at rest and with dipyridamole for the volunteers and for the normal and collateralized regions in the patients. At rest, there are no statistical differences between either collateralized or normally perfused regions in patients, compared with controls. Following dipyridamole however, the flow increment in the myocardium perfused by collaterals is significantly less than that in the myocardium of normal volunteers ($p<.005$). Although there was a tendency for lower flows in the normally perfused regions in patients at the high flow state, these were not statistically different from volunteers.

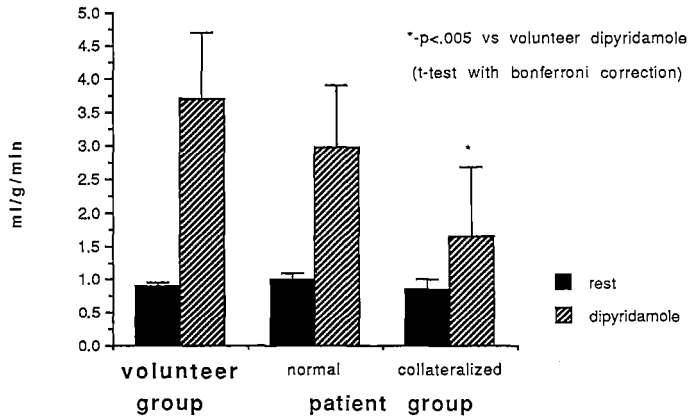

Figure 4: *Myocardial blood flow in patients with collateral dependent and normally perfused myocardium versus normal volunteers.*

Flow reserve in collateralized myocardium versus functional capacity:

We wished to determine if relative flow reserve in the collateralized regions could predict functional capacity with the graded exercise tests. Thus, the ratio of flow reserve in the collateral dependent and normally perfused regions was determined and is shown in Table 2 for each patient. These,then, are plotted against the exercise time and double product at peak exercise for each patient (figure 5a and 5b). The correlation between the relative differences in flow reserve between the two regions and total exercise time was good ($r=0.95$; $p<.01$) and between double product slightly weaker ($r=0.83$; $p=.08$).

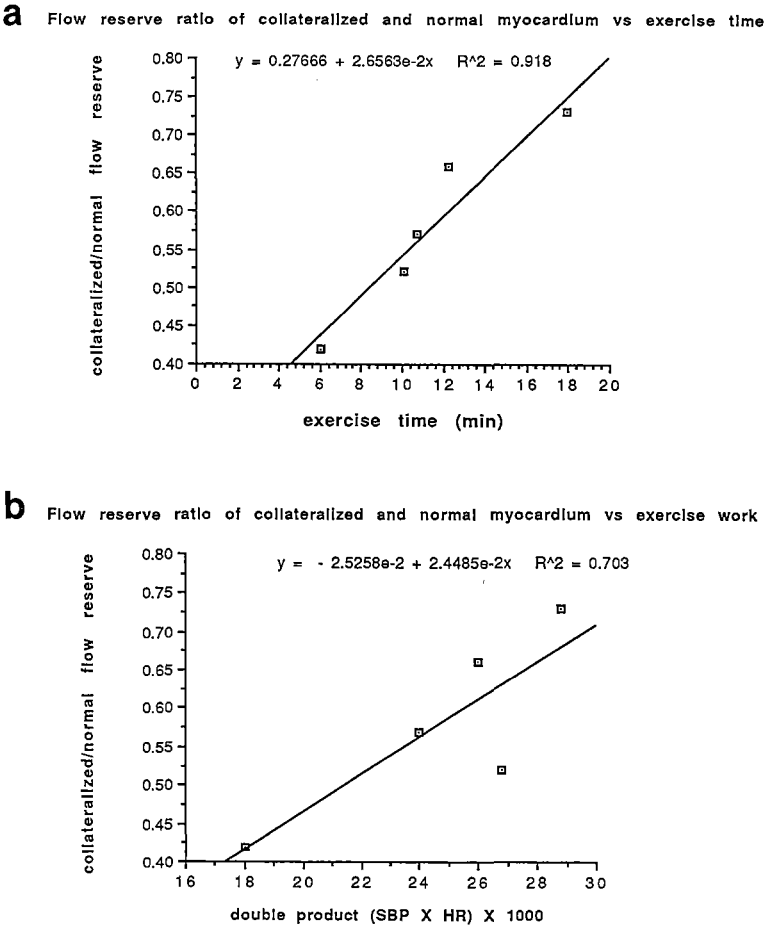

Figure 5: *The ratio of flow reserve in collateral dependent and normally perfused myocardium versus (a) total exercise time and (b) peak double product (systolic blood pressure X HR).*

Discussion

The physiological role of coronary collaterals is best studied in a group of patients with normal myocardial function and an occluded major epicardial artery perfused by non-jeopardized interarterial collaterals (24). In such patients, using positron emission tomography, we have quantitated myocardial blood flow at rest and with dipyridamole both in normally perfused and collateral dependent myocardium. Based on our results, we conclude that well developed collaterals can provide normal flow to noninfarcted myocardial regions during rest. The vasodilator reserve in the distribution of these collaterals following dipyridamole is limited however, and the degree of this alteration correlates well with exercise capacity. This helps explain why this group of patients continue to complain of angina at high work loads.

These results are consistent with the findings of other studies of myocardial collaterals, both in man and animal models. Well developed intramyocardial collaterals for instance, have been shown to provide enough perfusion at rest to preserve regional mechanical function. Nearly half of all patients with an occluded artery and angiographicaly demonstrable collaterals have normal wall motion compared with about 10% without obvious collaterals (4-8). The results of thallium uptake in such patients have also been well characterized. At rest, 40-50% of patients with collateral dependent myocardium maintain normal thallium perfusion, which corresponds well with the percentages reported to have normal wall motion (1-3). In chronic dog models, complete collateralization can be successfully induced by an ameroid constrictor over several weeks to months. In this model, rest flow is normal and myocardial function is preserved, despite complete arterial occlusion (10-13).

Although collaterals may provide enough flow at rest, their ability to increase perfusion during stress or with vasodilators is subject to many variables. In the dog model, the ability for collaterals to increase flow in response to exercise is not uniform and some dogs enhance flow more efficiently than others (10-13). In patients, this variability is confounded by insensitive methods of flow measurements. For instance, such patients have normal thallium perfusion with maximal exercise, implying that interarterial collaterals provide near maximal flow at high work load (1-3). On the other hand, It has been suggested that thallium perfusion during exercise is less sensitive than the electrocardiogram for detecting ischemia in patients with collaterals (25). Using the exercise EKG to test the efficiency of flow reserve via collaterals, patients with significant coronary artery disease and well visualized collaterals on angiography demonstrate no less ischemia when compared with an equally matched group without collaterals (14-17). Percutaneous transluminal angioplasty has offered some additional information about the physiological role of collaterals in attenuating ischemia. Patients with good collaterals distal to a severe stenosis experience less clinical and electrocardiographic evidence of ischemia and have higher great cardiac vein flow during balloon inflation than patients without collaterals (26). Distal perfusion pressure also seems to be higher during balloon inflation in these patients and thus would suggest greater myocardial perfusion to the myocardium at risk (27).

The novelty of these experiments is that we have combined relative vasodilator reserve

in regions of collateral flow with functional capacity to show that a good correlation exists. Vasodilator response to pharmacological agents in collateralized myocardium has been observed in animal models but its quantitation in respect to the normal vasculature is unknown. In a chronic dog model with collaterals induced by gradual coronary occlusion over an 8 week period, the conductance in the vasculature of collaterals was found to be 33% of that in the control bed, implying the presence of some reserve (28). In a similar model, the addition of nitrates during ischemia has been shown to dilate coronary collateral vessels and significantly improve contractile force in the ischemic region (29,30). Studies in humans however have shown minimal blood flow reserve in the distribution of intercoronary collateral vessels in response to isoproterenol infusion (18).

In the distribution of the normally perfused myocardium in patients, flow reserve was close to 3, which is consistant with that observed by coronary sinus thermodilution with equivalent doses of dipyridamole (31). It is lower, however than the results of our volunteers which was over 4. The latter value is consistent with flow reserve reported elsewhere with either positron emission tomography and rubidium-82 (32) or during intracoronary doppler recordings (33). One explanation for these differences may be the differences in hemodynamics following infusion of dipyridamole. In the control group, heart rate increased to a greater degree than in the patients, and thus may have caused a greater increase in myocardial oxygen consumption. Another possible reason for the differences is that the patients may have had small vessel coronary artery disease in "normally perfused" regions, and thus lower flow reserve values.

Conclusions

We have studied with positron emission tomography, a highly select group of patients to characterize vasodilator reserve in collateral dependent myocardium. Potential problems with our data include the small number of patients and the heterogeneity in flow measurements between patients. Despite these limitations, we have shown that well developed collaterals can supply normal coronary perfusion, to noninfarcted, collateral dependent myocardium during resting conditions. Pharmacological reserve however, is limited in these regions, and the degree of alterations correlates well with exercise capacity. This gives some understanding of why such patients with well developed intramyocardial collaterals experience ischemia with increasing work load.

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Chapter 3

The effect of anti-ischemic therapy on coronary flow reserve and the maximal coronary flow-pressure relationship in anesthetized swine.

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submitted for publication

Abstract

In 29 anesthetized swine, we determined the effect of 3 anti-ischemic drugs on coronary flow reserve and the maximal mean coronary flow-pressure relationship. The responses to 3 doses of either the β_1-selective adrenoceptor antagonist metoprolol, the β_1-selective adrenoceptor partial agonist epanolol or the calcium antagonist nifedipine were studied and compared with a control group ($n=6$/group). Intracoronary bolus infusions of adenosine (20 μg/kg/0.2 ml) were administered without and during 3 levels of coronary stenoses in each animal, prior to and following each dose of drug, to obtain maximal coronary blood flows. Absolute coronary flow reserve (the ratio of maximal to resting coronary blood flow) and relative coronary flow reserve (the ratio of maximal coronary blood flow with a stenosis to maximal coronary blood flow without a stenosis) were calculated for each group at equivalent levels of stenoses. In addition, the slope and the extrapolated pressure-intercept at zero flow of the maximal mean coronary flow-pressure relationship were obtained at baseline for each animal, and compared with subsequent measurements following each dose of drug. In the nifedipine group, absolute coronary flow reserve decreased from a baseline value of 4.5 ± 1.9 to 1.9 ± 0.3 ($p<0.05$) following the last dose, reflecting in part the increase in resting coronary blood flow with the drug. Relative coronary flow reserves at similar levels of stenoses however, increased from 0.33 ± 0.06 to 0.47 ± 0.10 ($p<0.05$) over the same time period, suggesting an altered maximal vasodilator response. The slopes of the maximal mean coronary flow-pressure relationships decreased from 2.27 ± 0.49 at baseline to 1.54 ± 0.51 ml/(min.mm Hg) following the final dose ($p<0.05$). No changes in any of the parameters were observed in the other three groups. In conclusion, nifedipine alters the maximal mean coronary blood flow response as manifested by a dose-dependent increase in relative coronary flow reserve values at equivalent levels of stenoses and a reduction of the slopes and extrapolated pressure-intercepts at zero flow of the maximal mean coronary flow-pressure relationships. No such effects were observed with either metoprolol, or epanolol. This nifedipine-adenosine interaction may have important implications for the drug's anti-ischemic effectiveness. It also should be a consideration in the interpretation of serial coronary flow reserve measurements in patients on nifedipine treatment.

Introduction

The concept of absolute coronary flow reserve has become a useful tool for understanding human coronary physiology. Defined as the ratio of maximal to resting coronary blood flow, it may be helpful in assessing the functional significance of coronary artery stenoses[1] as well as the need for revascularization in selected patients.[2] It has also provided insight into altered coronary vasoreactivity of small vessels in a number of pathophysiological states, including ventricular hypertrophy[3], heart failure[4], syndrome X[5] and early post coronary reperfusion.[6]

Because anti-ischemic drugs are commonly used in patients undergoing coronary flow reserve studies, it is important to determine whether these agents affect coronary flow reserve. Some evidence suggests that these drugs may alter vascular reactivity independent of changes in hemodynamics. Calcium antagonists, for instance, blunt the degree of reactive hyperemia following brief periods of coronary occlusion in dogs[7-9] as well as following injection of contrast in patients.[10] They have also been shown to attenuate the maximal coronary vasodilator response in dogs.[7] ß-adrenoceptor antagonists have also been shown to reduce peak hyperemic flow following brief coronary artery occlusions in dogs.[11,12]

The interpretation of absolute coronary flow reserve measurements can often be difficult in situations where resting coronary blood flow or systemic hemodynamics are altered.[13-16] Relative coronary flow reserve, the ratio of maximal coronary blood flow during a given stenosis to the maximal coronary blood flow without a stenosis has been introduced as a more sensitive measure of the maximal coronary blood flow capacity independent of such changes.[15] Likewise, the determination of the maximal mean coronary flow-pressure relationship over a wide range of perfusion pressures also can more accurately characterize the maximal coronary blood flow capactiy.[17-19]

The objective of this study was to study the effects of certain anti-ischemic agents on the maximal coronary blood flow capacity using measurements that are more independent of changes in resting coronary blood flow and systemic hemodynamics. Because absolute coronary flow reserve may be influenced by these factors, we also measured relative coronary flow reserve and the maximal mean coronary flow-pressure relationship. The effects of two classes of anti-ischemic agents on the maximal coronary blood flow capacity were studied. The studies were carried out in open chest anesthetized swine, testing three different drugs: the $ß_1$-selective adrenoceptor antagonist metoprolol, the $ß_1$-selective adrenoceptor partial agonist epanolol and the calcium antagonist nifedipine. Epanolol was chosen because unlike metoprolol, it exhibits its $ß_1$-adrenoceptor antagonistic properties at doses which have minimal effects on systemic hemodynamics.[20]

Methods

Preparation

Following an overnight fast, 29 cross-bred Landrace-Yorkshire pigs (HVC, Hedel, The Netherlands) of either sex (24-42 kg) were sedated with intramuscular 20 mg/kg ketamine (AUV, Cuijk, The Netherlands) and intravenous 5 mg/kg metomidate (Janssen Pharmaceutica, Beerse, Belgium). Animals were intubated and connected to a respirator for

intermittent positive pressure ventilation with a mixture of oxygen and nitrous oxide (1:2). Ventilator settings were adjusted during the experiment to maintain normal arterial pH (7.35-7.45), pCO2 (35-45 mm Hg) and pO2 (>100 mm Hg). The external jugular vein was cannulated with two 7F catheters for administration of haemaccel (Behringwerke A.G., Marburg, Germany) to replace blood withdrawn for sampling. The anesthetic regimen consisted of an intravenous bolus of 150 mg/kg α-chloralose (E. Merck, Darmstadt, Germany), dissolved in boric acid solution, and a continuous intravenous infusion of 5 mg/kg/h sodium pentobarbitone (Apharma, Arnhem, The Netherlands). Via the left carotid artery, a 7F Sensodyn micromanometer-tipped catheter (B. Braun Medical B.V., Uden, The Netherlands) was advanced into the left ventricle (LV) and used to monitor LV pressure and its first derivative (LVdP/dt). The femoral arteries were cannulated with 7F catheters for aortic blood pressure measurements and arterial blood gas collections. Rectal temperature was monitored throughout the experiment and maintained near 37° C with external heating pads.

Following intravenous administration of 4 mg pancuronium bromide (Organon Teknika B.V., Boxtel, The Netherlands), a midline thoracotomy was performed and the heart was suspended in a pericardial cradle. After ligation of the left mammary vessels, the second left rib was removed for ease of further instrumentation. The left anterior descending coronary artery (LAD) was dissected free of its adventitia, and an electromagnetic flow probe (2.25 or 2.50 mm; Skalar, Delft, The Netherlands) and a variable hydraulic occluder (R.E. Jones, Silver Spring, Maryland, USA) were placed proximally. Meticulous attention was paid to the positioning and stability of the flow probe and zero flows were checked regularly throughout the experimental protocol. Immediately distal to the probe and occluder, a small cannula (0.8 mm-outer diameter) was inserted into the LAD for administration of adenosine and measurement of coronary perfusion pressure. Reactive hyperemia following a coronary artery occlusion lasting 15 seconds was recorded pre- and post- cannula placement to ensure that no attenuation of maximal coronary blood flow had occurred due to obstruction of the vessel by the catheter.

Regional segment length shortening was measured with sonomicrometry (Triton, Technology Inc., San Diego, CA, USA) in the subendocardial and subepicardial layer of the area perfused by the LAD. Pairs of ultrasonic crystals (Sonotek Corporation, Del Mar, Ca, USA) were placed approximately 10 mm apart in each region. Systolic shortening in the two layers was calculated from the difference between lengths at end-diastole (time of onset of positive dP/dt) and end-systole (time of peak negative dP/dt) and expressed as a percent of end-diastolic length.[21] Baseline end-diastolic length measurements were later normalized to 10 mm.

Throughout the experimental period, recordings (50 mm/s) were made of aortic, left ventricular and coronary arterial pressures in addition to LV dP/dt_{max}, coronary blood flow and segment length. Studies were performed in accordance with the position of the American Heart Association on research animal use adopted November 11, 1984, and under regulations of the Erasmus University.

Experimental protocol

Four experimental groups, each with 6 animals were studied. In three groups, the effects of three intravenous doses of either metoprolol (0.1, 0.5, and 1.0 mg/kg in cumulative doses), epanolol (10, 50, 200 μg/kg in cumulative doses) or nifedipine (0.5, 1.0 and 2.0 μg/kg/min in continuous infusions) on coronary and systemic hemodynamics were studied. Metoprolol and epanolol were given as bolus injections over 2 min in a volume of 10 ml. The fourth group of animals received equivalent volumes of saline and served as controls to establish reproducibility of the measurements during the execution of the experimental protocol. Measurements were obtained during four experimental periods: baseline and 10 minutes following administration of each new dose of drug or saline. Hemodynamic recordings and measurements of mean and phasic maximal coronary blood flow were obtained in response to intracoronary bolus injections of adenosine. The maximal coronary blood flow response could always be obtained with 20 μg/kg adenosine in a volume of 0.2 ml injected over 5 seconds. This response was always greater than the peak hyperemic response to a coronary artery occlusion lasting 15 seconds. The maximal mean coronary flow-pressure relationship was further defined by repeating the adenosine injections during 3-4 levels of stenosis applied with the hydraulic occluder. These were in the autoregulatory range to prevent changes in subendocardial and subepicardial contractile function. Figure 1 is a representative example of the tracings prior to and immediately following adenosine without and with two levels of stenosis. Maximal mean coronary flow-pressure relationships were obtained following each dose of drug, within 45 minutes of its administration.

Because of changes in heart rate induced by the different drugs, a fifth group ($n=5$) was added to determine the effect of modest increases in heart rate on the maximal mean coronary flow-pressure relationship. Following baseline measurements of systemic hemodynamics, myocardial contractile function, and the maximal mean coronary flow-pressure relationship, the left atrium was paced at 40 beats/min above the control heart rate for 5 min and subsequently all measurements were repeated. This increase in heart rate was comparable to the changes in heart rate observed in the experimental groups.

Drugs

Adenosine (E. Merck, Darmstadt, Germany) was dissolved in saline to a final concentration of 100 μg/kg/ml. Epanolol ("Visacor", ICI-Pharmaceuticals, Macclesfield, Cheshire, UK) and metoprolol tartrate ("Selokeen", Astra Pharmaceuticals BV, Rijswijk, The Netherlands) were also dissolved in saline. Nifedipine ("Adalat", Bayer AG, Wuppertal, Germany) was dissolved in a mixture of polyethyleneglycol 400, glycerol and distilled water and diluted with saline to a final concentration of 1 μg/kg/ml. The drug was infused at rates of 0.5, 1.0 and 2.0 ml/min and protected from light exposure.

The doses of all three drugs were selected to represent therapeutic ranges. In two pilot experiments, the β_1-adrenoceptor antagonistic properties of epanolol were studied by determining the potency to inhibit the isoproterenol-induced increases in heart rate and LV dP/dt_{max}. The infusion of 10, 50 and 200 μg/kg of the drug in cumulative doses showed isoproterenol dose ratios of 10, 50 and 150, respectively, for both parameters.

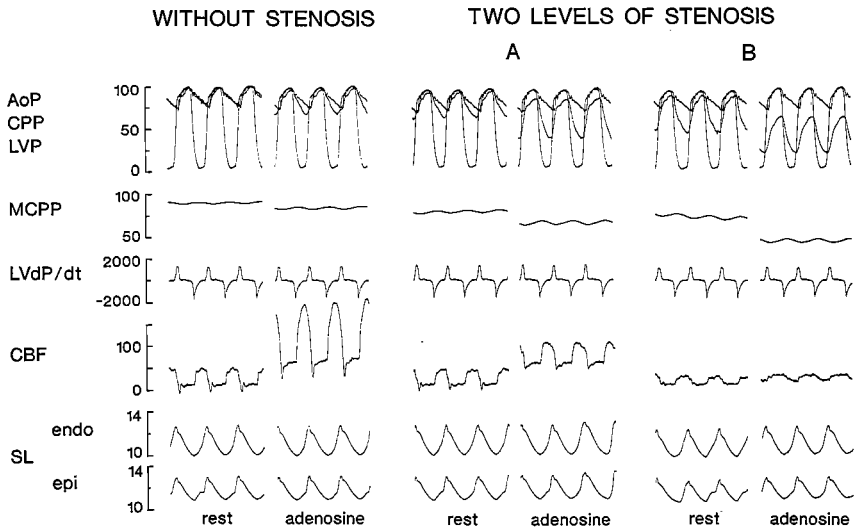

Figure 1. *Representative tracings from one animal showing the response to intracoronary adenosine without and during two levels of stenoses. Depicted are aortic pressure (AOP; mm Hg), coronary perfusion pressure (CPP; mm Hg), mean coronary perfusion pressure (MCPP; mm Hg), left ventricular pressure (LVP; mm Hg), maximal rate of rise of left ventricular pressure (LVdP/dt; mm Hg/s), coronary blood flow (CBF; ml/min) and subendocardial (endo) and subepicardial (epi) segment length (SL; mm).*

Data and statistical analyses

Maximal mean coronary flow-pressure relationships following adenosine were generated from the points collected during each sampling period, and correlations and regressions were determined by first order curve fitting. The maximal mean coronary flow-pressure relationship was described by the slope and the extrapolated pressure intercept at zero flow. Two levels of stenoses were also selected from each group with equivalent stenosis resistances. Resistances were determined from the pressure difference between mean arterial and mean coronary perfusion pressures divided by coronary blood flow at rest.

Absolute coronary flow reserve (ACFR) was calculated by the formula:

$$\text{ACFR} = \frac{\text{maximal coronary blood flow}}{\text{resting coronary blood flow}}$$

Relative coronary flow reserve (RCFR) was calculated at equivalent levels of stenoses by the formula (15):

$$RCFR = \frac{\text{maximal coronary blood flow in the presence of a stenosis}}{\text{maximal coronary blood flow without a stenosis}}$$

All data are expressed as means ± S.D.. Inter -and intragroup variability was tested for statistical significance by ANOVA (F-test; Fischer's PLSD). The changes in the pacing group were tested for significance by the paired t-test. Statistical significance was accepted at $p < 0.05$.

Results

Systemic hemodynamics (Table 1)

There were no statistical differences in the pre-drug baseline values between groups. In the control group, baseline mean arterial pressure was 91±20 mm Hg and remained stable throughout the experiment. Heart rate increased from 94±13 at baseline to 104±15 beats/min during the final experimental period ($p < 0.05$) and left ventricular end-diastolic pressure decreased from 8±2 to 5±2 mm Hg over the same period of time. Following the final dose of metoprolol, mean arterial pressure and heart rate had decreased by about 15%, and $LVdP/dt_{max}$ by nearly 50%. These changes were significantly different from those occuring in the control group at the same point of time. In the epanolol group, the changes in systemic hemodynamics were not different from those in the control group except that heart rate did not increase during the experiment. In the nifedipine group, mean arterial pressure decreased with each dose and was about 33% lower than baseline following the last dose. Heart rate increased with each dose but these changes were not significantly different from those occurring in the control group.

Coronary hemodynamics and absolute coronary flow reserve (Table 2)

There were no statistical differences between groups for any of the pre-drug baseline parameters. With increasing doses of metoprolol, coronary perfusion pressure was lowered causing a reduction in adenosine-induced maximal coronary blood flow. Baseline coronary blood flow was also reduced, which explains why absolute flow reserve at each dose remained unchanged. With increasing doses of epanolol, coronary blood flow, both at rest and following adenosine was not altered and absolute coronary flow reserve remained unchanged. With increasing doses of nifedipine, resting coronary blood flow was increased and maximal coronary blood flow decreased. Absolute coronary flow reserve was lower with each dose reflecting both the higher resting coronary blood flow and lower maximal coronary blood flow at lower perfusion pressures.

Relative coronary flow reserve (Table 3):

Because absolute coronary flow reserve may vary with changes in resting coronary blood flow and in systemic hemodynamics, we determined relative coronary flow reserve following each dose of drug at two equivalent levels of stenoses. The calculated stenosis resistances of the moderate (A) and severe (B) stenoses were 0.19±0.04 and 0.57±0.08 mm Hg.min/ml respectively, and were not different between groups during the experimental

Table 1: *Systemic Hemodynamics Following Administration of Anti-ischemic Agents*

	Baseline	Dose 1	Dose 2	Dose 3
CONTROL				
MAP	91 ± 20	89 ± 18	94 ± 15	91 ± 16
HR	94 ± 13	96 ± 12	101 ± 16	104 ± 15*
$LVdP/dt_{max}$	1750 ± 250	1710 ± 280	1740 ± 320	1680 ± 440
LVEDP	8 ± 2	7 ± 1	6 ± 2*	5 ± 2*
METOPROLOL				
MAP	93 ± 13	88 ± 17	86 ± 20	78 ± 16*#
HR	112 ± 15	104 ± 11	97 ± 6*	96 ± 7*#
$LVdP/dt_{max}$	1940 ± 540	1420 ± 230*	1190 ± 210*	1030 ± 180*#
LVEDP	8 ± 2	9 ± 2	9 ± 2#	9 ± 2#
EPANOLOL				
MAP	92 ± 5	88 ± 10	89 ± 13	88 ± 11
HR	101 ± 23	100 ± 13	100 ± 9	100 ± 6
$LVdP/dt_{max}$	1760 ± 370	1910 ± 460	1770 ± 500	1640 ± 480
LVEDP	7 ± 1	6 ± 1	6 ± 1	6 ± 1
NIFEDIPINE				
MAP	99 ± 8	89 ± 10*	72 ± 3*#	66 ± 7*#
HR	92 ± 18	97 ± 20	105 ± 26*	112 ± 26*
$LVdP/dt_{max}$	1780 ± 290	1650 ± 360	1570 ± 410	1470 ± 480
LVEDP	9 ± 1	9 ± 1	8 ± 2	9 ± 3

MAP, mean arterial pressure (mmHg); HR, heart rate (beats/min); $LVdP/dt_{max}$, maximum rate of rise of left ventricular pressure (mmHg/s); LVEDP, left ventricular end-diastolic pressure (mmHg). * $p<0.05$ versus baseline; # $p<0.05$ versus changes in the control group; see text for doses. Values are given as mean ± SD.

Table 2: *Coronary Hemodynamics and Absolute Coronary Flow Reserve Following Administration of Anti-ischemic Agents*

	Baseline	Dose 1	Dose 2	Dose 3
CONTROL				
CBF-rest	28 ± 14	26 ± 12	25 ± 12	24 ± 12
CBF-adenosine	114 ± 55	115 ± 51	116 ± 42	110 ± 45
MCPP-rest	91 ± 20	89 ± 18	94 ± 15	91 ± 16
MCPP-adenosine	83 ± 16	81 ± 14	85 ± 13	81 ± 14
ACFR	4.2 ± 0.4	4.5 ± 0.4	5.1 ± 1.6	4.9 ± 1.1
METOPROLOL				
CBF-rest	30 ± 9	26 ± 5	23 ± 5*	21 ± 4*
CBF-adenosine	109 ± 36	101 ± 41	99 ± 42	82 ± 24*
MCPP-rest	93 ± 13	88 ± 17	86 ± 20	78 ± 16*#
MCPP-adenosine	83 ± 11	79 ± 18	78 ± 17	72 ± 13*
ACFR	3.8 ± 1.5	4.1 ± 1.9	4.4 ± 2.2	4.2 ± 1.8
EPANOLOL				
CBF-rest	31 ± 12	30 ± 9	29 ± 9	26 ± 10
CBF-adenosine	116 ± 31	115 ± 25	122 ± 37	113 ± 37
MCPP-rest	92 ± 5	88 ± 10	89 ± 13	88 ± 11
MCPP-adenosine	84 ± 5	80 ± 10	78 ± 12*	74 ± 11*
ACFR	4.0 ± 1.1	4.1 ± 1.0	4.3 ± 0.8	4.5 ± 1.1
NIFEDIPINE				
CBF-rest	33 ± 13	44 ± 19*#	45 ± 19*#	40 ± 19
CBF-adenosine	130 ± 24	105 ± 28*#	88 ± 22*#	74 ± 20*#
MCPP-rest	99 ± 8	89 ± 10*	72 ± 3*#	66 ± 7*#
MCPP-adenosine	87 ± 7	80 ± 8*	69 ± 3*#	61 ± 7*#
ACFR	4.5 ± 1.9	2.6 ± 0.7#	2.1 ± 0.4*#	1.9 ± 0.3*#

CBF-rest, mean coronary blood at rest (ml/min); CBF-adenosine, mean coronary blood flow during adenosine (mml/min); MCPP-rest, mean coronary perfusion pressure at rest (mmHg); ACPP-adenosine, mean coronary perfusion pressure during adenosine (mmHg); **ACFR**, absolute coronary flow reserve; * $p<0.05$ versus own baseline; # $p<0.05$ versus control in same dose group; see text for doses. Values are given as mean ± SD

Table 3: *Relative Coronary Flow Reserves During Equivalent Moderate and Equivalent Severe Stenoses Following Administration of Anti-ischemic Agents*

	Baseline	Dose 1	Dose 2	Dose 3
CONTROL				
moderate	0.59 ± 0.14	0.52 ± 0.10	0.57 ± 0.18	0.60 ± 0.21
severe	0.35 ± 0.09	0.35 ± 0.14	0.32 ± 0.10	0.32 ± 0.10
METOPROLOL				
moderate	0.54 ± 0.12	0.57 ± 0.10	0.52 ± 0.14	0.56 ± 0.08
severe	0.39 ± 0.19	0.34 ± 0.12	0.36 ± 0.10	0.35 ± 0.10
EPANOLOL				
moderate	0.51 ± 0.08	0.56 ± 0.09	0.56 ± 0.16	0.57 ± 0.12
severe	0.33 ± 0.08	0.32 ± 0.07	0.33 ± 0.11	0.35 ± 0.17
NIFEDIPINE				
moderate	0.60 ± 0.08	0.73 ± 0.12*#	0.69 ± 0.11	0.73 ± 0.14*
severe	0.33 ± 0.06	0.40 ± 0.10	0.47 ± 0.11*#	0.47 ± 0.10*#

* $p<0.05$ versus baseline; # $p<0.05$ versus control in same dose group; see text for doses. Values are given as mean ± SD

periods. At baseline, relative coronary flow reserve in the nifedipine group was 0.60±0.08 and 0.33±0.06 for the moderate and severe stenoses respectively and were similar between the other groups. Relative coronary flow reserves remained constant during all doses in the control, metoprolol and epanolol groups, however in the nifedipine group, with the moderate and severe stenoses, they increased to 0.73±0.14 ($p<0.05$) and 0.47±0.10 ($p<0.05$) respectively following the final dose.

Maximal mean coronary flow-pressure relationships (Figure 2, Table 4)

Using the maximal mean coronary flow-pressure points, we generated lines to further characterize changes in maximal vasodilator capacity. Figure 2 shows representative examples of the maximal mean coronary flow-pressure relationships from an individual animal from each group, during each of the four experimental periods. Regression lines

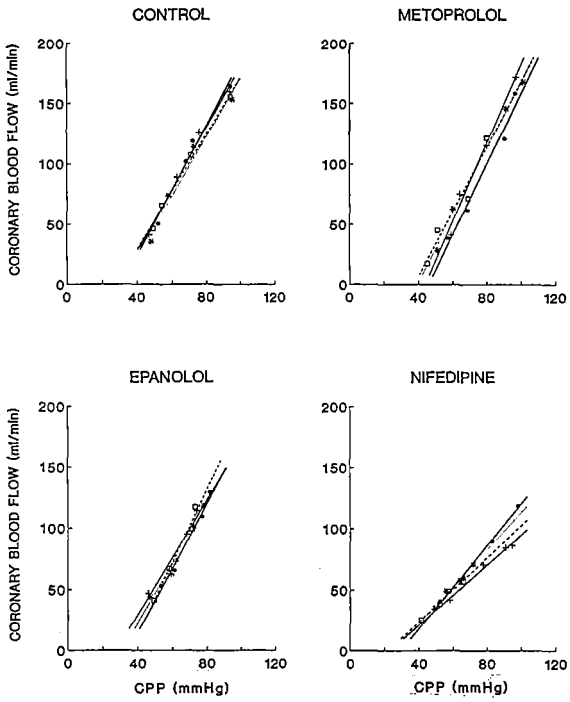

Figure 2. *Examples of maximal mean coronary flow-pressure relationships from each of the four experimental groups. Regression lines were generated from several coronary flow-pressure points following intracoronary administration of adenosine at baseline and following three doses of drug. Nifedipine altered the maximal response to adenosine with a reduction in the slope and extrapolated pressure-intercept at zero flow of the maximal mean coronary flow-pressure relationship.*

Table 4: *Slopes and Extrapolated Pressure-intercepts at Zero Flow of the Maximal Mean Coronary Flow-Pressure Relationships Following Administration of Anti-ischemic Agents*

	Baseline	Dose 1	Dose 2	Dose 3
CONTROL				
slope	2.14 ± 0.65	2.22 ± 0.69	2.18 ± 0.72	2.15 ± 0.63
extrapolated P_{zf}	29 ± 4	29 ± 10	34 ± 8	30 ± 4
METOPROLOL				
slope	2.05 ± 0.81	2.24 ± 0.99	2.05 ± 0.80	1.87 ± 0.61
extrapolated P_{zf}	29 ± 10	29 ± 10	28 ± 9	28 ± 11
EPANOLOL				
slope	2.30 ± 0.53	2.27 ± 0.13	2.43 ± 0.17	2.56 ± 0.62
extrapolated P_{zf}	34 ± 8	28 ± 3	30 ± 4	29 ± 8
NIFEDIPINE				
slope	2.27 ± 0.49	1.98 ± 0.62	1.86 ± 0.45*	1.54 ± 0.51*
extrapolated P_{zf}	30 ± 4	25 ± 4	21 ± 8*	20 ± 7*

extrapolated P_{zf}, extrapolated pressure-intercept at zero flow. *$p<0.05$ versus baseline; see text for doses. Values are given as mean ± SD

were determined by first order curve fitting, and the results of the correlations, slopes and extrapolated pressure intercepts at zero flow have been listed in Table 4. All the lines generated from these points showed significant linear correlations ($r^2 > 0.96$) and were highly reproducible in the control group (mean change in slope values of only $2\pm7\%$ between experimental periods). In the epanolol and metoprolol groups, there were no statistical differences in slopes or extrapolated pressure intercepts at zero flow following any of the doses, compared with pre-drug baseline values. Nifedipine on the other hand, decreased the slope of the maximal mean coronary flow-pressure relationship as well as the extrapolated pressure-intercept following each dose. The differences were significant following the last two doses.

Regional myocardial segment length changes: (Table 5, Figure 3)

None of the groups showed a significant change in subendocardial or subepicardial segment lengths with any of the three doses without a stenosis (Table 5). Function was recorded during all stenoses to ensure that the maximal mean coronary flow-pressure relationship was not altered due to an ischemia-induced change in systolic compressive forces. In Figure 3, subendocardial segment length changes are shown following the last dose for each of the groups, prior to and during the moderate and severe stenoses. As shown, function did not significantly change in any of the groups during these two levels of stenoses even with adenosine, indicating that differences in the maximal mean coronary flow-pressure relationship between groups were not due to a change in systolic compressive forces.

Heart rate and the maximal mean coronary flow-pressure relationship (Table 6)

Because heart rate between the experimental groups was different following drug administration, we included 5 additional animals to determine the effect of increasing heart rate in a similar range by atrial pacing. At baseline, heart rate was 96 ± 13 beats/min and mean arterial pressure was 91 ± 16 mm Hg. During adenosine, endocardial segment length shortening was $20\pm6\%$ without a stenosis and $20\pm7\%$ (NS) during the severest stenosis. Pacing at a heart rate of 40 beats/min above baseline did not affect systemic hemodynamics. With the pacing and during adenosine, subendocardial segment length shortening was $18\pm7\%$ without the stenosis and $16\pm9\%$ (NS) during the severest stenosis. The slope of the maximal mean coronary flow-pressure relationship was 3.96 ± 0.56 at baseline and during pacing was 3.79 ± 0.34 ml/(min.mm Hg) (NS). These data show that moderate increases in heart rate in the range observed in our experimental groups can not account for the differences in the maximal mean coronary flow-pressure relationship between the anti-ischemic agents.

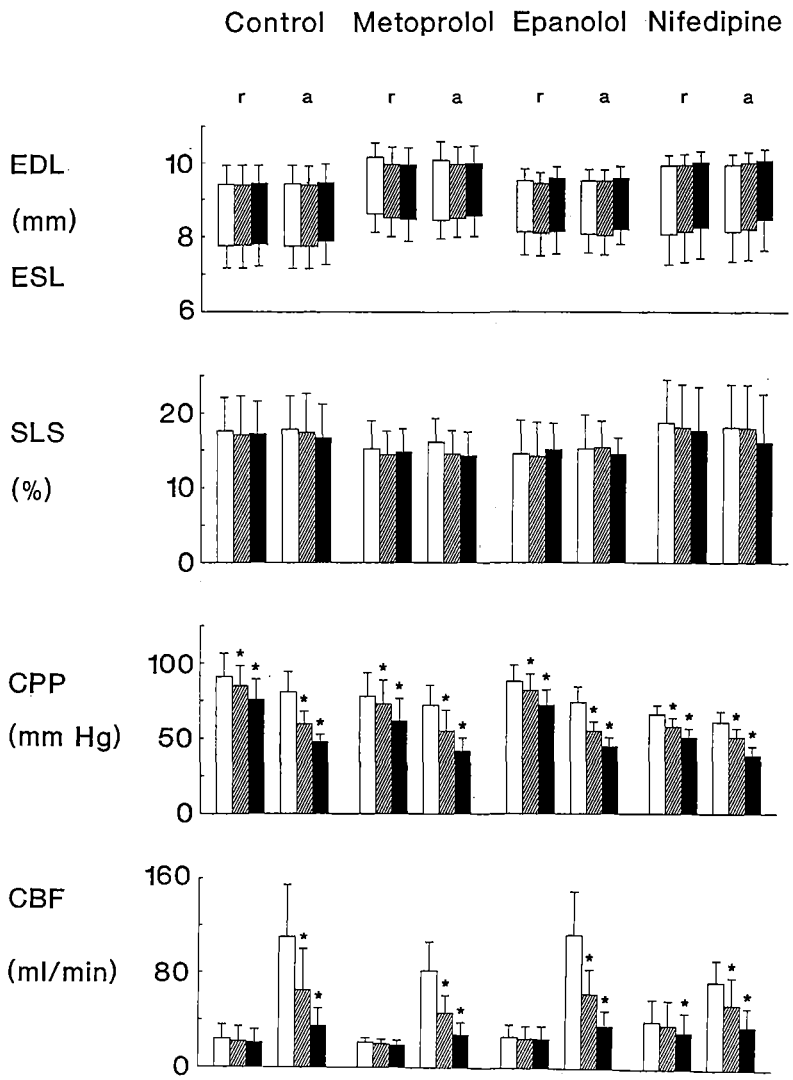

Figure 3. *Subendocardial end-diastolic length (EDL), end-systolic length (ESL), systolic segment length shortening (SLS), mean coronary perfusion pressure (CPP) and mean coronary blood flow (CBF) are shown following the final dose of drug in each group. The measurements were made without (□) and during the moderate (▨) and severe (■) stenoses. r, rest; a, adenosine). There were no significant differences in function for any of the groups, at these two levels of stenoses. Values are given as mean ± SD.*

Table 5: *Regional Myocardial Segment Length Changes Shortening Following Administration of Anti-ischemic Agents*

	Baseline	Dose 1	Dose 2	Dose 3
CONTROL				
Subepicardial segment length				
end-diastole (mm)	10.0	9.9 ± 0.2	9.9 ± 0.2	9.8 ± 0.5
end-systole (mm)	8.5 ± 0.3	8.4 ± 0.4	8.5 ± 0.3	8.4 ± 0.4
systolic shortening (%)	15 ± 3	15 ± 3	14 ± 2	14 ± 3
Subendocardial segment length				
end-diastole (mm)	10.0	9.8 ± 0.2	9.6 ± 0.2	9.4 ± 0.4
end-systole (mm)	8.0 ± 0.6	7.9 ± 0.5	7.9 ± 0.5	7.8 ± 0.6
systolic shortening (%)	20 ± 6	19 ± 6	18 ± 6	18 ± 4
METOPROLOL				
Subepicardial segment length				
end-diastole (mm)	10.0	10.2 ± 0.4	10.3 ± 0.5	10.4 ± 0.3
end-systole (mm)	8.8 ± 0.5	9.0 ± 0.5	9.1 ± 0.5	9.2 ± 0.4
systolic shortening (%)	12 ± 5	12 ± 4	12 ± 3	12 ± 2
Subendocardial segment length				
end-diastole (mm)	10.0	10.2 ± 0.2	10.2 ± 0.4	10.2 ± 0.3
end-systole (mm)	8.2 ± 0.3	8.4 ± 0.4	8.5 ± 0.4	8.6 ± 0.5
systolic shortening (%)	18 ± 3	18 ± 3	16 ± 4	15 ± 4
EPANOLOL				
Subepicardial segment length				
end-diastole (mm)	10.0	9.6 ± 0.2	9.7 ± 0.4	9.7 ± 0.3
end-systole (mm)	8.7 ± 0.2	8.4 ± 0.5	8.3 ± 0.6	8.5 ± 0.6
systolic shortening (%)	13 ± 2	13 ± 4	14 ± 4	12 ± 5
Subendocardial segment length				
end-diastole (mm)	10.0	9.7 ± 0.1	9.7 ± 0.2	9.5 ± 0.3
end-systole (mm)	8.3 ± 0.4	8.1 ± 0.5	8.1 ± 0.5	8.1 ± 0.6
systolic shortening (%)	17 ± 4	16 ± 5	16 ± 4	15 ± 5
NIFEDIPINE				
Subepicardial segment length				
end-diastole (mm)	10.0	9.8 ± 0.3	9.9 ± 0.2	10.0 ± 0.1
end-systole (mm)	8.1 ± 0.2	8.0 ± 0.2	8.1 ± 0.2	8.4 ± 0.5
systolic shortening (%)	19 ± 2	18 ± 3	18 ± 3	16 ± 4
Subendocardial segment length				
end-diastole (mm)	10.0	9.8 ± 0.3	9.9 ± 0.2	10.0 ± 0.1
end-systole (mm)	7.9 ± 0.4	7.8 ± 0.5	8.1 ± 0.4	8.1 ± 0.8
systolic shortening (%)	21 ± 4	21 ± 5	18 ± 5	19 ± 6

None of the changes reached statistical significance; see text for doses. Values are given as mean ± SD

Table 6: *Maximal Mean Coronary Flow-Pressure Relationship Pre -and Post-Atrial Pacing (n = 5)*

	Systemic hemodynamics				Coronary blood flow		Maximal mean coronary flow-pressure relationship	
	HR	MAP	$LVdP/dt_{max}$	LVEDP	Rest	Adenosine	Slope	Extrapolated P_{zf}
Control	96 ± 13	91 ± 16	1548 ± 169	9 ± 2	47 ± 8	201 ± 46	3.96 ± 0.56	26 ± 3
Pacing	136 ± 17*	89 ± 18	1536 ± 250	7 ± 1	48 ± 10	179 ± 47*	3.79 ± 0.34	28 ± 4

HR, heart rate (beats/min); MAP, mean arterial blood pressure (mmHg); $LVdP/dt_{max}$, maximal rate of rise of left ventricular pressure (mmHg/s); LVEDP, left ventricular end-diastolic pressure (mmHg); Coronary blood flow (ml/min); Slope (ml/(min.mmHg)); Extrapolated P_{zf}, extrapolated pressure-intercept at zero flow (mmHg); * $p<0.05$ versus baseline. Values are given as mean ± SD

Discussion

This study was designed to characterize the effects of three anti-ischemic agents on absolute and relative coronary flow reserve and the maximal mean coronary flow-pressure relationship. We have shown that metoprolol and epanolol had no effect on relative coronary flow reserve or the slope and extrapolated pressure-intercept at zero flow of the maximal mean coronary flow-pressure relationship. Nifedipine on the other hand, not only reduced maximal coronary blood flow by its effects on perfusion pressure but in addition, altered the maximal coronary blood flow response to adenosine over the range of perfusion pressures tested. This is best illustrated in Figure 3 which demonstrates that increasing doses of the drug reduced the slope and extrapolated pressure-intercepts of the maximal mean coronary flow-pressure relationship.

The interpretation of pharmacological interventions on absolute coronary flow reserve has inherent limitations.[19] We attempted to minimize the effects of changes in systemic hemodynamics on maximal mean coronary blood flow by determining the maximal mean coronary flow-pressure relationship over a range of pressures. A number of factors can still alter the maximal vasodilator capacity in response to vasodilators, independent of their effect on vasomotor tone.[14] Preload changes, for instance, alter the maximal coronary vascular conductance and pressure-zero flow values in a capacitance free model in anesthetized dogs.[17] This was not responsible for the observed findings in the nifedipine group however, because no changes in left ventricular end-diastolic pressure were noted. In the control group, left ventricular end-diastolic pressure was reduced from 8 mm Hg to 5 mm Hg over the course of the experiments but the maximal vasodilator response to adenosine was unchanged. This suggests that small changes in preload have little effect on the maximal vasodilator capacity. These data support recent studies in humans where maximal coronary blood flow measurements were unchanged when pulmonary capillary wedge pressure was increased from 9 to 16 mm Hg with rapid saline loading.[26] Changes in heart rate may also affect maximal coronary blood flow.[14] In conscious dogs, a significant attenuation in maximal hyperemic flow to adenosine has been shown when heart rate is increased from 150 to 200 beats/min.[16] In the nifedipine group, heart rate increased modestly from 92 to 112 beats/min and could conceivably have been responsible for the altered maximal mean coronary flow-pressure relationship. However, we have included an additional group of animals to show the effect of the maximal mean coronary flow-pressure relationship following similar changes in heart rate. As shown in Table 6, it is unlikely that the changes in heart rate in the nifedipine group were in a range that would independently alter maximal coronary blood flow. These data suggest that small changes in heart rate in a lower physiological range have a negligible effect on the maximal mean coronary flow-pressure relationship. Observations in patients, for example, have shown that maximal coronary blood flow is unchanged when heart rate is increased from 76 to 120 beats/min with atrial pacing.[26]

Absolute coronary flow reserve measurements were included in the present study, but are not necessarily specific to changes in the coronary vasculature. They may be altered because of changes in resting coronary blood flow or systemic hemodynamics. In the metoprolol group, absolute flow reserve remained constant due to proportionate reductions

in both resting and adenosine-induced maximal coronary blood flows. In the nifedipine group, absolute coronary flow reserve was lowered because of both the increase in resting and reduction in maximal coronary blood flows. The latter not only reflected the reduced perfusion pressure during increasing doses of nifedipine but also the altered response to adenosine. Relative coronary flow reserve has been shown to be a more reliable predictor of vasodilator capacity during a fixed stenosis, independent of changes in systemic conditions.[15] During equivalent levels of stenoses, we showed an increase in relative flow reserve with increasing doses of nifedipine, which is consistent with the findings of the lower slopes and leftward shift of the extrapolated pressure-intercepts at zero flow of the maximal mean coronary flow-pressure relationship.

The effects of calcium antagonists on the maximal coronary vasodilator capacity have been reported in several models and the results are consistent with our findings. In open chest dogs, nifedipine was shown to blunt both coronary reactive hyperemia following a 30 second occlusion and the response to intracoronary infusions of adenosine.[7] In conscious dogs, coronary reactive hyperemia to coronary artery occlusions lasting 10 seconds, was attenuated 2 hours after the administration of nifedipine when coronary blood flow and systemic hemodynamics had returned to pretreatment values.[8] Similarly, in 12 patients undergoing coronary angiography, contrast-induced reactive hyperemia was shown to be reduced following an infusion of intravenous diltiazem, suggesting that the effect is not limited to nifedipine.[10] Because coronary perfusion pressures were not measured in any of these studies, it is difficult to determine whether the reduced flow responses were specific to changes in the coronary vasculature or a result of changes in systemic hemodynamics.

The effects of ß-adrenoceptor antagonists on resting and maximal coronary blood flow have also been well studied. In anesthetized dogs, these drugs blunt the degree of peak reactive hyperemia following brief periods of occlusion.[11,12] In conscious animals as well as humans, they increase resting end-diastolic coronary resistance but do not alter maximal coronary blood flow.[22-25] The latter results are more consistent with our findings where neither metoprolol nor epanolol affected the maximal mean coronary flow-pressure relationship. The altered reactive hyperemia observed following administration of ß-adrenoceptor antagonists is probably more influenced by a lower oxygen demand during the occlusion period and therefore may not be representative of intrinsic changes in maximal vasodilator capacity.

We have shown that nifedipine alters the maximal mean coronary flow-pressure relationship to adenosine. The application of these data are important in the interpretation of clinical flow reserve measurements as an index of stenosis severity in patients using the drug. In addition, this specific nifedipine-adenosine interaction may have important therapeutic implications. It has been suggested that adenosine receptors in different tissues also bind nifedipine and its dihydropyridine analogs.[7] By inhibiting adenosine receptors in the myocardium during ischemia, it is possible that a regional redistribution of flow away from the subendocardium is prevented by nifedipine.[8] This may explain why nifedipine improves subendocardial perfusion during moderate levels of stenosis.[9] It is not clear whether the interaction of nifedipine and adenosine also applies to other calcium antagonists and also to other smooth muscle vasodilators. For example, intracoronary Doppler measurements of maximal flow measurements in in response to papaverine have not been

altered following infusions of diltiazem.[28]

Limitations of the study

In the present study, we calculated the extrapolated pressure-intercept at zero flow of the maximal mean coronary flow-pressure relationship. This parameter helps to define the maximal mean coronary flow-pressure relationship, but cannot be used as an actual pressure-intercept at zero flow and should therefore not be interpreted as such. In view of the capacitance effects that occur throughout the cardiac cycle and which are enhanced during increasing severity of stenosis due to the increased diastolic and systolic coronary perfusion pressure variations (Figure 1), we chose to present only mean coronary blood flow As a consequence we cannot differentiate between diastolic and systolic effects of the anti-ischemic drugs.

Conclusions

The results of this study in anesthetized swine show that the calcium antagonist nifedipine alters the maximal mean coronary flow-pressure relationship following intracoronary adenosine, an effect that was not observed with either the β_1-selective adrenoceptor antagonist metoprolol or the β_1-selective adrenoceptor partial agonist epanolol. The effects of nifedipine may have important implications for its anti-ischemic actions. It also should be a consideration when serial coronary flow reserve measurements are used to assess stenosis severity in patients using the drug.

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Chapter 4

The importance of vasomotor tone to myocardial function and regional metabolism during constant flow ischemia in swine.

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Importance of vasomotor tone to myocardial function and regional metabolism during constant flow ischaemia in swine

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Abstract

Study objective – The aim was to test the hypothesis that the release of vascular tone with adenosine during constant flow ischaemia alters both transmural function and regional metabolism in a detrimental way.

Design – In one group of anaesthetised swine, the effects of graded reductions of flow on segmental left ventricular function, myocardial oxygen consumption (MVo_2), and lactate production in the distribution of the left anterior descending coronary artery (LAD) were determined. In a second group, a model of constant flow ischaemia was induced to test how altering vascular tone with adenosine changed the relationship of flow, function, and metabolism.

Experimental material – The experiments were performed in 20 open chest, anaesthetised swine. Protocol A consisted of 11 animals and protocol B of nine animals.

Measurements and main results – In protocol A, during graded ischaemia, reductions in flow, % systolic wall thickening (WTh), normalised MVo_2 and % lactate extraction (%LE) correlated well with reductions in coronary perfusion pressure when fitted with 3rd order polynominal curves ($r = 0.78$, 0.87, 0.85 and 0.81 respectively; $p<0.00001$). In protocol B, during constant flow ischaemia, at control, % WTh was 33 (SD 11)%, mean coronary artery pressure was 72(10) mm Hg, mean LAD transmural flow was 0.99(0.43) $ml \cdot min^{-1} \cdot g^{-1}$, and % LE was +14(9)%. With inflation of a hydraulic occluder on the LAD, perfusion pressure was lowered to 38(5) mm Hg and transmural flow dropped to 0.76(0.31) $ml \cdot min^{-1} \cdot g^{-1}$ (intact vasomotion). During an infusion of intracoronary adenosine with flow held constant (absent vasomotion), %WTh was further reduced from 27(9) to 13(10) ($p<0.001$), and %LE from –18(42) to –70(61) ($p<0.05$). MVo_2 with and without vasomotion did not differ significantly at 3.14(0.75) and 3.18(0.86) $ml \cdot min^{-1} \cdot g^{-1}$ respectively.

Conclusion – In swine coronary circulation, reductions in regional function, MVo_2 and lactate production correlate well with reductions in flow and perfusion pressure during ischaemia with vasomotor tone intact. The effect of adenosine on vascular tone during constant flow ischaemia caused dramatic reductions in function and lactate extraction without altering MVo_2. This emphasises the important role of vascular tone in protecting both transmural function and regional metabolism during moderate ischaemia.

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During myocardial ischaemia, the degree of preservation of perfusion to subendocardial layers is an important determinant of the degree of functional and metabolic alterations.[1–4] This in turn is dependent on multiple factors, one of which is coronary perfusion pressure.[5–17] Another factor which affects subendocardial perfusion is vasomotor tone, which may differ within regions of the myocardium at various levels of ischaemia. An infusion of adenosine during moderate ischaemia in dogs, for instance, has been shown to reduce vascular tone primarily in the subepicardium, with a redistribution of flow away from the subendocardium.[18 12–14] In both anaesthetised and awake dogs, adrenergic innervation to the subepicardial regions of the myocardium has also been shown to play an important role in maintaining subendocardial perfusion during flow reductions.[19] The loss of this α mediated vascular tone causes a maldistribution of flow along with a reduction in wall thickening.[20 21]

Although altering vasomotion during ischaemia has been shown to alter function, the effects on regional metabolism in a model of constant flow ischaemia have not been shown. Therefore, in anaesthetised swine, we first determined the relationship of perfusion pressure to wall thickening, lactate production, and myocardial

oxygen consumption with intact vasomotion over a wide range of ischaemic flow reductions. We then studied the effects of modifying vasomotor tone with adenosine, at two levels of ischaemia during which flow was held constant. The data support the hypothesis that during ischaemia, vascular tone in the subepicardium plays an important role in preserving both transmural wall thickening and correlates of metabolism, including myocardial oxygen consumption and lactate production.

Methods

ANIMAL PREPARATION

We performed two sets of experiments. The preparation was similar in both, and has been described previously.[22 23] Domestic swine of either sex weighing between 31 and 47 kg were studied. Premedication consisted of an initial intramuscular dose of xylazine (2 $mg \cdot kg^{-1}$) and ketamine (12 $mg \cdot kg^{-1}$). Anaesthesia was then provided by a bolus of intravenous morphine sulphate (2 $mg \cdot kg^{-1}$) and ketamine (10 $mg \cdot kg^{-1}$). A constant intravenous infusion of morphine sulphate (1 $mg \cdot kg^{-1} \cdot h^{-1}$) and ketamine (10 $mg \cdot kg^{-1} \cdot h^{-1}$) was maintained throughout the experiment.[24] d-Tubocurarine, 2 ml intravenously, was given during the surgical preparation to minimise muscular activity during the use of electrocautery. Animals were intubated, placed on a piston respirator, and ventilated with oxygen enriched air. The respirator was adjusted to keep arterial pH between 7.35 and 7.45, P_{CO_2} at 4.7-6.0 kPa, and P_{O_2} above 13.3 kPa. Rectal temperature was monitored and kept between 36.7 and 38.3°C. Through a cutdown, the femoral vein and artery were cannulated. A midline sternotomy was performed and the heart suspended in a pericardial cradle. A catheter was placed in the left atrium and one in the apex of the left ventricle for recording pressure and its first derivative. The left anterior descending coronary artery was dissected free from surrounding tissue and a Statham flow probe (2.0-2.5 mm diameter) followed by an inflatable hydraulic occluder were placed proximally. Distally, a small silicone catheter (inside diameter 0.3 mm, outside diameter 0.6 mm) was inserted and used to monitor distal coronary pressure. Using a similar technique, a catheter was inserted into the distal great cardiac vein for sampling regional venous lactate and oxygen saturation from the ischaemic left anterior descending artery bed. On the left ventricular free wall served by the left anterior descending artery, epicardial and endocardial ultrasonic crystals (5 MHz) were placed perpendicular to each other and their proper position confirmed at the conclusion of each experiment. End diastolic wall thickness was measured at the onset of positive dP/dt, and end systolic wall thickness was measured 20 ms before peak negative dP/dt. The extent of wall thickening was calculated as the difference in mm between end systolic and end diastolic dimensions and expressed as a percentage change from end diastolic wall thickness.

Phasic and mean coronary flow and pressure, electrocardiogram, systolic left ventricular wall thickness, and left ventricular pressure and its first derivative were recorded during each study on an eight channel Mark 200 Brush recorder. Pressure was measured using Statham P-23 Gb pressure transducers calibrated against a mercury manometer. Flow probe zero readings by transient left anterior descending artery occlusion were obtained at the conclusion of each ischaemic period. Oxygen (O_2) content was determined on a Lex-O_2-Con and the $\%O_2$ extraction was computed by dividing the arterial-coronary venous O_2 content difference (vol %) by the arterial O_2 content and multiplying by 100. Myocardial oxygen consumption (MVo_2) was determined by multiplying O_2 content differences by the flow probe reading in the first protocol (A) and microsphere flows in the second protocol (B).

Lactate concentrations were determined from plasma by an electrode technique[18] and lactate extraction calculated by dividing the difference of arterial and cardiac vein lactate concentration by the arterial concentration and multiplying by 100.

Studies were performed in accordance with the

Table I Functional and metabolic variables at control and during graded reductions in flow (protocol A). Data are means (SD)

	Control (n=11)	*During flow reduction*						
Pressure (mm Hg)	77(13)	50 (n=7)	45 (n=6)	40 (n=3)	35 (n=4)	30 (n=5)	25 (n=4)	20 (n=3)
Coronary flow ($ml \cdot min^{-1}$)	31(16)	25(14)	27(13)	15(5)	18(9)	12(8)	10(3)	5(3)
Normalised flow	100	86(7)	79(13)	82(11)	59(9)	41(34)	42(16)	19(14)
Wall thickening (%)	30(11)	25(11)	23(9)	31(16)	21(5)	6(8)	7(10)	1(5)
Normalised % wall thickening	100	83(11)	80(13)	61(22)	55(17)	16(27)	19(46)	12(26)
% Oxygen extraction	83(7)	89(6)	87(8)	92(4)	91(3)	93(4)	93(4)	93(4)
MVo_2 ($ml \cdot min^{-1}$)	3.2(1.5)	2.8(1.6)	2.7(0.7)	1.9(0.6)	1.8(0.7)	1.1(0.7)	1.1(0.5)	0.9(0.5)
Normalised MVo_2	100	89(7)	85(13)	86(7)	63(11)	44(35)	51(18)	31(16)
Lactate extraction (%)	+11(16)	−6(36)	+2(26)	−123(125)	−210(177)	−160(107)	−296(150)	−362(25)

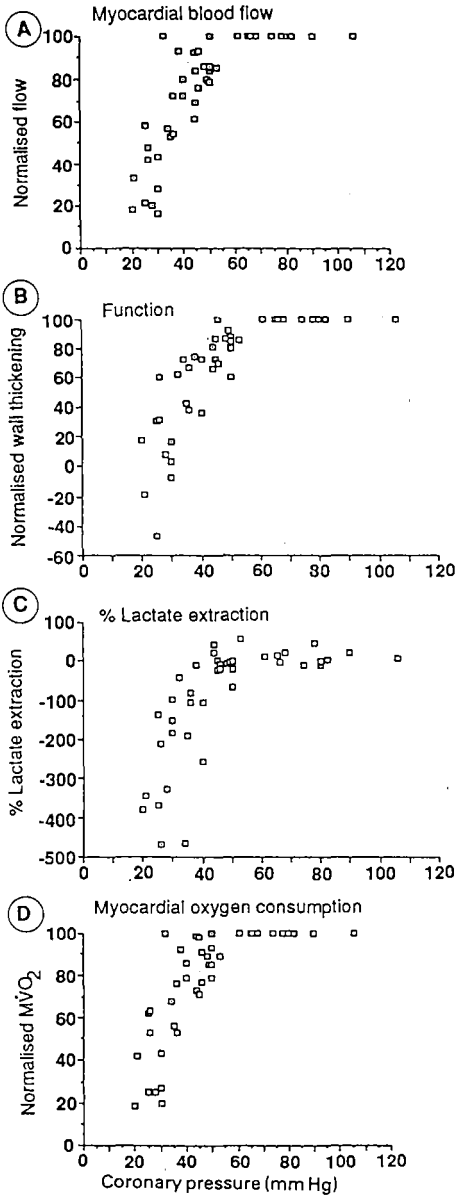

Figure 1 Coronary perfusion pressure versus (A) normalised flow, (B) normalised wall thickening, (C) normalised $M\dot{V}O_2$, and (D) % lactate extraction at control and during variable ischaemia in 11 swine (protocol A).

position of the American Heart Association on research animal use adopted November 11, 1984, and under the regulations of the Animal Care Committee of the Oregon Health Sciences Center.

EXPERIMENTAL DESIGN

Protocol A — The objective of this protocol was to obtain steady state functional and metabolic data at control and over a range of ischaemic flow reductions with intact vasomotion. The value 50 mm Hg was chosen as the upper limit of the ischaemic range because this represents the point where autoregulation begins to fail.[22] At the beginning of each experiment, control measurements were made of pressure, flow, and wall thickness and simultaneous arterial and regional cardiac vein samples were drawn for determining oxygen content and lactate concentration. By use of a screw clamp attached to the occluder, coronary pressure was then lowered and measurements and blood samples obtained between 2 and 5 min of a steady state. The pressures were selected at 5 mm Hg intervals between 20 and 50 mm Hg with the order randomly assigned. Pressure was allowed to return to normal after each set of measurements and the animal allowed to recover for at least 15 min. It was required that function return to 90% of the original control value in order to include the results of subsequent measurements. This, we believe, eliminated any residual effects of prior ischaemic pressures on subsequent measurements.

Protocol B — The objective in this protocol was to test how altered vascular tone during constant ischaemic flow affects transmural function and regional metabolism. We wished to induce two levels of ischaemia, with identical total flow but altered vascular tone, by using an infusion of intracoronary adenosine. The animals were prepared as described above with an additional left anterior descending artery catheter inserted proximal to the flow probe for infusion of adenosine.[26] As in protocol A, initial measurements and blood samples were obtained for each animal and in addition regional myocardial blood flow was measured at control and at each subsequent ischaemic pressure. Radioactive microspheres (New England Nuclear, (9-11 micron) labelled with either ^{141}Ce, ^{51}Cr, ^{103}Ru or ^{95}Nb were injected into the left atrium over 30 s while a reference sample was withdrawn from the aorta at a constant rate of 10 $ml \cdot min^{-1}$ for 2 min. Microspheres were suspended in 0.9% saline and agitated for 15 min prior to injection. Approximately 2-3 million spheres were injected for each measurement.

Coronary flow was then lowered with the occluder to a level that reduced systolic wall thickening by 15-20%. The same measurements and samples as listed for the control period were obtained within 2-5 min of achieving steady state and microspheres were injected. Adenosine (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was then infused into

the proximal left anterior descending artery catheter resulting in a rise in coronary flow. The occluder was adjusted so that coronary flow measured by flow probe was equal to the initial ischaemic flow. In a steady state, measurements and samples were again obtained and another set of microsphres was injected.

After removal of the heart, the distribution of each coronary artery was identified by injecting 20 ml of different coloured dyes at equal pressures into each coronary artery. The heart was then placed in 10% buffered formalin. After 3-7 d, the tissue perfused by the left anterior descending artery was separated from the remaining left ventricle, and both were divided into inner, mid, and outer regions. The entire heart was cut up into approximately one gram tissue samples and each sample counted for 5 min using a programmable Nuclear Data ND600/600 multichannel analyser connected to a Packard Instrument Changer. Transmural and regional flows were calculated.[8] Since the objective was to perfuse the myocardium at two different pressures at a constant ischaemic transmural flow, we established prospectively that microsphere transmural flows at the two ischaemic periods were within 15% of each other.

STATISTICS

In the initial graded ischaemia studies (protocol A), flow, systolic wall thickening, and regional MVo_2 were normalised to the control values at each ischaemic pressure. Scatter plots for coronary pressure were made with normalised flow, wall thickening, and MVo_2 and % lactate extraction, and fitted with third order polynominal curves. For the constant flow experiments (protocol B), comparisons of the two ischaemic groups of data were made with paired *t* tests. All data are expressed as means (SD).

Results

HAEMODYNAMIC VARIABLES

Eleven animals were studied in protocol A. During the control state, the left ventricular systolic and diastolic pressures were 99(13) mm Hg and 8(2) mm Hg respectively, and the heart rate was 94(13) beats·min^{-1}. During the experiment, a maximum change of 9% (mean) was observed in the heart rate or systolic blood pressure at any of the low pressures compared with control.

Twelve animals were used in protocol B but the data from three are not included because transmural flow was not constant as specified by the design. At control, heart rate was 94(14) beats·min^{-1} and systolic and diastolic pressures were 96(11) and 10(2) mm Hg respectively. There were no statistical differences in these variables between the control and two ischaemic periods.

GRADED FLOW REDUCTIONS WITH INTACT VASOMOTION (PROTOCOL A)

In the 11 animals, measurements were obtained at control and at a total of 32 pressures over the ischaemic range. Due to sustained effects on function from prior periods of ischaemia, the number of measurements in any given animal was limited. As discussed, we believe the arbitrary 90% recovery of function between

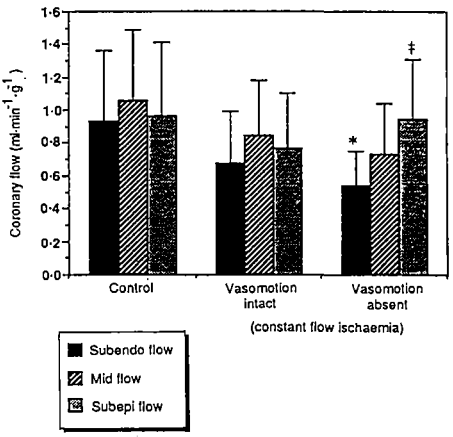

*Figure 2 Regional coronary flow at control and during constant flow ischaemia, with and without adenosine (protocol B). *p<0.05; ‡p<0.001 v control.*

Table II Constant flow ischaemia (protocol B). Data are means (SD). Statistical comparisons are between two ischaemic periods

		Constant flow ischaemia		
	Control	*Vasomotion intact*		*Vasomotion absent*
LAD flow (ml·min^{-1}·g^{-1})	0.99(0.43)	0.76(0.31)	NS	0.76(0.28)
Inner/outer flow ratio	0.96(0.08)	0.91(0.26)	p<0.01	0.59(0.16)
MVo_2 (ml·mm^{-1}·100 g^{-1})	4.23(1.21)	3.14(0.75)	NS	3.18(0.86)
Wall thickening (%)	33(11)	27(9)	p<0.05	13(10)
Lactate extraction (%)	+14(9)	−18(42)	p<0.05	−70(61)
LAD pressure (mm Hg)	72(10)	38(5)	p<0.01	33(4)

LAD=left anterior descending coronary artery; MVo_2=myocardial oxygen consumption

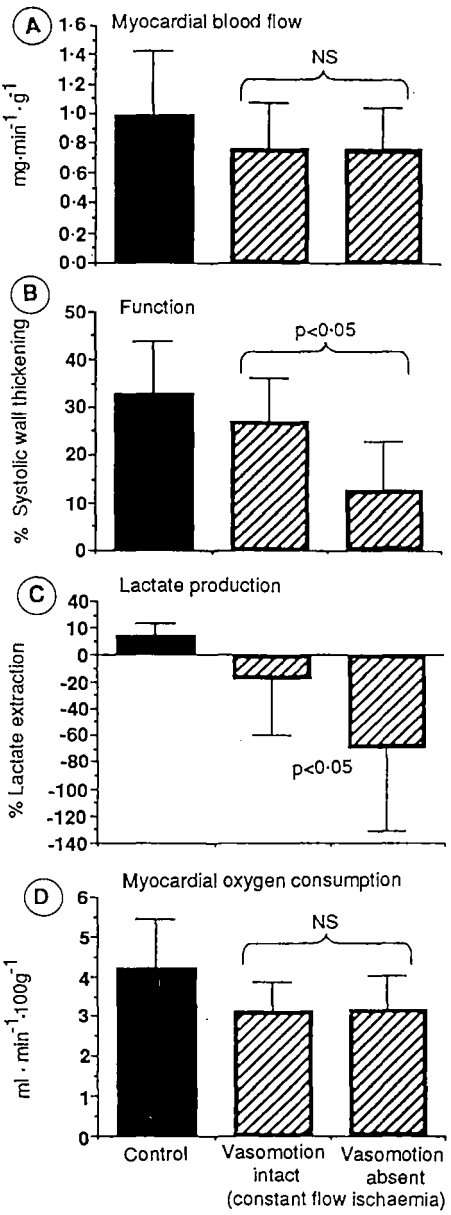

Figure 3 (A) transmural flow, (B) % wall thickening, (C) % lactate extraction, and (D) myocardial oxygen consumption at control and during constant flow ischaemia, with and without adenosine (protocol B).

measurements allowed an accurate assessment of the effects of each ischaemic pressure and eliminated residual changes from prior measurements.

Coronary flow was obtained by flow probe in these experiments and myocardial oxygen consumption (MVo_2) expressed in $ml \cdot min^{-1}$. Because of interanimal variability in % wall thickening, flow, and MVo_2, these values were normalised to control values for each low pressure. All the data for the graded reductions in pressure protocol are listed in table I.

Figure 1 shows perfusion pressure at control for the 11 animals, and for each ischaemic period plotted against: (a) normalised flow, (b) normalised % wall thickening, (c) normalised MVo_2, and (d) % lactate extraction. As shown, reductions in flow, wall thickening, MVo_2, and lactate extraction correlated well with reductions in coronary pressure when fitted with third order polynominal curves ($r = 0.78, 0.87, 0.85, 0.81$ respectively; $p<0.00001$). These curves emphasise that during ischaemia with intact vasomotor tone, transmural function and regional metabolism are directly dependent on flow and perfusion pressure once autoregulation fails.

CONSTANT FLOW ISCHAEMIA WITH ALTERED VASOMOTION (PROTOCOL B)

The results of this protocol are shown in table II. At control, transmural flow was 0.99(0.43) $ml \cdot min^{-1} \cdot g^{-1}$ and with vasomotion intact was then reduced to 0.76(0.31) $ml \cdot min^{-1} \cdot g^{-1}$. This was associated with a modest reduction in wall thickening and lactate extraction, which was consistent with the findings from protocol A for a 20-25% reduction in flow (table I). Vasomotor tone was then released with the infusion of adenosine, and flow was held constant. Figure 2 shows the transmural blood flow in the subendo-, mid, and subepicardial layers at control and during the two ischaemic periods. The only region which increased flow in response to adenosine was the subepicardium. This release of vasomotor tone resulted in a significant reduction in inner/outer ratios and was associated with a 5 mm Hg drop in perfusion pressure (table II). The changes in function, lactate production, and MVo_2 at control and during ischaemia with and without vasomotor tone are further illustrated in fig 3. Panel (A) shows transmural flow, which was unchanged between the two ischaemic periods. Percent wall thickening and % lactate extraction were 33(11) and +14(9) respectively at control. With the loss of subepicardial vasomotor tone during ischaemia, % wall thickening was reduced from 27(9) to 13(10) (table II). This change is represented in panel (B). Percent lactate extraction during the same period was also reduced from −18(42) to −70(61) ($p<0.05$) and is shown in (C). Myocardial oxygen consumption at control was 4.23(1.21) $ml \cdot min^{-1} \cdot 100\ g^{-1}$. It is interesting that despite the reductions in wall thickening and lactate extraction with the loss of vasomotor tone, oxygen

consumption remained unchanged with and without vasomotion (fig 3D).

Discussion

The most important finding in this study is that during moderate ischaemia, subepicardial vascular tone plays an important role in preserving regional myocardial metabolism, as well as transmural function. With total coronary flow held constant during the two ischaemic periods in protocol B, the effect of adenosine on subepicardial resistance reduced wall thickening and increased lactate production dramatically. In addition, the reduction in wall thickening with the infusion of adenosine was not associated with a parallel reduction in myocardial oxygen consumption, as might have been predicted from protocol A (fig 1C). This suggests that subepicardial vascular tone during ischaemia has an additional protective effect of minimising the cost to the myocardium by limiting the amount of oxygen consumed.

Our results of the graded flow reduction experiments in protocol A are consistent with the findings of other workers. In open chest anaesthetised dogs, reductions in total coronary flow beyond 15-25% correlated well with reductions in regional function in a near linear fashion.[27-29] More recently, it has been shown that reductions in regional wall shortening during variable ischaemic flows are better correlated with subendocardial flow than with either total or subepicardial flow. Weintraub *et al*[6] observed in anaesthetised dogs that below a perfusion pressure of 50 mm Hg, reductions in transmural flow were linearly related to diastolic pressure. Normalised % systolic shortening, however, was best correlated with subendocardial blood flow when fit by a sigmoidal equation. In unanaesthetised dogs, similar relationships between graded subendocardial flow and transmural function have been observed. Gallagher *et al*[2] found a mildly curvilinear but highly significant correlation between normalised wall thickening and subendocardial flow during six groups of variable ischaemic flows, with only a weak fit between total or subepicardial flow. In a similar model, Vatner[3] has described a significant exponential relationship between reductions in segmental length and subendocardial flow with graded flow reductions.

These studies have extended the work of others in regard to oxygen consumption and lactate production during graded reductions in flow. We have shown that the degree of anaerobic glycolysis as measured by lactate extraction is inversely related to the degree of reduction in pressure over the ischaemic range. Figure 1B shows the magnitude of change in lactate extraction over a wide range of pressures during ischaemia which parallels the reductions in function. Stowe *et al*[30] studied the effects of various degrees of flow reduction in open chest swine and found only a weak correlation between reductions in flow and lactate extraction. In their study, only one ischaemic measurement was made per animal 15-60 min after the onset of reduced flow. The large degree of interanimal variability in lactate extraction limits its use as an indicator of ischaemia[31 32] and together with the extended time of ischaemia may have influenced their results. The fact that we obtained multiple measurements per animal within 2-5 min of ischaemia may explain the differences in correlation between studies.

Subendocardial perfusion during ischaemia has been shown to be dependent upon coronary perfusion pressure.[5-16] It is also dependent on factors in the subepicardium which maintain vascular tone. During moderate degrees of ischaemia in anaesthetised animal preparations for instance, adenosine has a greater vasodilator effect in subepicardial regions, and may induce a maldistribution of flow away from the subendocardium.[12 18] The recent observations of α adrenergic coronary vasoconstriction during ischaemia has also emphasised the importance of subepicardial tone to transmural flow distribution.[19] In anaesthetised dogs, Nathan *et al* showed that subendocardial perfusion during constant ischaemic flow was lower in the presence of α blockade, thus showing the protective effects of α mediated constrictor tone in the subepicardium.[21] The same effect has been shown during exercise in dogs, where intact α receptors maintained uniform transmural blood flow distribution.[20]

The relationship of MVo_2 to regional function during and following ischaemia is also of great interest. In the graded ischaemia protocol, reductions in MVo_2 paralleled the changes in mechanical function over the ischaemic range of pressures and flows (fig 1C). During the constant ischaemic flow protocol, however, wall thickening was reduced but MVo_2 remained unchanged (fig 3D). This implies that the myocardium consumes oxygen inefficiently for any level of work load when vasomotor tone is altered during ischaemia. There are several potential explanations for this. It is possible that with the release of vasomotor tone in the subepicardium during ischaemia, areas of myocardium are perfused which are unable to contribute to overall transmural thickening. A similar phenomenon may be present in regions of stunned myocardium where oxygen consumption is heterogeneous, despite an overall decrease in function.[33 34] An equally plausible reason for observing no change in MVo_2 during the two ischaemic flows is that wall thickening is only a minor determinant of overall oxygen consumption. Therefore, during constant flow ischaemia, reductions in wall thickening during adenosine infusion have little effect on myocardial oxygen consumption. It is also possible that changes in MVo_2 are more dependent on changes in flow than oxygen extraction, and therefore little variation would be expected during constant ischaemic flow.

In summary, we have shown that during graded

reductions in flow, with vasomotion intact, reductions in regional wall thickening, myocardial oxygen consumption, and lactate extraction correlate well with reductions in coronary perfusion pressure. In a model of constant ischaemic flow, adenosine releases vasomotor tone, primarily in the subepicardial regions, and reduces transmural wall thickening while increasing lactate production without a change in MVo_2. Therefore, subepicardial tone during moderate ischaemia plays an important role in preserving both function and metabolism.

LIMITATIONS OF THE STUDY

A major criticism of this study is the use of anaesthesia, which may independently alter coronary vascular tone. Our coronary dissections were minimal and we can only assume that our findings would apply to awake animals. In support of this is the fact that multiple studies have used adenosine to alter coronary reactivity and have shown similar effects in both anaesthetised and unanaesthetised animals. The use of adenosine to stimulate maximum vasodilatation has been universal but deserves some comment. At variable heart rates, its effect on the degree of transmural vasodilatation may not be uniform.[6] Because no statistical difference in heart rate was observed between the ischaemic groups, this was not a problem. Of importance is the site of action of the agent relative to the region of the myocardium. Crystal *et al*[35] studied the effects of coronary adenosine infusion in anaesthetised dogs and showed at a constant non-ischaemic pressure, equivalent increments in flow transmurally. They also showed no effects on myocardial oxygen consumption, small vessel blood volume, or packed cell volume. The potential negative inotropic effects of adenosine also deserve comment. In isolated atrial muscle preparations, adenosine in physiological concentrations has been shown to inhibit catecholamine mediated increases in myocardial contractility.[36] This is unlikely to have contributed to the drop in function in our experiments since adenosine in doses sufficient to cause maximal coronary vasodilatation has not been shown to have negative inotropic effects.[13]

Finally, the use of regional atriovenous gradients for the determination of myocardial consumption during ischaemia may be criticised as being heterogeneous, particularly in non-steady-state conditons.[37] We believe that our measurements were obtained after a long enough period to allow for equilibration of regional flows. In terms of its sensitivity for regional changes in flow, table I shows that oxygen and lactate extraction were quite sensitive to alterations in perfusion pressure over a range of even 5 mm Hg.

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Chapter 5

Coronary vasodilation reserve persists despite tachycardia and myocardial ischemia.

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Coronary vasodilator reserve persists despite tachycardia and myocardial ischemia

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BRISTOW, J. DAVID, EDWARD O. McFALLS, CHERYL G. ANSELONE, AND GEORGE A. PANTELY. *Coronary vasodilator reserve persists despite tachycardia and myocardial ischemia.* Am. J. Physiol. 253 (Heart Circ. Physiol. 22): H422–H431, 1987.—During myocardial ischemia, we tested whether coronary blood flow would increase in response to tachycardia, thereby employing known coronary flow reserve. We instrumented the left anterior descending (LAD) coronary circulation in anesthetized pigs and performed three sets of experiments while coronary pressure was controlled and several heart rate increases were produced. *1*) Pacing-induced tachycardia at normal LAD pressure was characterized by increased LAD flow and myocardial oxygen consumption, without production of lactate. *2*) Tachycardia at a mean LAD pressure of 38 mmHg was associated with a lower, fixed coronary flow and oxygen consumption. At average heart rates of 90 and 150 beats/min, LAD flow was 19.6 and 19.4 ml/min and corresponding myocardial blood flows were 0.59 and 0.54 $ml \cdot g^{-1} \cdot min^{-1}$. Lactate was produced at all rates and local myocardial function declined progressively. *3*) Coronary flow at low LAD pressure doubled during tachycardia when intracoronary adenosine was added. The increase to the subepicardium was >100%, whereas subendocardial flow changed little. There is persistent coronary flow reserve during moderately severe myocardial ischemia, even when metabolic demand is increased by tachycardia. This reserve, however, is predominantly subepicardial.

coronary circulation; myocardial oxygen consumption; lactate metabolism; adenosine

DESPITE MYOCARDIAL ISCHEMIA resulting from moderately low coronary arterial pressure, the coronary vascular bed does not vasodilate fully. Several studies have shown that considerable coronary flow reserve is inducible by adenosine at ischemic levels of pressure (1, 6, 10, 24). Such increases in myocardial blood flow do not seem to improve local myocardial function and metabolism significantly in the ischemic zone, suggesting that the region has been set at a new relationship among flow, function, and metabolism. It is not clear why this reserve of vasodilation exists in the presence of ischemia, or what the responses to an increase in local myocardial metabolic demand might be in this situation. Prior investigations of the effect of increased demand on coronary dynamics, myocardial performance, and metabolism at low pressure levels have been few and have led to divergent conclusions (16, 29).

Our study was designed to investigate the effects of increased heart rates at a controlled, low coronary artery pressure on coronary flow dynamics, local left ventricular mechanical function, and metabolism. We postulated that the demands imposed by the increased metabolic requirements of heart rate would result in coronary vasodilation, using known flow reserve. This would be expressed as an increase in total coronary blood flow, or, at the least, in the level of coronary flow during late diastole.

Our experiments demonstrated that this was not the case, however. Coronary blood flow and myocardial oxygen consumption ($M\dot{V}O_2$) proved to be remarkably constant at low coronary pressure, despite considerable increases in heart rate and in the face of coronary flow reserve, demonstrated by intracoronary adenosine. This pharmacologically induced increase in flow was distributed mainly to the subepicardium of the left ventricle, and the subendocardium received much less of an increment.

METHODS

Preparation

Seventeen domestic pigs were studied; weights ranged from 33 to 50 kg. The preparation was similar to that previously reported (24–26) and is summarized below. Premedication was given with xylazine (2 mg/kg) and ketamine (10 mg/kg) intramuscularly. Anesthesia was provided with morphine sulfate (2 mg/kg) and ketamine (6 mg/kg) intravenously, followed by a constant infusion of morphine at 1 $mg \cdot kg^{-1} \cdot h^{-1}$ and ketamine 10 $mg \cdot kg^{-1} \cdot h^{-1}$; chondrocurine was given as required when anesthesia level was assured.

Ventilation with oxygen-enriched air was produced with a piston ventilator so as to produce an arterial pH between 7.35 and 7.45, partial arterial pressure of CO_2 (Pa_{CO_2}) between 35 and 45 and partial arterial pressure of O_2 (Pa_{O_2}) above 100 mmHg. Rectal temperature was monitored and maintained above 98°F. After a midline sternotomy was performed, catheters were inserted into the left ventricular apex and left atrium, as well as the aorta and inferior vena cava from the femoral region.

The proximal left anterior descending coronary artery (LAD) had the following placed in or around it. A small Silastic catheter (0.3 mm ID, 0.6 mm OD) was inserted

in the mid-LAD for recording pressure; its response characteristics have been described (24). An electromagnetic flow probe (2.0 or 2.5 mm) was placed on the LAD proximally, immediately followed by an inflatable fluid-filled occluder around the vessel. Another Silastic catheter was inserted into the anterior cardiac vein as it coursed parallel to the LAD, at the same level as the LAD occluder. It was used to sample coronary venous blood. For *protocol 3*, a second LAD catheter was inserted proximally for infusion of adenosine.

Meticulous attention was paid to the positioning and stability of the electromagnetic flow probe. The zero base line for the system was set during complete occlusion of the LAD, produced by inflation of the occluder. Once placed on the LAD, the transducer was not moved or adjusted during an experiment. No recordings were used if problems developed with the probe, i.e., all data are from experiments with stable, continuous flowmeter operation without adjustment of its position. Zero flow was checked briefly after each experimental period. Measurements were made of mean flow and that at end diastole, defined as 30 ms before the onset of the QRS complex.

Myocardial blood flow was measured with radioactive microspheres (11 ± 0.3 μm diam, New England Nuclear) labeled with ^{141}Ce, ^{51}Cr, ^{103}Ru, and ^{95}Nb (18). A reference sample was withdrawn from the aorta at a rate of 10 ml/min for 2 mins. Left atrial injection of spheres spanned 30 s and was begun shortly after the start of the reference sample collection. Preparation and counting of spheres was performed as we previously reported (24).

A pair of ultrasonic crystals (5 MHz) was placed in the anterior wall of the left ventricle in the region served by the LAD (27). One crystal was sewn to the epicardium and the second was inserted obliquely to its subendocardial position. The crystals were used to measure diastolic wall thickness and systolic thickening, with timing of mechanical systole as described by Gallagher (12). After the hearts had been removed, different colored dyes were injected simultaneously at the same rate in the LAD and right coronary arteries to define the myocardial region served by the LAD and to assure that the dimension crystals were well in the center of it.

Pressures were recorded with Statham P23 Gb transducers. Pressures, electromagnetic flow, ultrasonic crystals, and electrocardiographic signals were recorded on an eight-channel Mark 200 Brush pen recorder. Oxygen content of arterial and coronary venous samples was determined in duplicate with the Lex-O_2-Con.

Plasma lactate concentration was determined in triplicate using the method of Clark (8, 9). Blood samples were drawn into iced syringes containing heparin fluoride. They were promptly centrifuged, and the plasma was then decanted and frozen for later analysis. Lactate consumption or production was expressed in micromoles per minute, ignoring red blood cell (RBC) content of lactate.

Small electrodes were clipped to the lateral wall of the right atrial appendage and used for atrial pacing with a clinical pacemaker, generally set at 5 mA.

Protocols

A total of 17 swine was studied. There were seven animals in *protocol 1*, used to study the effects of an increase in heart rate at normal coronary pressure. Ten animals were included in *protocol 2*, which studied the effects of increased heart rate at low coronary pressure. Five of these animals participated in both *protocols 1* and *2*. In these, stable hemodynamics were present after the pacing protocol at normal LAD pressure, and the pacing study at low pressure was performed. Five other animals were studied in *protocol 3*; none of these participated in other protocols.

Protocol 1: pacing at normal coronary pressure. This set of experiments was performed to confirm that our preparation would respond to atrial pacing at normal LAD pressure with an increase in mean and end-diastolic coronary flow, and in $M\dot{V}O_2$. Approximately 30 min after the completion of the surgical procedure, control measurements were made. Atrial pacing was then begun, by choosing a heart rate slightly above the spontaneous rate, which consistently produced capture (the control-paced rate). This averaged 12 beats/min faster then the spontaneous rate. Increases in heart rate were then studied at 10-beat/min increments above that, up to 60 beats/min above the paced control, but with return to the control-paced rate between each one. Before the next increase in rate was studied, stable control findings were obtained. The sequence of the increases in heart rate was varied, although the most extreme increase in rate (+60 beats/min) was reserved for last.

Blood samples for oxygen and lactate analyses were drawn in the control and paced control periods, and with increments of 20, 40, and 60 beats/min. Sampling was performed after 3 min of stability at a given rate.

Protocol 2: pacing at low LAD pressure. These experiments were the primary focus of our study. We wished to determine whether an increase in coronary and myocardial blood flow and $M\dot{V}O_2$ could be induced with atrial pacing at moderately low LAD pressure. We studied several moderate increases in rate, so as not to miss an increment in flow that might be lost at faster rates, with progressive loss of diastolic time.

After control measurements at the spontaneous heart rate, a paced heart rate was produced that provided consistent capture of the heart; this control paced rate averaged 90 beats/min. After measurements were made at that rate, the coronary hydraulic occluder was inflated to reduce LAD pressure and flow. The extent of reduction desired was that which would decrease systolic left ventricular wall thickening by ~20–35%. This LAD mean pressure level was then employed for all subsequent experimental periods in that animal. Once that level was determined, and held for 3 min, blood samples were obtained and measurements recorded. The heart rate was then continued at the control-paced setting, and the coronary occlusion released. After a period of stability, the occlusion was set at the same level of mean pressure as in the preceeding period, and a different heart rate produced at increments of 10 beats/min up to a total of 60 beats/min above the control-paced rate. Between each of these experimental periods, the occlusion was released, and the control-paced rate reproduced. The rest interval between each experimental period varied from 5 to 25 min.

Blood samples for oxygen and lactate studies were obtained in all experimental periods (control, control paced, control paced at low pressure, and low pressure plus 10, 20, 30, 40, 50, and 60 beats/min above the control-paced rate) after 3 min of stability. As in *protocol 1*, the sequence of heart rates was varied so as to avoid a systematic change, but the 60 beat/min heart rate increase was done last.

Ten animals had four microsphere determinations of myocardial blood flow, all of which were performed during low pressure. In five animals these determinations were made during the control-paced rate and at increments of 10, 20, and 30 beats/min. In the other five animals, measurements were made at the control-paced rate as well as with 20-, 40-, and 60-beat/min increments.

Protocol 3: pacing at low LAD pressure with and without intracoronary adenosine. In five other animals, measurements were first made at the control and control-paced heart rates. As in *protocol 2*, the LAD pressure was then lowered and measurements made at the control-paced rate. The rate was then increased by 20, 40, and 60 beats/min above the control-paced rate, whereas mean LAD pressure was kept constant. After 3 min at a given rate, sampling was done. Then intracoronary adenosine was begun at a dose of 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Measurements were repeated after stable readings were present. A period of normal LAD pressure and control-paced rate was provided between the different increased rates, but not between measurements with and without adenosine, which was added at a given rate. Microsphere flow measurements were made at low LAD pressure with and without adenosine during heart rate increments of 20 and 60 beats/min.

Calculations

$M\dot{V}O_2$ was calculated for the region served by the LAD beyond the flow transducer as the product of mean LAD flow (flowmeter) and the arteriocoronary venous oxygen difference.

Lactate consumption or production was calculated on the basis of plasma values by the formula: lactate, μmol/min = (1 − hematocrit)(coronary flow)(AV lactate difference).

Systolic wall thickening was the difference between systolic and diastolic thickness, expressed as a percent of diastolic thickness (12).

Statistical Analysis

Results are given as means and standard deviations. Most of the variables were tested by one-way analysis of variance with repeated measures. Range testing was done with Tukey's test. For some of the microsphere flow determinations, two-way analysis of variance was done to compare effects of heart rate change within the LAD and non-LAD distributions. For the evaluation of some relationships, regression analysis was performed.

RESULTS

Representative tracings are presented in Fig. 1.

Protocol 1: Normal LAD Pressure

We wished to be certain that our preparation behaved as expected in response to increased heart rate at normal pressure. The spontaneous heart rate averaged 81 beats/min, the control paced rate was 93, and the average paced maximum in this group was 153. End-diastolic LAD flow rose with each increment in rate and increased substantially overall, more than doubling between rates of 81 and 153 beats/min (35.3–73.4 ml/min, $P < 0.0005$, Figs. 1 and 2). There was a strong positive correlation between heart rate and the average values for end-diastolic flow at each rate ($r = 0.96$). Mean LAD flow increased less, with an overall change of 38%.

Increases in heart rate were associated with increased diastolic thickness, reflecting a smaller diastolic volume (4, 27). This relationship was systematic, with correlation coefficients for the relationship between heart rate and diastolic thickness in individual experiments which averaged 0.79 and were between 0.84 and 0.93 in six of the seven studies. There was a modest decrease in systolic thickening with increased heart rate, from 38% in the control state to 28% at a heart rate of 153 ($P < 0.0005$, Fig. 3).

$M\dot{V}O_2$ rose with increases in heart rate; the maximum change was 38% ($P < 0.0005$). The rise in oxygen consumption varied in a linear fashion with the increase in heart rate ($r = 0.81$).

The amount of lactate consumed did not change with pacing; net lactate production did not occur. Thus evidence of anaerobic metabolism was not found in this preparation at the heart rates produced at uncontrolled (normal) LAD pressure.

Protocol 2: Low LAD Pressure

Results are presented in Tables 1 and 2 and Figs. 1–3.

Heart rate and LAD pressure. The spontaneous heart rate averaged 74 beats/min, and the control-paced rate was 90; the maximum paced rate averaged 150 beats/min. The standard deviation of ~9 beats/min at each paced rate reflects the different rates in different animals, not variation in the paced rate in each animal. Mean LAD pressure was lowered after measurements were made at the control-paced rate. The mean pressure chosen in each animal produced a ~25% decrement in systolic wall thickening at the control-paced rate. The pressures to achieve this ranged from 28 to 50 mmHg in different animals. Once chosen, a given mean pressure was used consistently throughout the remainder of the rates in that animal. The standard deviation for LAD pressure of ~7 mmHg during the low pressure periods reflects different levels in different animals, rather than variation within an animal. The degree to which low mean LAD pressure could be reproduced during the experiments was shown by the mean difference of subsequent periods from the original control-paced-low pressure period, of 1.1 ± 2.2 (SD) mmHg.

End-diastolic and mean LAD flow. When LAD pressure was lowered at the control-paced rate, mean LAD flow fell 26% and LAD end-diastolic flow fell 54% ($P <$ 0.0005). Thereafter, at rates up to 60 beats/min above

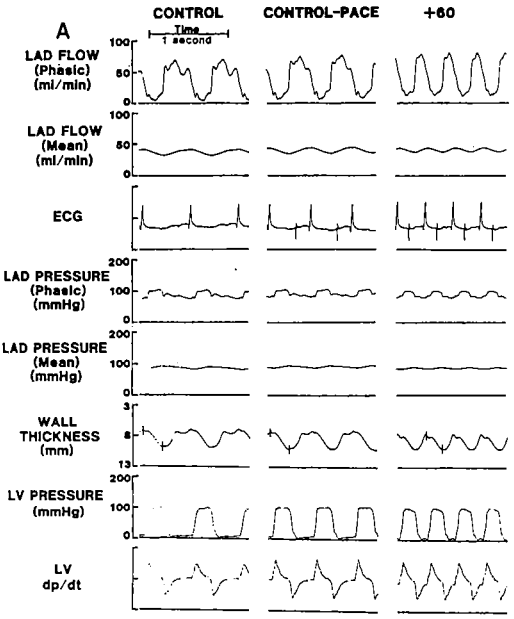
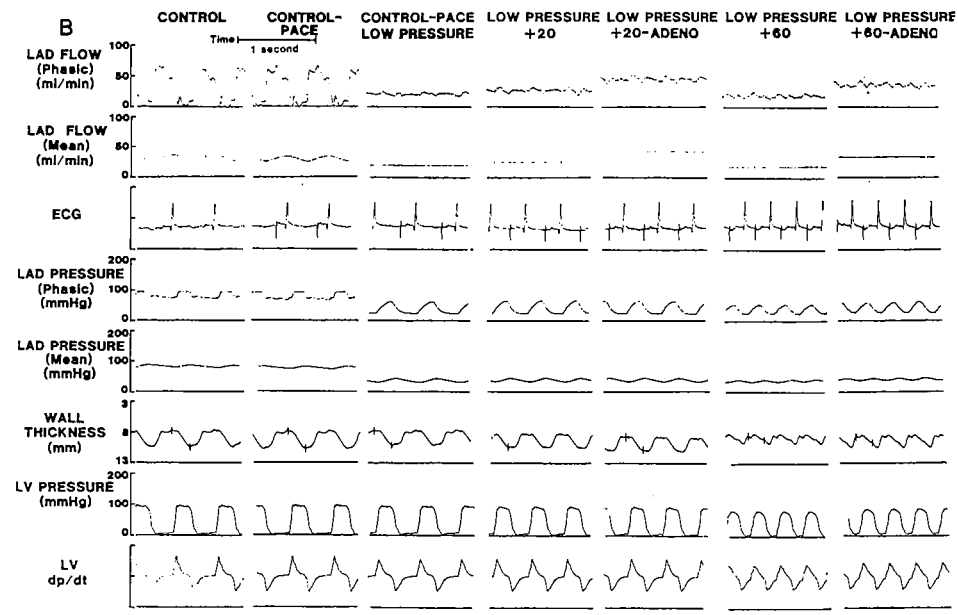


FIG. 1. *A*: record from *protocol 1* with induced tachycardia at normal left anterior descending artery (LAD) pressure. An overall 15% increase in end-diastolic LAD flow is associated with the increase in rate. Control systolic left ventricular (LV) wall thickening of 38% decreases to 34% with a control-paced rate of 110 beats/min. It then declines slightly to 28% with a further increment of 60 beats/min. Timing for calculation of thickening is shown by *short vertical lines*. *B*: same animal, during *protocol 2*. When LAD pressure is lowered, thickening declines to 31%, associated with a 50% fall in mean LAD flow. When tachycardia is produced, function decreases to 25% (20-beats/min increment) and then to 7% (60-beats/min increment). Addition of adenosine (adeno) at each paced rate substantially increased mean LAD flow, demonstrating previously unused coronary reserve. ECG, electrocardiogram.



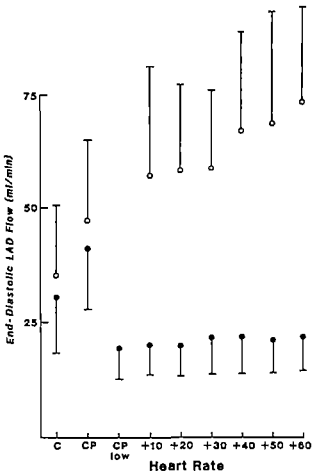


FIG. 2. Values are means ± SD for end-diastolic left anterior descending artery (LAD) flow. *Open circles*, measurements during pacing-induced tachycardia at normal LAD pressure; *closed circles, protocol 2* low LAD pressure group; C, control at normal pressure; CP, paced-control heart rate at normal pressure; CP low, low LAD pressure at control-paced rate. +10, +20, etc., increments in heart rate at low LAD pressure. End-diastolic flow rises sharply in normal pressure group. First two *closed circles* represent control and control-paced data at normal LAD pressure in *protocol 2*. Thereafter, LAD pressure was kept constantly low; flow fell and did not rise with tachycardia.

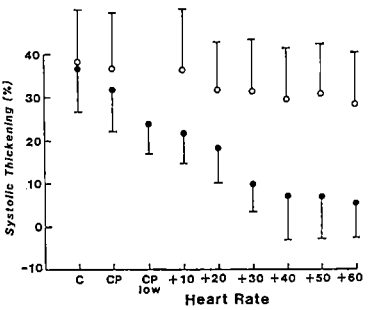


FIG. 3. Values are means ± SD for systolic function in myocardial zone perfused by LAD. Format and abbreviations are same as in Fig. 2.

the paced control rate, neither mean nor end-diastolic flows significantly increased. For example, mean LAD flow was 19.6 ml/min at the control-paced rate during low pressure and was 19.4 ml/min at 60 beats/min faster.

Myocardial blood flow by microsphere determinations. Two sets of data were obtained, each from five animals. One set was at the low-pressure control rate and 10, 20, and 30 beats/min above it; the other was at increments of 20, 40, and 60 beats/min. All points were obtained during low LAD pressure. Results are summarized in Table 2.

In the LAD region flow did not increase significantly in either series with increases in heart rate at low LAD pressure. In fact, at the faster rates, there was a downward trend in subendocardial flow, and a decrease in the subendocardial-to-subepicardial ratio, as demonstrated by regression analysis ($r = -0.63$).

In the non-LAD region, which was exposed to normal coronary pressure, increases in transmural flow occurred in the 20, 40, 60 series ($P < 0.01$). Increases in all three layers were suggested by regression analysis. The average endocardial-to-epicardial flow ratios remained between 1.10 and 1.31 in the two series of rates.

Every measurement in every animal showed a lower value for flow in the LAD region than the non-LAD region and this change was significant by two-way analysis of variance ($P < 0.05$ in the + 20, 40, 60 series and $P < 0.01$ in the + 10, 20, 30 series).

Left ventricular wall function. In the control-paced low pressure period, LV systolic wall thickening had declined by design. Further increases in heart rate produced progressively more severe reductions in function (Fig. 3). At a rate of 60 beats/min above the paced-control rate, the value for systolic thickening had declined from an average of 36.7 to 5.1%, and the wall was dyskinetic in some animals.

There was no consistent relationship between heart rate and end-diastolic thickness, as seen in *protocol 1*. The average correlation coefficient between these variables was 0.26 from the 10 experiments.

The relationship between end-diastolic wall thickness and systolic thickening was also quite different than in *protocol 1*, wherein a consistent negative relationship was found between the average values for these two variables. At low pressure, systolic thickening was more or less completely dissociated from end-diastolic thickness, i.e., resting thickness changed little while thickening declined markedly.

Oxygen and lactate data. Comparing $M\dot{V}O_2$ during the paced low-pressure control period with the six subsequent increases in heart rate, no increase was found. At an average rate of 90 beats/min, 2.1 ml/min was measured, vs 2.05 at rate 150. There was a strong, positive correlation between oxygen consumption per beat and systolic wall thickening ($r = 0.97$). Oxygen extraction from arterial blood increased from 76.4% in the original control period to 89.4 with low pressure. This value gradually increased modestly to 92.7% extraction at the fastest heart rate.

Lactate consumption was present in the control and control-paced period, promptly changed to production at low pressure and stayed negative. Looked at as simply positive or negative quantities, there is shift of extraction from positive to negative at low LAD pressure as shown by Cochran's Q test ($P < 0.001$). Lactate consumption shifted to production with low pressure and progressively rose ($P < 0.0005$, Table 1).

Protocol 3: Low LAD Pressure and Adenosine

Although adenosine-mediated changes in coronary blood flow have been well documented at spontaneous heart rates, it was not known if vasodilation reserve was

TABLE 1. *Pacing-induced tachycardia during ischemic LAD pressure*

	Control	Control Pace	Low Pressure Pace	Δ Heart Rate, beats/min			P
				+20	+40	+60	
Heart rate, beats/min	74.1±13.4	89.9±9.4	90.1±9.2	110.0±9.4	130.3±9.1	150.2±9.5	<0.0005
Mean LAD pressure, mmHg	75.0±12.7	77.2±14.6	37.3±7.6	38.5±7.2	39.0±7.7	38.3±6.8	<0.0005
End-diastolic LAD pressure, mmHg	63.7±10.4	70.1±14.1	22.2±4.6	22.3±4.5	23.6±4.5	25.8±4.5	<0.0005
Mean LAD flow, ml/min	23.9±6.8	26.4±7.2	19.6±5.5	20.5±6.6	20.1±7.1	19.4±5.8	<0.0005
End-diastolic LAD flow, ml/min	30.2±11.9	41.2±13.3	18.9±7.0	19.8±6.5	21.9±7.8	21.9±7.2	<0.0005
Systolic wall thickening, %	36.7±10.4	31.6±9.7	23.9±6.5	18.2±8.3	6.9±10.3	5.1±7.6	<0.0005
End-diastolic wall thickness, mm	8.65±1.1	9.02±1.1	8.86±1.1	9.00±1.1	8.85±1.2	9.08±1.2	NS
$M\dot{V}O_2$, ml/min	2.11±0.74	2.34±0.72	2.10±0.69	2.13±0.86	2.19±1.00	2.05±0.87	NS
Lactate consumption, μmol/min	4.4±5.4	5.1±6.1	−3.4±12.4	−4.8±11.3	−19.4±20.8	−10.6±12.6	<0.0005
LV systolic pressure, mmHg	92.4±18.1	95.6±16.9	92.7±17.8	86.8±14.8	88.1±14.6	81.0±10.7	<0.0005
LV diastolic pressure, mmHg	8.7±3.0	6.9±1.9	7.0±1.4	6.1±2.0	6.4±2.2	6.9±2.4	NS

Values are means ± SD. LAD, left anterior descending artery; Δ heart rate, increases of 20, 40, and 60 beats/min in control pace heart rate; $M\dot{V}O_2$, myocardial O_2 consumption; LV, left ventricular; NS, not significant.

TABLE 2. *Microsphere determinations of myocardial blood flow during pacing at ischemic LAD pressure*

	LAD Distribution						Non-LAD Distribution					
	Low pressure control	+10	+20	+30	ANOVA	Correlation (probability)	Low pressure control	+10	+20	+30	ANOVA	Correlation (probability)
Subendocardial flow, $ml \cdot min^{-1} \cdot g^{-1}$	0.41 ±0.17	0.40 ±0.11	0.49 ±0.21	0.45 ±0.14	NS	0.12 (NS)	0.66 ±0.15	0.67 ±0.25	0.86 ±0.28	0.86 ±0.26	NS	0.11 (NS)
Midwall flow, $ml \cdot min^{-1} \cdot g^{-1}$	0.54 ±0.19	0.54 ±0.15	0.62 ±0.16	0.62 ±0.17	NS	0.07 (NS)	0.65 ±0.18	0.66 ±0.24	0.82 ±0.24	0.80 ±0.32	NS	0.02 (NS)
Subepicardial flow, $ml \cdot min^{-1} \cdot g^{-1}$	0.54 ±0.16	0.50 ±0.21	0.66 ±0.24	0.67 ±0.24	NS	0.01 (NS)	0.60 ±0.12	0.58 ±0.21	0.74 ±0.23	0.75 ±0.23	NS	0.03 (NS)
Ratio	0.76 ±0.28	0.86 ±0.17	0.76 ±0.24	0.68 ±0.10	NS	0.22 (NS)	1.10 ±0.13	1.16 ±0.11	1.16 ±0.10	1.15 ±0.09	NS	0.47 (<0.05)
Total flow, $ml \cdot min^{-1} \cdot g^{-1}$	0.51 ±0.16	0.49 ±0.17	0.60 ±0.19	0.60 ±0.19	NS	0.07 (NS)	0.63 ±0.13	0.64 ±0.23	0.79 ±0.24	0.80 ±0.27	NS	0.01 (NS)
	LAD Distribution						Non-LAD Distribution					
	Low pressure control	+20	+40	+60	ANOVA	Correlation (probability)	Low pressure control	+20	+40	+60	ANOVA	Correlation (probability)
Subendocardial flow, $ml \cdot min^{-1} \cdot g^{-1}$	0.58 ±0.17	0.53 ±0.15	0.45 ±0.21	0.41 ±0.20	NS	−0.45 (<0.05)	0.84 ±0.11	1.02 ±0.23	1.00 ±0.19	0.97 ±0.20	NS	0.45 (<0.05)
Midwall flow, $ml \cdot min^{-1} \cdot g^{-1}$	0.61 ±0.10	0.60 ±0.12	0.53 ±0.26	0.53 ±0.25	NS	−0.29 (NS)	0.85 ±0.20	0.98 ±0.25	1.02 ±0.19	0.97 ±0.19	NS	0.58 (<0.01)
Subepicardial flow, $ml \cdot min^{-1} \cdot g^{-1}$	0.59 ±0.13	0.68 ±0.10	0.59 ±0.13	0.62 ±0.10	NS	0.10 (NS)	0.65 ±0.11	0.81 ±0.17	0.81 ±0.14	0.78 ±0.14	<0.001	0.58 (<0.01)
Ratio	0.99 ±0.27	0.77 ±0.21	0.73 ±0.29	0.64 ±0.23	NS	−0.63 (<0.01)	1.31 ±0.09	1.25 ±0.11	1.24 ±0.18	1.26 ±0.30	NS	0.10 (NS)
Total flow, $ml \cdot min^{-1} \cdot g^{-1}$	0.59 ±0.12	0.63 ±0.09	0.54 ±0.18	0.54 ±0.15	NS	−0.24 (NS)	0.75 ±0.13	0.92 ±0.20	0.92 ±0.16	0.88 ±0.15	<0.01	0.57 (<0.01)

Values are means ± SD; $n = 5$. All determinations were made during low LAD pressure. +10, +20, etc., increments in heart rate above the paced control; ratio, subendocardial-to-subepicardial flow ratio. ANOVA, analysis of variance. See Table 1 for abbreviations. Correlation coefficients (r) were calculated from the regression of all flow data points on heart rate.

still present at high heart rates, as well. Thus *protocol 3* was performed at three rate increases with and without intracoronary adenosine. Table 3 summarizes the results.

Heart rate and LAD pressure. The changes in heart rate were similar to the other two protocols. The average spontaneous rate of 93 beats/min was captured with the paced rate of 100; the average maximum produced in this protocol was 164 beats/min. The mean LAD pressure that produced an 18% decrement in systolic wall thickening was 44 mmHg at the control-paced rate.

End-diastolic and mean LAD flow. Changes at the control-paced rate at low pressure were as seen in *protocol 2*. When intracoronary adenosine was added at each of the four rates (control paced, +20, +40, +60) marked increases in end-diastolic flow occurred ($P < 0.0005$). Mean flow values demonstrated similar changes. At the control-paced rate at low pressure, adenosine increased the mean flow from 30.2 to 78.2 and at 60 beats/min faster rate, 27.6 changed to 67 ml/min. Thus, despite the encroachment on diastolic time by tachycardia, marked

TABLE 3. *Results from pacing-induced tachycardia during low LAD pressure, before and during intracoronary adenosine*

	Control	Control Pace	Low Pressure Pace	Low Pressure Pace + Adeno	Δ Heart Rate, beats/min						ANOVA
					+20	+20 (Adeno)	+40	+40 (Adeno)	+60	+60 (Adeno)	
Heart rate, beats/min	93.2 ±18.8	100.2 ±12.5	102.0 ±9.0	102.6 ±9.8	120.0 ±12.2	120.0 ±12.2	142.0 ±8.4	142.0 ±6.4	164.4 ±8.9	164.4 ±8.9	<0.0005
Mean LAD pressure, mmHg	82.0 ±10.8	83.8 ±11.9	44.0 ±5.9	43.6 ±4.8	44.4 ±4.9	43.6 ±4.7	44.0 ±4.7	42.6 ±5.5	42.4 ±4.4	43.4 ±5.0	<0.0005
End-diastolic LAD pressure, mmHg	71.8 ±9.3	73.5 ±6.6	25.8 ±2.1	26.3 ±2.2	28.3 ±4.8	28.0 ±3.9	34.5 ±6.2	31.0 ±4.4	35.8 ±5.3	36.8 ±5.0	<0.0005
Mean LAD flow, ml/min	37.8 ±9.5	37.4 ±8.7	30.2 ±8.4	78.2 ±30.0	28.0 ±7.6	54.6 ±23.0	27.6 ±7.4	62.0 ±23.4	27.6 ±5.5	67.0 ±29.7	<0.0005
End-diastolic LAD flow, ml/min	53.6 ±12.3	63.0 ±15.3	31.2 ±8.0	85.4 ±32.9	26.6 ±8.7	57.4 ±29.3	29.4 ±9.9	69.6 ±32.0	29.4 ±7.0	70.8 ±37.4	<0.0005
Systolic wall thickening, %	34.2 ±17.5	31.2 ±15.7	25.8 ±13.5	26.8 ±12.8	14.4 ±16.0	18.4 ±9.8	13.4 ±3.8	14.6 ±5.7	6.4 ±6.8	9.0 ±3.9	<0.0005
$M\dot{V}O_2$, ml/min	3.95 ±1.00	3.93 ±0.87	3.47 ±0.87	3.80 ±1.01	3.40 ±1.16	3.58 ±1.22	3.42 ±1.03	3.78 ±1.19	3.32 ±0.51	4.60 ±1.16	<0.05
O_2 extraction, %	75.6 ±12.3	77.2 ±9.8	84.4 ±6.9	39.2 ±13.1	87.4 ±7.8	48.0 ±9.4	86.8 ±4.1	44.0 ±8.3	86.6 ±5.7	53.2 ±17.0	<0.0005
LAD subendocardial flow, $ml \cdot min^{-1} \cdot g^{-1}$					0.57 ±0.12	0.64 ±0.09			0.48 ±0.16	0.50 ±0.10	<0.05
LAD midwall flow, $ml \cdot min^{-1} \cdot g^{-1}$					0.63 ±0.13	0.88 ±0.07			0.59 ±0.17	0.77 ±0.17	<0.05
LAD subepicardial flow, $ml \cdot min^{-1} \cdot g^{-1}$					0.61 ±0.14	1.28 ±0.23			0.74 ±0.31	1.66 ±0.44	<0.001
Ratio					0.95 ±0.22	0.51 ±0.14			0.58 ±0.13	0.31 ±0.06	<0.0005
Total flow, $ml \cdot min^{-1} \cdot g^{-1}$					0.58 ±0.14	0.97 ±0.17			0.61 ±0.25	1.07 ±0.21	<0.0005

Values are means ± SD. At each rate during low LAD pressure, intracoronary adenosine (adeno) was added. See Table 1 for abbreviations. ANOVA, analysis of variance.

flow reserve was easily demonstrable.

Myocardial blood flow. The results are shown in Table 3 and Fig. 4. Total transmural LAD flow rose each of the times adenosine was added (+20, and +60 beats/min, $P < 0.01$ by range testing). The increments were 67 and 75%, somewhat less than seen by flowmeter. By range testing, the greatest adenosine-induced changes were in the subepicardial layer, wherein flow more than doubled with the addition of adenosine at the two paced rates. The actual subendocardial flow changed little with adenosine. The inner-to-outer ratio fell markedly because of the subepicardial increase. Flow to the subepicardium of the LAD region with adenosine exceeded flow to the subepicardium of the non-LAD region, which was not exposed to adenosine or low pressure.

Left ventricular wall function. There was a progressive fall in systolic wall thickening produced by the tachycardia. Addition of intracoronary adenosine and the attendant increases in coronary blood flow did not alter the depressed function during the 5–10 min over which measurements were made. None of the changes with addition of adenosine was significant compared with its own control.

Oxygen and lactate data. A striking change was the decrease in oxygen extraction from arterial blood during adenosine infusion. Thus, despite persistent reductions in local systolic wall thickening, extraction fell from the low pressure control value of 84–39% and from 87 to 53% at the paced rate of 60 beats/min increase. $M\dot{V}O_2$ tended to be higher when adenosine was added; in each case the adenosine value was higher than the value before it. The increases at the four low pressure heart rates were 10, 5, 11, and 39%. Only the last change, at an increment at 60 beats/min was significant ($P < 0.05$).

Significant improvement in anaerobic metabolism was not seen in the lactate results. The statistically meaningful changes were between the normal pressure data (first two of the experimental periods) and the low pressure data, (the remaining part of the protocol). There was a trend toward worsening of lactate metabolism with increased heart rate, but the variations were great and lactate extraction was noted during one ischemic period.

DISCUSSION

The primary purpose of this study was to determine, during myocardial ischemia, whether increases in coronary blood flow could be stimulated by increased heart rate, thus using flow reserve known to be present in the coronary circulation. We recognized that such changes in mean or late diastolic flow would be the result of complex interactions among coronary pressure, diastolic and systolic time, coronary vasomotor tone, and changes in demand related to changes in mechanical function.

We first demonstrated increased LAD flow and $M\dot{V}O_2$ with pacing at normal pressure, which were expected findings (2, 3, 5, 28, 33). We then tested the response of the system to tachycardia at a LAD pressure that had been reduced to the point at which a modest decrement in systolic wall function occurred. The average decrease in coronary flow and systolic function corresponded to

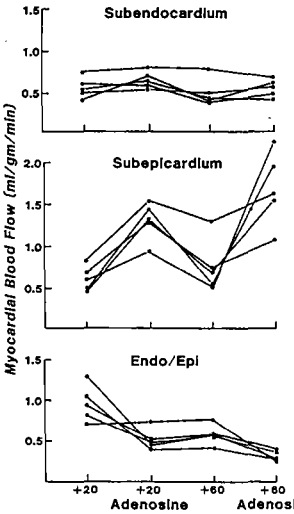


FIG. 4. Results of microsphere determinations of myocardial blood flow during two levels of pacing-induced tachycardia, at low left anterior descending artery (LAD) pressure, before and during intracoronary adenosine infusion. Data points from each animal are shown. Marked increases in subepicardial flow are produced by adenosine, with little change in subendocardial flow, resulting in a decline in the subendocardial-to-subepicardial ratio.

the same degree as observed by others (12, 30). Tachycardia produced further, progressive worsening of left ventricular function in the zone perfused by the LAD, but flow did not increase significantly whether measured at end diastole or as mean flow (flowmeter and microspheres). Accordingly, $M\dot{V}O_2$ was also a fixed quantity, given the already high degree of oxygen extraction with low pressure perfusion.

Two adjustments were associated with the constant $M\dot{V}O_2$. First, left ventricular systolic wall thickening decreased progressively in the ischemic segment, reflecting less oxygen usage per beat. Second, left ventricular systolic pressure decreased with pacing at low pressure, perhaps from failure of the ischemic segment to contribute adequately to global left ventricular function. The lactate extraction ratio and values for consumption to extraction clearly showed a switch to an anaerobic state with low LAD pressure, with and without the additional increments in heart rate by pacing. The problem worsened as rate was accelerated, as noted by others (31).

The adenosine experiments then showed the persistence of considerable flow reserve, not used despite the increased demand due to tachycardia; dramatic reductions of oxygen extraction to levels of less than half of the preadenosine values occurred with adenosine administration. Thus, when spontaneous regulatory mechanisms were allowed to prevail, net flow was not maximal during tachycardia as myocardial demand was forced upward.

We found that the inducible flow reserve was limited to the midwall and subepicardium at high heart rates, consistent with other studies of coronary stenosis with adenosine that have shown preferential flow increases to the outer layers (14). At a rate increase of 20 beats/min with low LAD pressure, the flow increase with adenosine in the subendocarcium averaged 12%. No change was seen at +60 beats/min. In the subepicardium the corresponding increases were 110 and 124%, and the midwall increases were 40 and 31%. Thus there is a gradient of flow reserve across the left ventricular wall during increased metabolic demand at low pressure, as suggested by Grattan et al. and by Warltier et al. (15, 32). The subendocardium has greater need, yet less reserve. The subendocardial reserve previously demonstrated at the spontaneous heart rate is apparently exhausted as rate is increased, even to the level of our control-paced periods.

Previous efforts to evaluate the response of coronary flow to tachycardia during ischemia have provided variable results. Studies in humans with angina pectoris demonstrated that coronary sinus flow increased with accelerating heart rate, up to the point of coronary insufficiency (34). At first, this might suggest vasodilation in low pressure regions of the circuit. However, downstream coronary pressures are not available in such studies and coronary sinus flow may represent a mixture of ischemic and nonischemic segments. In some animal experiments, it has been concluded that tachycardia in the presence of coronary stenosis results in a redistribution of flow from subendocardial to subepicardial layers, and some have observed an absolute decrease in flow to the inner region (22, 23). A decrease in total, transmural flow has also been observed (29).

Recently, Grover and Weiss reported a 53% increase in $M\dot{V}O_2$ in a region served by a stenotic coronary artery with pacing (16). This is quite different from our results. Differences between their report, others, and ours could be explained by several factors. In many of the prior studies, distal coronary pressure was not measured and not controlled as an independent variable. Thus coronary resistance is difficult to estimate. In our experiments, the low mean LAD pressure was controlled, as was heart rate, with flow as the test variable. In addition, most prior studies have been in dogs, which have substantial epicardial collaterals capable of delivering blood to an ischemic zone. The pig, in contrast, has virtually no exchange between the major coronary branches (13). Thus flow could increase, at a given low pressure, via collaterals in the dog but not in the pig. Alternatively, the pig and dog coronary circulations could be intrinsically different in their response to ischemia.

It is of further interest that the increased myocardial blood flow produced by adenosine did not significantly improve the depressed mechanical function of the ischemic LV wall segment, although there was a modest trend upward each of the four times adenosine was added. This is consistent with our earlier observations (24). One might argue that the persistence of depressed function is due to inadequate flow increase to the subendocardium, i.e., the increased flow is not sufficient to make a difference. There are other tenable explanations, however, as

the increment in flow to much of the LV wall was not trivial, and oxygen extraction from LAD blood fell remarkably with adenosine-induced vasodilation. The possibility remains that depression of contraction is due to metabolic inhibitors of function, such as tissue lactate levels resulting from anaerobic metabolism, as proposed by Neely, requiring more time to be washed out (21).

The progressive fall in systolic wall thickening with increasing heart rate during low LAD pressure demonstrates that the response of the heart to ischemia is graded. With a constant LAD flow, tachycardia disturbs the relationship between supply and demand in a progressive manner. Each heart-rate step upward was accompanied by a further decrement in function. There was not a threshold value for flow or demand at which function was suddenly markedly depressed. This is reasonable, since supply was constant, demand increasing, and supply of oxygen per beat to support function must fall. Still not explained are how these adjustments are mediated, why the full reserve of vasodilation is not employed, nor why there is such a disparity between subendocardial and subepicardial flow reserve.

There is considerable recent evidence of the importance of α-adrenergic tone in the coronary bed during exercise, hypoperfusion, and tachycardia (2, 7, 17, 19, 20). Some studies found that blockade of such effect results in increased coronary flow and higher $M\dot{V}O_2$ (17, 19). Nathan and Feigl (20) concluded, however, that adrenergically mediated tone is necessary to insure adequate subendocardial perfusion, and to avoid a steal of blood to the outer layers. Our study was not designed to address these issues, but we can say that with tachycardia, low LAD pressure, and then addition of maximal pharmacological vasodilation, subendocardial flow did not fall. The decrease in inner-to-outer flow ratio that was found was due to the large increase in subepicardial flow.

Conclusions

Our experiments have shown that, at the low coronary pressures and range of heart rates studied, myocardial blood flow and oxygen consumption are remarkably constant. As a consequence of the increased metabolic demand produced by tachycardia, there is a progressive fall in local ventricular systolic wall function with stepwise increases in rate. This occurs despite substantial coronary flow reserve throughout the spectrum of heart rates tested. The observations were made in a vascular bed that does not have significant collateral interchange with other coronary vessels. The additional flow induced by adenosine was largely distributed to the subepicardium, however, and metabolic need was presumably greater in the subendocardium. The subendocardial flow reserve previously demonstrated at spontaneous heart rate by several laboratories appears to be quickly exhausted when demand is increased.

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Chapter 6

Relation of lactate production to postischaemic reduction in function and myocardial oxygen consumption after partial coronary occlusion in swine.

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Relation of lactate production to postischaemic reduction in function and myocardial oxygen consumption after partial coronary occlusion in swine

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SUMMARY Postischaemic myocardial dysfunction (stunning) induced by partial occlusion of the left anterior descending coronary artery and its relation to lactate production during reperfusion were studied in nine swine. A 40% reduction in regional left ventricular wall thickening, as measured by ultrasonic crystals, was prospectively defined as stunning. A perfusion pressure of 20 mmHg was maintained with a hydraulic occluder for each ischaemic period and was monitored by a distal arterial catheter. To achieve a 40% reduction in function, four animals required three ischaemic periods (mean ischaemic flow reduction 73%), four two (86% flow reduction), and one one (93% flow reduction). At 25 min of reperfusion transmural flow was slightly reduced from 0.67 $ml \cdot g^{-1} \cdot min^{-1}$ at control to 0.58 $ml \cdot g^{-1} \cdot min^{-1}$ ($p<0.05$), whereas regional flow endocardial to epicardial flow ratio was unchanged. At 60 min reperfusion, percentage systolic wall thickening was reduced to 25% from a control of 39% ($p<0.01$) and parallel reductions in regional myocardial oxygen consumption from 4.3 $ml \cdot min^{-1}$ to 2.7 $ml \cdot min^{-1}$ occurred ($p<0.01$). Lactate extraction was depressed at 15 min reperfusion (–4.0% compared with control +18.0% ($p<0.05$)) but returned to control values by 30 min. It is concluded that postischaemic myocardial dysfunction (stunning) can be induced by partial coronary occlusions and that the extent of dysfunction depends on the degree of flow reduction. The reductions in myocardial oxygen consumption parallel those of wall thickening during reperfusion after stunning. Finally, lactate production occurs during early reperfusion but does not persist with the postischaemic reductions in function and myocardial oxygen consumption.

The recovery of myocardial function after a brief episode of ischaemia, in the absence of infarction, can be delayed.[1] This transient reversible dysfunction (stunned myocardium) has been shown to occur despite the return of normal endocardial blood flow after ischaemia.[2–7] Current hypotheses to explain this phenomenon include depletion of energy substrate,[2 8] generation of oxygen free radicals during reperfusion,[4 9] an attenuated myocardial response to the sympathetic nervous system,[10] and accumulation of cellular calcium or glycolytic byproducts.[11–15] Lactate production during ischaemia may be a major cause of myocardial dysfunction,[11] and its presence in tissue during reperfusion[16] may play a role in postischaemic dysfunction.

The animal models used to study postischaemic dysfunction thus far have used complete occlusion of an artery during ischaemia. However, spasm and thrombosis leading to brief partial coronary occlusion are common clinical findings and the subsequent myocardial response is equally important.

Therefore, we wished to determine (*a*) if severe reductions in flow resulted in postischaemic dysfunction and, if so, (*b*) the relation between

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changes in lactate production during reperfusion and myocardial dysfunction.

Material and methods

Ten swine (weight 35-54 kg) of either sex were premedicated with xylazine (2 $mg \cdot kg^{-1}$ im) and ketamine (12 $mg \cdot kg^{-1}$ im) followed by a bolus of morphine sulphate (2 $mg \cdot kg^{-1}$ iv) and ketamine (10 $mg \cdot kg^{-1}$ iv). This was followed by a continuous intravenous infusion of morphine sulphate (1 $mg \cdot kg^{-1} \cdot h^{-1}$) and ketamine (10 $mg \cdot kg^{-1} \cdot h^{-1}$) that was used throughout the duration of the experiment. The animals were intubated and ventilated with oxygen enriched air using a piston respiratory pump. Tidal volume and respiratory rate were regulated in each animal to maintain physiological arterial pH (7.37-7.45), Pao_2 (>100 mmHg (13.3 kPa)), and $Paco_2$ (35-45 mmHg (4.7-6.0 kPa). Once adequate anaesthesia was administered, tubocurarine (2 ml iv) was given to facilitate use of electrocautery. The femoral artery and vein were exposed through a cutdown and catheters were placed in each. The heart was exposed via a midline sternotomy and suspended in a pericardial cradle. A catheter was placed in the left atrium for injecting microspheres and one in the apex of the left ventricle for recording pressure and its first derivative. The left anterior descending artery (LAD) was exposed and instrumented using an electromagnetic flow probe and hydraulic occluder proximally, and a small silastic catheter (internal diameter 0.3 mm, external diameter 0.6 mm) was positioned in the mid-portion of the vessel to record pressure. Our previous experience with this catheter size has shown no effect in altering coronary distribution, flow reserve, or resting lactate production.[17 18] Another small silastic catheter was inserted into the great cardiac vein draining the ischaemic zone for determining regional venous lactate and oxygen saturation. Ventricular function was measured by epicardial and endocardial ultrasonic crystals and was expressed as percentage systolic fractional thickening.[17] The correct position of the crystals was confirmed at the conclusion of each study. Measurements of oxygen content were made on a LEX O_2 CON analyser and of plasma lactate by an electrode technique.[19]

Regional myocardial blood flow was measured by injecting 9-11 μm microspheres labelled with a gamma emitting radionuclide (^{141}Ce, ^{51}Cr, ^{103}Ru, or ^{95}Nb) through the left atrial catheter. Reference samples of arterial blood were drawn over 2 min during each injection, and regional blood flow per gram of tissue calculated as reported previously.[17] At the conclusion of the experiment, Evans blue and acid red dyes were injected into the right and LAD arteries respectively. Flow was then determined in the endocardial, mid and epicardial regions of the LAD and non-LAD arteries. After surgical preparation was complete heparin (10 000 units iv) was given to prevent clotting of coronary catheters.

PROTOCOL

Five minutes of complete arterial occlusion have been shown to induce a 40% reduction in wall thickening with up to 30 min of reperfusion.[5] Therefore, we prospectively chose a 40% reduction in postischaemic function as our definition of stunning with up to three ischaemic periods to achieve this. The hydraulic occluder was inflated to reduce distal coronary pressure to 20 mmHg for 5 min. If postischaemic function was not reduced by 40% after 30 min of reperfusion the ischaemic period was repeated. A pressure of 20 mmHg was chosen since this is about 10-13 mmHg above the pressure at which flow stops in swine,[18] allowing some flow to occur. Our previous work has also shown this pressure drop to be severe enough to induce some level of dysfunction.[20] During final reperfusion, simultaneous arterial and coronary venous samples were obtained at 3, 15, 30, 45, and 60 min for measuring lactate concentration and oxygen content. Calculations included: MVO_2 = (total LAD flow by flow probe) × (arterial minus venous oxygen content); % lactate extraction = [(arterial lactate minus venous lactate)/arterial lactate] × 100; % wall thickening = [(end systolic thickness minus end diastolic thickness)/end diastolic thickness] × 100. Regional blood flow by microspheres was determined before ischaemia (control) and 25 min after the final ischaemic period.

STATISTICS

All data are expressed as mean(SEM). Multiple comparisons were made by one way analysis of variance (ANOVA) with repeated measures and range testing by Tukey's procedure. Single comparisons were analysed by Student's *t* test.

Results

Ten animals were studied initially. Ventricular fibrillation occurred in three animals; of these, two were converted to sinus rhythm within 1 min with paddles placed on the chest wall. The third animal could not be converted promptly and is not included in the results.

HAEMODYNAMIC DATA

Table 1 shows mean heart rate, systolic blood pressure, and double product (systolic blood pressure multiplied by heart rate) at control and 60 min reperfusion after the final ischaemic period. A

TABLE 1 *Haemodynamic data. Values are mean(SEM)*

		Reperfusion time (min)							
	Control	*3*	*6*	*9*	*12*	*15*	*30*	*45*	*60*
Heart rate	79(4)	86(4)*	86(4)*	87(4)*	87(4)*	86(4)*	87(4)*	89(4)*	89(4)*
Systolic blood pressure	102(3)	100(7)	97(8)	93(5)	96(3)	94(3)	92(3)	93(3)	89(3)
Double product†	8082(380)	8504(588)	8292(655)	8031(410)	8238(350)	8037(323)	7913(209)	8208(434)	7937(335)

†Heart rate times systolic blood pressure.
*$p<0.05$ compared with control.

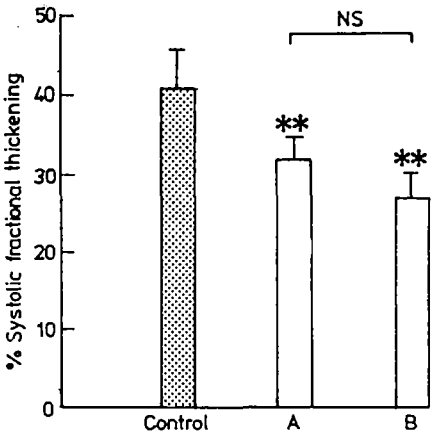

FIG 1 *Percentage systolic fractional thickening at control and 30 min after two consecutive periods of low pressure (A and B). Values are mean(SEM); n=8. **$p<0.01$ vs control.*

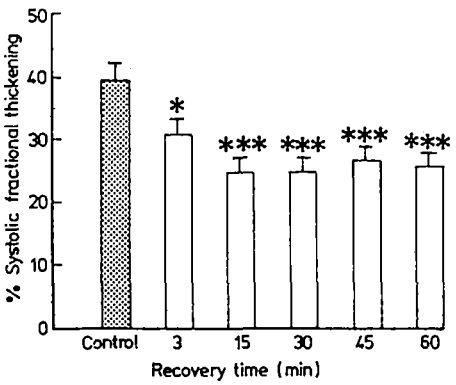

FIG 2 *Percentage systolic fractional thickening at control and after stunning (recovery time). Values are mean(SEM); n=7. *$p<0.05$, ***$p<0.001$ vs control.*

significant rise in heart rate and lowering of systolic blood pressure were noted over the course of the experiments despite attention to the level of anaesthesia and fluid administration. The double product, however, remained unchanged over the same period, and therefore myocardial oxygen consumption remained relatively constant.

POSTISCHAEMIC DYSFUNCTION

All nine animals showed at least a 40% reduction in wall thickening with up to three ischaemic periods.

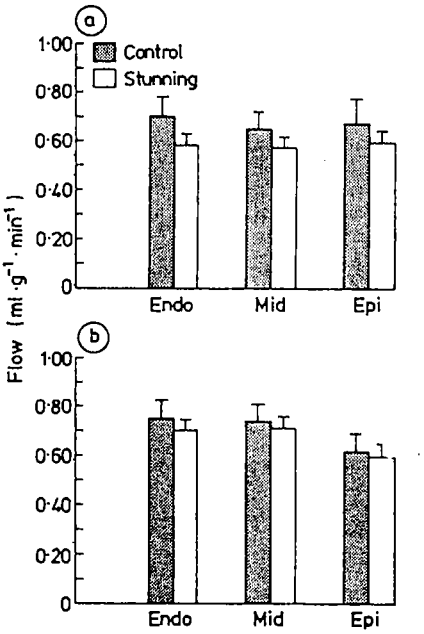

FIG 3 *Regional blood flow at control and 25 minutes after stunning in (a) LAD and (b) non-LAD areas. Endo=endocardial; mid=mid endocardial; Epi=epicardial.*

The degree of dysfunction induced was dependent on the amount of flow reduction during the ischaemic period. Four animals required three ischaemic periods to achieve a 40% functional decrement and their flow was reduced to a mean of 73% at 20 mmHg. The four swine requiring two periods had an 86% mean reduction in flow at the same pressure and the one animal receiving one ischaemic period a 93% flow reduction during ischaemia.

Figure 1 shows the reduction in percentage wall thickening in those eight animals that received at least two ischaemic periods. As shown, each ischaemic period induced a subsequent loss of function during reperfusion which was cumulative. However, the degree of functional reduction was greatest after the initial ischaemic period.

TABLE 2 *Myocardial flow ($ml \cdot g^{-1} \cdot min^{-1}$) at control and 25 min into reperfusion. Values are mean(SEM); n=9*

Myocardial flow	*Control*	*25 min reperfusion*
LAD:		
Transmural	0.67(0.08)	0.58(0.05)*
Endocardial:epicardial ratio	1.07(0.08)	1.03(0.06)
Non LAD:		
Transmural	0.70(0.07)	0.67(0.05)
Endocardial:epicardial ratio	1.16(0.03)	1.23(0.04)

*$p<0.05$ *vs* control.

Figure 2 shows the reductions in wall thickening during 60 min of reperfusion after the final ischaemic period compared with the control period. As shown, it is during the initial 15 min reperfusion period that the greatest reductions in function occurred. Dysfunction was sustained for at least 60 min into reperfusion.

REGIONAL BLOOD FLOW

Figure 3 and table 2 show the regional changes in flow in the LAD and non-LAD bed before and after stunning. Total transmural flow in the LAD region was reduced by only 8% ($p<0.05$) at 25 min into reperfusion compared with control. In the non-LAD bed there was a slight but insignificant reduction in transmural flow over the same period. The ratio of endocardial to epicardial flow, however, remained unchanged in both the LAD and non-LAD areas 25 min into reperfusion compared with the control period (table 2). This normalisation of flow distribution occurred at a time when function remained depressed.

MYOCARDIAL OXYGEN CONSUMPTION AND LACTATE EXTRACTION

Complete data were not available in two of the animals during reperfusion and the following results pertain to the remaining seven. Figure 4 shows the relation of myocardial oxygen consumption and percentage lactate extraction at control and 60 min into reperfusion. Commensurate with the reductions in

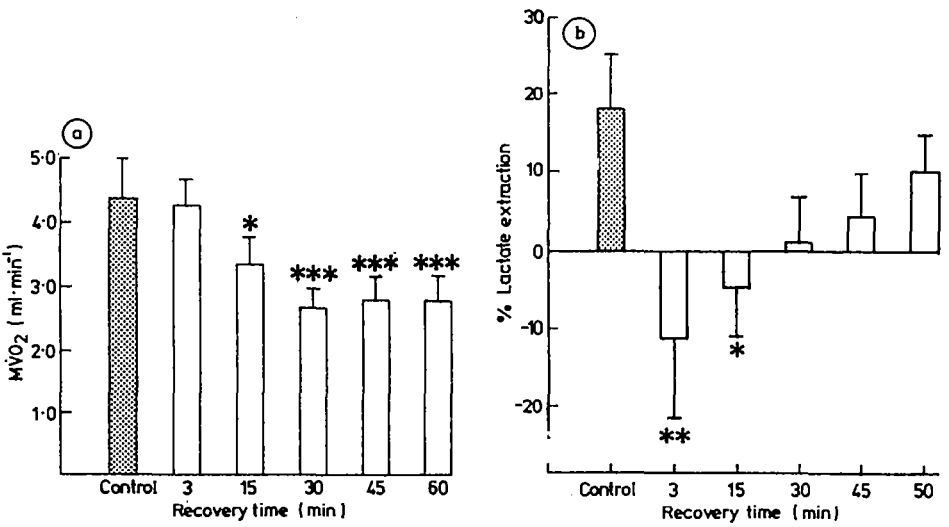

FIG 4 *(a) Myocardial oxygen consumption (MVO_2) and (b) percentage lactate extraction at control and after stunning (recovery time). Values are mean(SEM); n=7. *$p<0.05$; **$p<0.01$; ***$p<0.001$ vs control.*

wall thickening is a parallel reduction in myocardial oxygen consumption over the same period that remains statistically lower than control at 60 min. Percentage lactate extraction, on the other hand, is significantly different from control with a net production of lactate at least 15 min into reperfusion. By 30 min, however, percentage lactate extraction has returned toward control values with overall net extraction at a time when function and oxygen consumption still remain depressed.

Discussion

The phenomenon of stunned myocardium refers to reversible dysfunction following total coronary artery occlusion unassociated with myocardial necrosis.[1-10 21] A unique aspect of our model was the use of a constant arterial pressure during ischaemia, which induced variable flow reductions in each animal. We have shown that incomplete coronary artery occlusion does induce postischaemic dysfunction to an extent that appears to be dependent on the degree of baseline flow reduction. The most severe reduction in wall thickening occurred in the animal with a greater than 90% flow reduction during ischaemia. This 5 min ischaemic period achieved a 40% reduction in function during a 30 min reperfusion period, which is equivalent to the functional reductions observed with 5 min of complete arterial occlusion.[5] Those animals that had smaller baseline flow reductions at the ischaemic pressure of 20 mm mmHg had less postischaemic dysfunction. Therefore, the extent of postischaemic dysfunction is dependent on both the time of ischaemia[5 21] and the degree of baseline flow reduction.

We have also confirmed that the reductions in function after multiple incomplete coronary occlusions are cumulative, with the greatest decrement following the initial ischaemic episode. This has been shown in open chest dogs after up to 16 five minute complete arterial occlusions.[6 22] This is contrary to Lange's finding of no cumulative deterioration of regional function seen with up to three consecutive arterial occlusions.[5] Weiner and colleagues also showed no cumulative functional impairment after three successive 20 min occlusions in dogs.[23] The major discrepancy appears to be in the reflow period allowed between occlusions. Those studies showing a cumulative effect have shorter reflow periods, whereas others without such an effect use prolonged reperfusion periods (of more than 35 min).

Persistent coronary flow redistribution after ischaemia does not apper to be the primary mechanism in maintaining the stunned myocardium. We have shown that postischaemic dysfunction following partial arterial occlusions is not associated with regional flow disturbances as evidenced by the return of endocardial and epicardial flow towards control values (table 2). Heyndrickx and colleagues induced transient myocardial dysfunction that lasted for at least 24 hours after a 15 min arterial occlusion with persistent endocardial flow reductions at least three hours into reperfusion.[21] Subsequent studies, however, have shown a return of regional flow after a brief arterial occlusion at a time when myocardial dysfunction is sustained.[2-10] This dissociation of flow and functional recovery has led to other theories of stunning such as depletion of high energy phosphates,[2 8] accumulation of cellular calcium or glycolytic byproducts,[11-15] and generation of free radicals during reperfusion.[4 9] The transmural flow reduction was slight but significant at 25 min into reperfusion and is consistent with other studies.[6 7] This reduction could be explained by the observed postischaemic reductions in myocardial oxygen consumption and subsequent demand.

If limited flow during reperfusion was the cause of persistent reductions in function and myocardial oxygen consumption anaerobic glycolysis and lactate production would continue. We therefore compared the changes in percentage lactate extraction during reflow with changes in function and myocardial oxygen consumption. Although considerable variability in resting control values for percentage lactate extraction does exist, the myocardium under normal conditions should extract lactate.[24] We have shown that 20 min into reperfusion, lactate extraction returns to control values at a time when wall thickening and myocardial oxygen consumption remains depressed. Smith studied lactate extraction in open chest dogs after a 10 min arterial occlusion and found an insignificant reduction after 20 min of reperfusion.[7] In a similar model in pigs, after coronary occlusion, lactate extraction had not normalised by 5 min of reperfusion.[25] It is clear from our work that persistent anaerobic glycolysis was not present with persistent reduction in function with the stunned myocardium.

It is interesting that at least 15 min into reperfusion lactate extraction in the stunned myocardium remains significantly depressed. Taegtmeyer and colleagues have shown that after 5 min of ischaemia in isolated rat hearts, tissue lactate concentrations remain raised until 40 min into reperfusion.[16] this would imply that our findings represent more than just delayed lactate washout. The early postischaemic reflow period may demonstrate accentuated anaerobic glycolysis at a time when flow has returned to normal. Some support of this has been shown with positron emission tomography in patients with postischaemic exercise tests. By use of radiolabelled fluoro-2-deoxyglucose and rubidium-82, studies have shown increased

uptake of exogenous glucose in the distribution of postischaemic myocardial segments 15 min after exercise.[24] This would suggest that the stunned myocardium preferentially metabolises glucose early in reperfusion, despite adequate oxygen delivery.

POTENTIAL LIMITATIONS OF STUDY

Lactate production has been a reliable index for the presence of anaerobic glycolysis,[26] and percentage lactate extraction has been shown to correlate well with tissue gradients of lactate.[27] However, with severe ischaemia lactate production often declines owing to inhibition of glycolytic enzymes, failure of cellular release of lactate, and substrate depletion. This accounts for some of the problems with lactate concentrations as a reflection of degree of ischaemia during severe reductions in flow.[28] On the other hand, the reperfused myocardium is different from severely ischaemic myocardium where regeneration of anaerobic substrate occurs. Allison and colleagues have shown that by 20 minutes after a 20 min occlusion glycogen and glycolytic enzymes have returned to normal.[3]

Inherent in the definition of stunned myocardium is the absence of necrosis as the cause of dysfunction following occlusion. One study has shown that more than 14 consecutive 5 min arterial occlusions can lead to some necrosis.[29] Others have used several 5 min occlusions 10 minutes apart and have shown minimal irreversible change.[5 6 22] Moreover, none of the studies of postischaemic dysfunction cited have found necrosis as the potential cause of the phenomenon.[2-10 21 22] In our laboratory,[30] as in others,[31] substantial necrosis in tissue has not been found after up to 15 min of arterial occlusion. We therefore feel justified in assuming that our brief incomplete arterial occlusions were not associated with significant necrosis.

CONCLUSION

We have shown that postischaemic myocardial dysfunction (stunning) occurs when a coronary artery is subtotally occluded with the degree dependent on the extent of baseline flow reduction. Although cumulative, the initial postischaemic reductions are significantly greater than subsequent ones. The reduction in systolic wall thickening seen during reperfusion parallels the reduction in regional myocardial oxygen consumption, and both are sustained long after regional coronary flow has returned towards baseline. Finally, anaerobic glycolysis, as measured by percentage lactate extraction, appears to be enhanced during early reperfusion but returns to normal by 30 min before the return of function. It is therefore unlikely that the accumulation of lactate during reperfusion or the continuation of anaerobic glycolysis are associated with the persistence of postischaemic dysfunction.

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Chapter 7

Endothelial-dependent vasodilation following brief ischemia and reperfusion in anesthetized swine.

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Abstract

Study objective- The aim was to compare the responses of intracoronary infusions of ATP, an endothelial-dependent vasodilator with adenosine following brief ischemia (10 minutes) and reperfusion in a model of myocardial stunning.

Design- In group 1 (n=6), coronary blood blood flow and endocardial (endo) and epicardial (epi) % segment length shortening (SS) were measured in the distribution of the left anterior descending artery before and during maximal intracoronary infusions of either adenosine or ATP (20 ug/kg/min). Measurements were obtained pre- and post-myocardial stunning both at control HR and during atrial pacing (150 beats per minute). In group 2 (n=6), myocardial blood flows by microspheres and arterial-venous lactate and oxygen differences were determined following the same ischemia-reperfusion protocol to characterize transmural changes in blood flow and metabolism in this model of stunning.

Experimental material- The experiments were performed in 12 anesthetized swine.

Measurements and main results- In group 1, baseline endo and epi SS were 16$\pm$3% and 14$\pm$6% and following reperfusion were reduced to 10$\pm$4% and 8$\pm$6% respectively ($p<.05$). Prior to stunning, minimal coronary resistances during adenosine and ATP were 0.81$\pm$.40 and 0.76$\pm$.25 mm Hg-min/ml respectively and following reperfusion were 0.86$\pm$.31 (NS) and 0.85$\pm$.23 (NS) mm Hg-min/ml respectively. Infusion of either vasodilator enhanced function by 30% following reperfusion whereas no such effect was observed prior to ischemia. In group 2, no maldistribution of blood flow was observed following the same ischemia-reperfusion protocol to account for this vasodilator enhancement in function. % lactate extraction values were 29$\pm$11% and 25$\pm$14% at pre-ischemic control and paced heart rates respectively and following reperfusion were lowered to 0$\pm$12% without pacing ($p<.05$) and -1$\pm$34% during pacing ($p<.05$).

Conclusion- Brief ischemia and reperfusion in swine induces myocardial stunning without altering the vasodilator responses of either ATP, an endothelial-dependent vasodilator or adenosine. Recruitment in post-ischemic shortening was observed during infusions of both vasodilators at a time when maldistribution of flow by microspheres was not observed. Possible mechanisms include either enhanced washout of lactate from the reperfused myocardium or greater utilization of substrates during higher blood flows.

Introduction

It has now become widely accepted that regional myocardial function can be reversibly depressed following brief periods of ischemia and reperfusion (1). Although sustained abnormalities in transmural blood flow as measured by radioactive microspheres are not the cause of this entity called "myocardial stunning", evidence is accumulating that coronary perfusion during the recovery phase is important. Several groups for instance, have demonstrated that infusion of vasodilators such as adenosine can improve post-ischemic function during late reperfusion (2-7). The mechanism of this vasodilator-enhancement in function may be a result of either improved oxygen delivery to heterogeneously reperfused regions or a washout of certain metabolic byproducts from the ischemic period (8,9). The coronary vasculature in the distribution of regionally stunned myocardium may also be altered both structurally and functionally. One hour of occlusion and reperfusion in dogs for instance, induces morphological changes in the endothelial cells including focal injury and partial detachment from the underlying cell matrix (10). The same degree of ischemia and reperfusion has been shown to alter the vasodilator response of several endothelial-dependent vasodilators including acetylcholine, bradykinin, arachidonic acid and ATP (11-14).

These findings, often referred to as vascular "stunning", have been induced after prolonged ischemic times, which may also cause more permanent alterations. Such irreversible changes as necrosis and "no-reflow" have been documented after 20 minutes of coronary flow reduction (15). In this study, we wished to determine whether myocardial and vascular stunning could be dissociated following a shorter period of ischemia. Vascular function prior to and following ischemia-reperfusion was tested by comparing the vasodilator responses of intracoronary ATP, an endothelial-dependent vasodilator, with adenosine (13,16). Because the effect of increased work load or shear stress could enhance any differences in endothelial-dependent mechanisms, the agents were infused both at baseline heart rates and during atrial pacing. In a second group of animals, the same ischemia-reperfusion protocol was repeated to determine the effects on transmural coronary blood flow and regional arterial-venous oxygen and lactate differences.

Methods

GENERAL PREPARATION

Following an overnight fast, 12 cross-bred Landrace-Yorkshire pigs of either sex (25-39 kg) were sedated with intramuscular ketamine (20 mg/kg) and intravenous (iv) metomidate (5 mg/kg). Animals were intubated and connected to a respirator for intermittent positive pressure ventilation with a mixture of oxygen and nitrous oxide (1:2). Ventilator settings were adjusted during the experiments to maintain normal arterial pH (7.35-7.45), pCO2 (35-45 mm Hg) and pO2 (>150 mm Hg). The external jugular vein was cannulated with two 7F catheters for administration of anesthetics and saline. The anesthetic regimen consisted of a bolus of iv alpha chloralose (150 mg/kg) dissolved in boric acid solution and a continuous infusion of low dose sodium pentobarbitone (5 mg/kg/h). The femoral arteries were cannulated with 7F catheters and used for either aortic blood pressure measurements, arterial blood gas collections or reference sampling for microsphere determination. Rectal temperature was monitored throughout the experiment and maintained near 37 degrees C with external heating pads. Pancuronium bromide (4 mg) was given iv, and following a midline thoracotomy, the heart was suspended in a pericardial cradle. The left mammary vessels were ligated and the second left rib was removed for ease of further instrumentation. The left anterior descending coronary artery (LAD) was dissected free of its adventitia, and an electromagnetic flow probe (2.25 or 2.50 mm; Skalar, Delft, The Netherlands) and hydraulic occluder were placed proximally. Meticulous attention was paid to the positioning and stability of the flow probe and zero flows were checked regularly throughout the experimental protocol. Pacing leads were attached to the left atrial appendage and connected to a pacing stimulator.

SPECIFIC INSTRUMENTATION

Group 1 (n=6). The purpose of this group was to test the vasodilator responses to intracoronary adenosine and ATP (Boehringer; Mannheim, W. Germany), prior to and following myocardial stunning at two different heart rates (baseline and during atrial pacing of 150 beats/min). Immediately distal to the probe and occluder, a small cannula (0.8 mm-outer diameter) was inserted into the LAD for administration of the vasodilators. A microtipped catheter (7F Millar) was advanced into the left ventricle (LV) via the left carotid artery and used to monitor LV pressure and its first derivative (LVdP/dt). To measure regional segment length shortening in the distribution of the LAD, pairs of ultrasonic crystals (Triton, Technology Inc., San Diego, CA, USA) were placed in the endocardial and epicardial layers, approximately 10-15 mm apart in each region. Systolic shortening in the two layers was calculated from the difference between lengths at end-diastole (time of onset of positive dP/dt) and end-systole (time of peak negative dP/dt) and expressed as a percent of end-diastolic length (17).

Group 2 (n=6). The purpose of this group was to determine transmural coronary blood flow at baseline heart rate and during atrial pacing (150 beats/min), before and after the same ischemic-reperfusion protocol as in group 1. In addition, arterial and LAD coronary venous samples were obtained for oxygen and lactate concentrations during each of the interventions. Instrumentation in this group was limited, with only a small cannula

inserted into the LAD coronary vein for sampling and a catheter inserted into the left atrium for administration of radio-labeled microspheres.

Studies were performed in accordance with the position of the American Heart Association on research animal use adopted November 11, 1984 and under the regulations of the Animal Care Committees of the VA Medical Center, Minneapolis MN, USA and Erasumus University, Rotterdam, The Netherlands.

EXPERIMENTAL PROTOCOL

Group 1. Following a 30 minute stabilization period, baseline measurements were taken of mean aortic and left ventricular pressures, coronary blood flow, LV dP/dt and endocardial and epicardial segment length changes. Maximal vasodilator responses for both adenosine and ATP were determined prior to each experiment and those doses used for the remainder of the protocol (20-40 ug/kg/min). The maximal dose of intracoronary adenosine was infused continuously and recordings obtained after 5 minutes of steady state. The infusion was discontinued and following a 5 minute washout period, the maximal dose of ATP was begun and measurements obtained after 5 minutes. The protocol was then repeated during atrial pacing at 150 beats per minute. 10 minutes of ischemia was then induced by adjusting the hydraulic occluder so that coronary blood flow was reduced by greater than 80% of the baseline value. We have previously found that this degree of partial coronary blood flow reduction induces myocardial stunning with less ventricular fibrillation compared with that of complete coronary occlusions (18). The heart was reperfused for 30 minutes, and recordings were repeated prior to and following intracoronary infusions of adenosine and ATP, without and during repeat pacing.

Group 2. In this protocol, transmural blood flow was determined at baseline heart rate and during atrial pacing, both before and after the same ischemia-reperfusion protocol as in group 1. Microspheres were injected prior to and during each pacing intervention as well as during the partial coronary occlusion. In addition, simultaneous arterial and LAD coronary venous samples were obtained for oxygen saturation and lactate concentrations during the same sampling periods.

REGIONAL MYOCARDIAL BLOOD FLOW

For each flow measurement in group 2, 1-2 million microspheres (15 microns) of either 141-Ce, 113-Sn, 103-Ru, 95-Nb, or 46-Sc (NEN-TRAC, New England Nuclear, Boston, Mass.) were injected into the left atrium. Reference arterial blood samples were withdrawn from the femoral artery catheter at a fixed rate of 10 ml/min, from 15 seconds prior to until 1 minute after microsphere injection. At the conclusion of the experiment, the animal was sacrificed and the heart excised. The distribution of the post-ischemic myocardium was identified by injecting patent violet dye into the LAD coronary artery at the level of the occluder. Hearts were fixed in 10% formalin for 48 hours, and separated into LAD and nonLAD regions. Each was then divided into 3 equal layers (inner, mid, and outer) and placed in 1-2 gram samples. Myocardial and reference blood samples were counted in a multichannel analyzer (Gamma Counter-5000; Packard Instrument Inc. USA) and regional blood flows determined (19).

ARTERIOVENOUS OXYGEN AND LACTATE

In group 2 animals, oxygen saturation was determined from arterial and LAD cardiac venous samples by an OSM2 system (Radiometer; Copenhagen, Denmark). Blood for lactate determinations were collected in iced syringes containing heparin, and promptly centrifuged for later analysis by the enzymatic technique (20). Oxygen content was determined from the formula (1.34 x Hgb x % oxygen saturation). Oxygen consumption in the distribution of the reperfused myocardium was then calculated by the product of microsphere flow and the arterial-coronary venous oxygen difference. Percent lactate extraction was calculated by the arterial-coronary venous lactate difference divided by arterial lactate X 100.

STATISTICS

Results are expressed as arithmetic means $\pm$ standard deviations. Pre- and post-ischemia-reperfusion differences were tested for significance at the $p<.05$ level by analysis of variance with repeated measurements (Fisher's PLSD and F-test). In addition, microsphere flows during each intervention in group 2 were compared in the LAD and nonLAD regions by unpaired t-testing.

Results

SYSTEMIC HEMODYNAMICS (GROUP 1)

Table 1 summarizes the systemic hemodynamics during all interventions in group 1. Except for the increase in heart rate, there were no changes in any of the parameters following reperfusion compared with pre-ischemic values.

CORONARY HEMODYNAMICS AND REGIONAL FUNCTION (GROUP 1)

Coronary blood flows and endocardial and epicardial segment length changes are summarized in Table 2. Figure 1 shows a representative tracing in one animal. Following reperfusion (baseline-2), coronary resistances (mean arterial pressure/mean coronary blood flow) were higher compared with pre-ischemic values at both heart rates. Minimal resistances achieved with either adenosine or ATP however were unchanged compared with pre-ischemic values.

Following reperfusion, endocardial and epicardial segment length shortening was lower than pre-ischemic values at each heart rate. Figure 2 shows the effect of infusions of adenosine and ATP on (a) endocardial and (b) epicardial function prior to and following myocardial stunning at control heart rate. As shown, both vasodilators enhanced % segment length shortening in the two layers following reperfusion but had no effect prior to ischemia-reperfusion. The effect was the same at both heart rates. Because end-diastolic lengths and systolic pressures were similar during these interventions, it is unlikely that the functional changes were a result of changes in the loading conditions.

MYOCARDIAL BLOOD FLOW, OXYGEN AND LACTATE (GROUP 2)

Heart rate was 75 ± 20 beats per minute prior to ischemia and increased to 81 ± 29 ($p<.05$) following reperfusion. Mean arterial pressure decreased slightly but insignificantly from 87 ± 16 to 79 ± 16 mmHg during the same interval. During initial atrial pacing, mean

Table 1 : *Systemic Hemodynamics Pre-ischemia and 30 Minutes Post-reperfusion (Group 1)*

	Pre-ischemia			30 Minute Reperfusion		
Control HR	Baseline-1	Adenosine	ATP	Baseline-2	Adenosine	ATP
MAP	78 ± 17	75 ± 17	74 ± 21	78 ± 12	78 ± 13	71 ± 13
HR	102 ± 6	101 ± 8	100 ± 12	116 ± 8*	116 ± 9*	117 ± 13*
LVEDP	7 ± 1	7 ± 2	7 ± 3	7 ± 2	7 ± 2	6 ± 1
$LVdP/dt_{max}$	1579 ± 394	1481 ± 337	1434 ± 366	1392 ± 416	1430 ± 390	1480 ± 446
Pacing HR						
MAP	76 ± 16	76 ± 18	75 ± 18	76 ± 13	81 ± 12	76 ± 12
HR	150	150	150	150	150	150
LVEDP	8 ± 2	8 ± 2	8 ± 2	6 ± 2	5 ± 3	5 ± 1
$LVdP/dt_{max}$	1516 ± 351	1377 ± 402	1479 ± 284	1548 ± 218	1610 ± 429	1645 ± 192

MAP, mean arterial pressure (mmHg); HR, heart rate (beats/min); LVEDP, left ventricular end-diastolic pressure (mmHg); $LVdP/dt_{max}$ (mmHg/s); mean ± SD; * $P<0.05$ vs Baseline-1.

Table 2: *Coronary Hemodynamics and Regional Function Pre-ischemia and 30 Minutes Post-reperfusion (Group 1)*

	Pre-ischemia			30 Minute Reperfusion		
Control HR	Baseline-1	Adenosine	ATP	Baseline-2	Adenosine	ATP
LAD Coronary Artery						
Blood Flow	30 ± 13	114 ± 59*	111 ± 54*	21 ± 8	98 ± 32+	90 ± 33+
Resistance	3.06 ± 1.14	0.81 ± 0.40*	0.76 ± 0.25*	4.11 ± 1.12*	0.86 ± 0.31+	0.85 ± 0.23+
Segment Lengths						
Endo ES	9.1 ± 1.5	9.1 ± 1.4	9.1 ± 1.6	9.5 ± 1.4*	9.3 ± 1.3+	9.4 ± 1.5+
Endo ED	10.8 ± 2.0	10.8 ± 2.0	11.0 ± 2.1	10.6 ± 1.9	10.7 ± 1.8	10.8 ± 2.0
Endo Shortening (%)	16 ± 3	16 ± 3	17 ± 3	10 ± 4*	13 ± 4+	13 ± 4+
Epi ES	9.5 ± 1.0	9.5 ± 1.0	9.3 ± 1.1	10.1 ± 1.1*	9.9 ± 1.1+	9.7 ± 1.1+
Epi ED	11.1 ± 1.6	11.0 ± 1.6	10.9 ± 1.8	11.0 ± 1.4	11.0 ± 1.5	10.9 ± 1.6
Epi Shortening (%)	14 ± 6	14 ± 6	14 ± 5	8 ± 6*	9 ± 7+	10 ± 7+
Pacing HR						
LAD Coronary Artery						
Blood Flow	30 ± 11	106 ± 56*	97 ± 47*	23 ± 5	102 ± 22+	87 ± 32+
Resistance	2.88 ± 1.28	0.86 ± 0.38*	0.90 ± 0.39*	3.55 ± 0.29*	0.81 ± 0.15+	0.95 ± 0.29+
Segment Lengths						
Endo ES	9.8 ± 1.2	9.6 ± 1.1	9.5 ± 1.2	9.2 ± 1.3*	9.3 ± 1.4+	9.4 ± 1.4+
Endo ED	10.8 ± 1.5	10.8 ± 1.5	10.7 ± 1.5	10.6 ± 1.5	10.6 ± 1.4	10.4 ± 1.6
Endo Shortening (%)	13 ± 2	12 ± 4	11 ± 3	9 ± 4.5*	11 ± 4+	11 ± 4+
Epi ES	10.4 ± 0.8	10.2 ± 0.8	10.1 ± 0.8	9.7 ± 0.6*	9.5 ± 0.9+	9.6 ± 0.7+
Epi ED	11.2 ± 0.8	11.1 ± 0.8	11.1 ± 0.8	11.0 ± 0.9	10.7 ± 1.1+	10.7 ± 1.0+
Epi Shortening (%)	10 ± 5	10 ± 3	8 ± 4	6 ± 6*	7 ± 6+	8 ± 6+

LAD coronary blood flow (ml/min); LAD coronary resistance (mmHg/min/ml); Endo, Endocardial; Epi, Epicardial; ED, end-diastolic ES, end-systolic; mean ± SD; * $P<0.05$ vs baseline-1; + $P<0.05$ vs baseline-2.

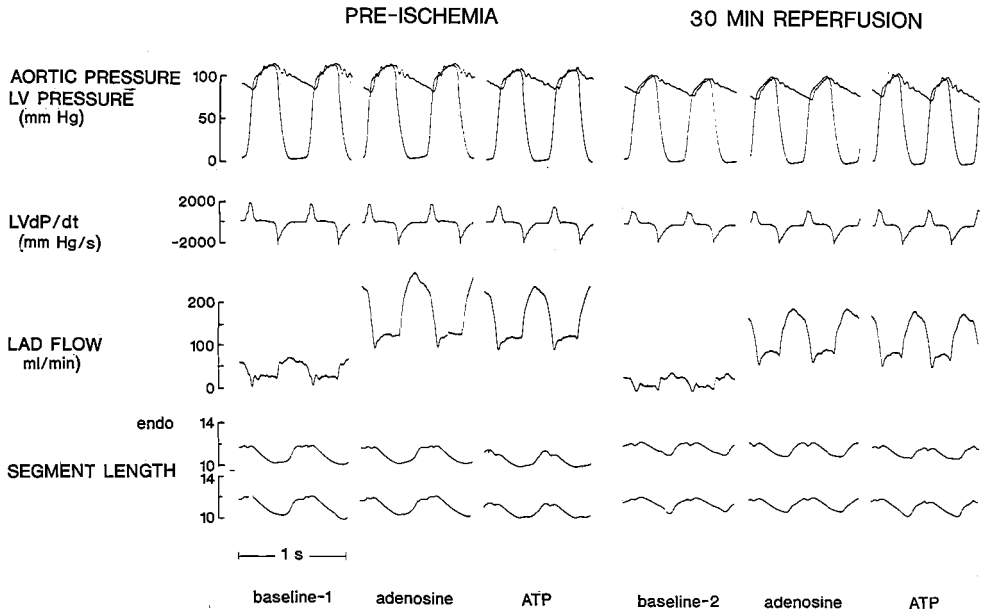

Figure 1: *Representative tracing from an animal in group 1 prior to ischemia and following reperfusion. AOP-aortic pressure (mmHg), LVP-left ventricular pressure (mmHg), LVdp/dtmax-first derivative of left ventricular pressure (mmHg/sec), CBF-coronary blood flow (ml/min), endocardial (Endo) and epicardial (Epi) segment length (SL) changes (mm).*

Myocardial blood flows in the LAD and nonLAD regions are shown in Table 3. During ischemia, blood flow in the LAD region was reduced to 20% of baseline ($p < .05$), while in the nonLAD region, the reduction was not statistically significant. Following 10 minutes of ischemia and 30 minutes of reperfusion, transmural flows returned to pre-ischemic baseline values in all layers. During pacing, transmural flow in the reperfused regions did not increase to the same degree as in the normal region and was statistically lower in the subendocardial layer.

Myocardial oxygen consumption in the LAD region is also shown in Table 3. Although values tended to increase with pacing and decrease following reperfusion, the differences were not statistically significant. Lactate extraction during each intervention was measured from the reperfused bed and is demonstrated in figure 3. Following 30 minutes of reperfusion, lactate production was greater at both heart rates compared with pre-ischemic baseline. It is interesting to note that lactate extraction following reperfusion remained unchanged at the two heart rates despite relative subendocardial hypoperfusion in the LAD region. This suggests that the lower flows were not associated with ischemia.

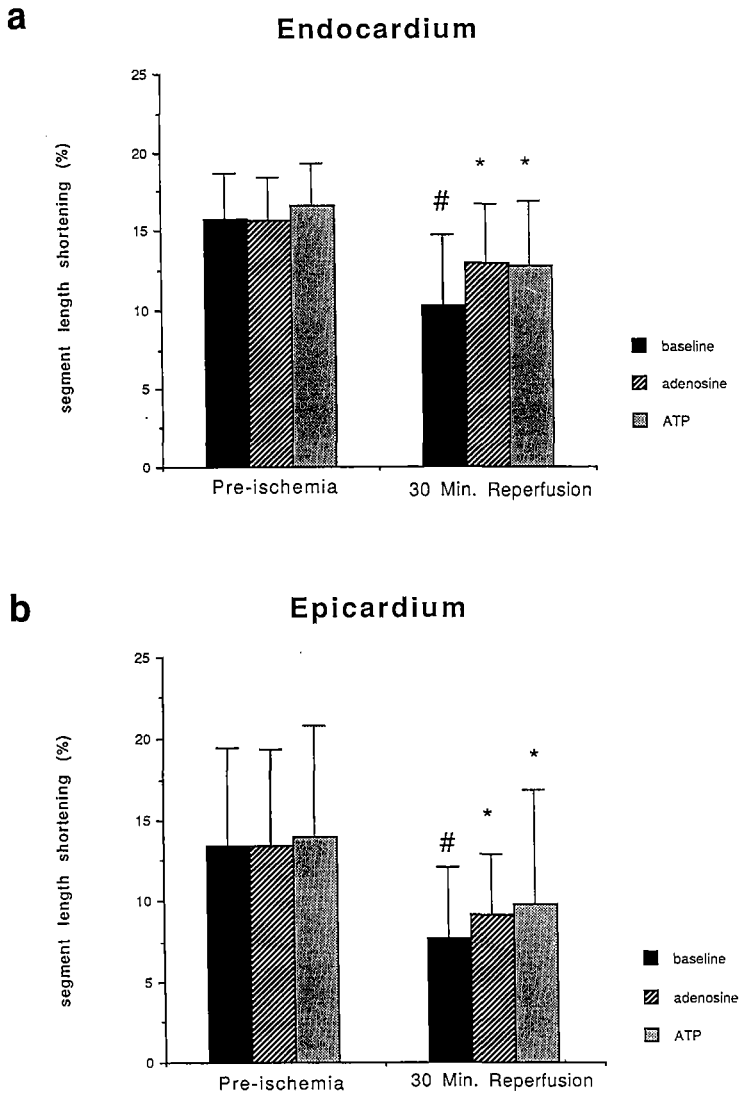

Figure 2: *(a) endocardial systolic segment length changes (%) pre-ischemia and post-reperfusion at control heart rate in group 1. (b) epicardial systolic segment length changes (%) during the same interventions. (n=6;means±sd) #-p<.05 vs baseline pre-ischemia; *-p<.05 vs baseline post-ischemia arterial pressure was 79±10 mm Hg and was 76±11 mm Hg following ischemia-reperfusion (NS).*

Table 3: *Myocardial Blood Flow and Oxygen Consumption Pre-ischemia and 30 Minutes Post-reperfusion (Group 2)*

	Baseline	Pacing	Ischemia	30 Min reperfusion	30 Min reperfusion + pacing
			Myocardial Blood Flow		
Transmural					
LAD	0.92 ± 0.32	1.17 ± 0.69	0.18 ± 0.22*	0.89 ± 0.39	0.89 ± 0.32
nonLAD	1.00 ± 0.29	1.23 ± 0.49	0.82 ± 0.34	0.99 ± 0.37	1.10 ± 0.25
Endocardium					
LAD	0.88 ± 0.26	1.18 ± 0.62	0.23 ± 0.30*	0.89 ± 0.32	0.84 ± 0.24+
nonLAD	1.06 ± 0.31	1.23 ± 0.49	0.87 ± 0.37	1.04 ± 0.43	1.13 ± 0.19
Midmyocardium					
LAD	0.93 ± 0.30	1.25 ± 0.75	0.18 ± 0.23*	0.97 ± 0.41	0.96 ± 0.32
nonLAD	1.10 ± 0.37	1.28 ± 0.48	0.88 ± 0.43	1.00 ± 0.35	1.13 ± 0.23
Epicardium					
LAD	0.96 ± 0.43	1.12 ± 0.73	0.16 ± 0.18*	0.86 ± 0.48	0.87 ± 0.41
nonLAD	0.96 ± 0.31	1.16 ± 0.54	0.85 ± 0.39	0.95 ± 0.40	1.05 ± 0.34
			LAD Myocardial Oxygen Consumption		
O_2-ex	7.94 ± 2.55	7.56 ± 2.52		6.64 ± 2.66	7.77 ± 2.85
MVO_2	6.46 ± 1.80	8.11 ± 2.46		5.04 ± 3.02	5.93 ± 1.58

*$P<0.01$; +$P<0.05$ vs nonLAD region (unpaired t-test); myocardial blood flow-ml/g/min; O_2-ex (myocardial oxygen extraction)-ml O_2/100 ml blood; MVO_2 (myocardial oxygen consumption)-ml O_2/100 g/min

Discussion

In this model of myocardial stunning, we have shown that the vasodilator responses of intracoronary infusions of ATP and adenosine are not altered following brief ischemia and reperfusion. Unlike adenosine, ATP requires an intact endothelium for vasoactivity and thus has been used as a marker of altered endothelial cell function (13,16). Other endothelial dependent vasodilators such as acetylcholine, prostacycline and bradykinin have also been studied in various models of ischemia-reperfusion. In anesthetized dogs for instance, their vasodilator capacity is attenuated following 45-60 minutes of complete coronary occlusion (11-14). This "vascular stunning" has primarily been demonstrated after prolonged periods of ischemia, which may also induce more permanent changes such as

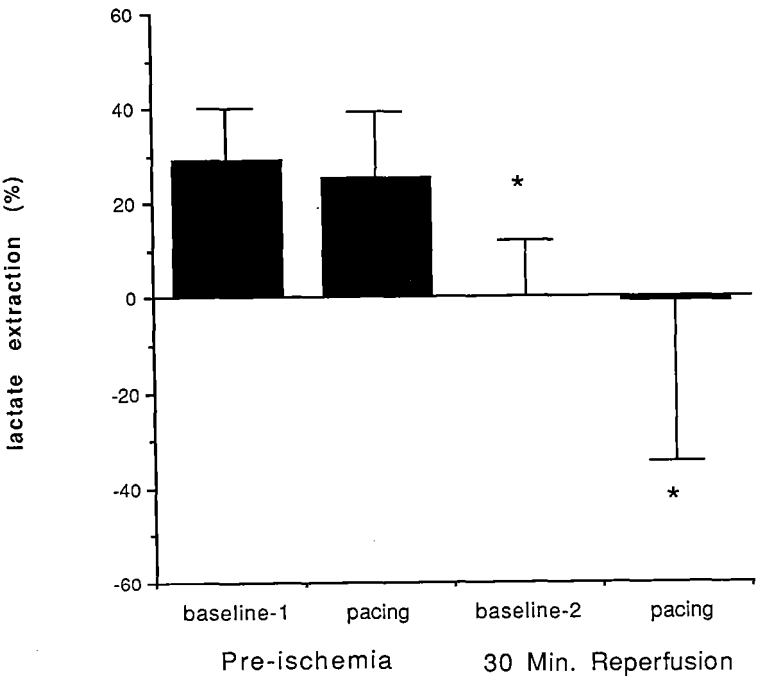

Figure 3: *Lactate extraction (%) at control heart rate and during atrial pacing (150 beats per min), prior to and following ischemia-reperfusion in group 2. (n=6;means±sd).*
**-p<.05 vs baseline pre-ischemia*

necrosis and "no-reflow" (15). Our results are consistent with a more recent study which shows no differences in vascular reactivity between ADP and adenosine following brief ischemia (21). We have extended these findings to ATP and adenosine and in addition, have

measured the effect on function during vasodilation. The present study dissociates mechanical stunning during reperfusion from functional changes in the vasculature at a time when permanent changes are unlikely.

Following reperfusion, minimal coronary resistances during intracoronary infusions of either ATP or adenosine were not different from the pre-ischemic baseline values. This is consistent with the findings in both conscious and open-chest dogs, where the vasodilator response to adenosine following 10 minutes of ischemia and reperfusion were not altered (22,23). In anesthetized dogs, Bolli et al (6) have recently reported that coronary resistance was higher in regionally stunned myocardium, 4 hours after a 15 minute occlusion. They also reported that minimal coronary resistances during intravenous infusions of adenosine or papaverine were higher in the reperfused regions compared with normally perfused myocardium and proposed that microvascular stunning existed. It is possible that our model of 10 minutes of partial coronary occlusion is not severe enough to achieve the differences that they found after 15 minutes of complete occlusion. Johnson et al (24) however, have studied maximal flow-pressure lines during intracoronary adenosine infusions in anesthetized swine and showed no change in slopes following 20 minutes of complete occlusion and 15-45 minutes of reperfusion. This would suggest that functional alterations in the vasculature are minimal when ischemia is less than 20 minutes in duration.

Microvascular stunning is particularly interesting in light of the observations that both vasodilators in group 1 enhanced segment length shortening in the reperfused myocardium. This flow dependent improvement in function was not observed prior to ischemia-reperfusion and thus is different from the original observations made by Gregg (25). In group 2 animals, transmural flow distribution returned to normal following the same ischemia-reperfusion protocol as in group 1. Although pacing following reperfusion was associated with greater differences in flow between the two regions, lactate production did not increase and thus it seems unlikely that these changes in flow resulted in ischemia. Others have also reported improved function with vasodilation in models of myocardial stunning. Following twelve 5 minute ischemic periods in dogs, Stahl et al (2) infused three different vasodilators, dipyridamole, papaverine and nitroglycerine and showed a selective improvement in segment length following reperfusion. They postulated that the improved function resulted from an increase in flow to incompletely reperfused regions. With longer periods of ischemia and reperfusion, others have shown that adenosine not only improves function but also limits reperfusion injury. The proposed mechanism is a result of adenosine's ability to reduce microvascular plugging of leukocytes and thus prevent endothelial cell damage and "no-reflow" (3-5).

Mechanisms other than microvascular "no-reflow" have been investigated to elucidate this flow dependent recruitment in function following stunning. Although adenosine and ATP can provide substrate for ATP production during reperfusion, this does not seem to be the explanation for the improved function. In isolated rat hearts exposed to 3 hours of hypothermic arrest, functional recovery improved when reperfused with adenosine but ATP levels did not increase (7). It is possible that increased flow during reperfusion improves function by the wash-out of metabolic byproducts from the ischemia. In isolated working rat hearts, intermittent reperfusion during 40 minutes of global ischemia improved functional recovery compared with hearts exposed to no reperfusion (8). The authors hypothesized that

intracellular calcium homeostasis was maintained in this model by preventing the buildup of intracellular sodium. This might also explain why mitochondrial function is improved when dogs have been reperfused with adenosine following brief ischemia (9).

Our data show that lactate was continuously produced even 30 minutes after reperfusion. Because the distribution of coronary blood flow as measured by microspheres had returned to pre-ischemic values at that time, it is unlikely that the myocardium was still ischemic. It is possible that the higher flows with ATP and adenosine improved contractile function by the wash-out of lactate in the reperfused regions. Neely et al (26) have shown that lactate production during anaerobic glycolysis may be responsible for ventricular dysfunction. In isolated rabbit myocardium, tissue lactate levels have been shown to be elevated following brief ischemia and reperfusion and are lower when adenosine is included in the reperfusate (27). An alternative explanation to these observations is that vasodilation during reperfusion improves contractile function by increasing oxygen delivery and enhancing substrate utilization (7).

In summary, we have shown that alterations in regional function induced by 10 minutes of partial coronary occlusion and 30 minutes of reperfusion in swine are not associated with altered responses to the endothelial-dependent vasodilator ATP or adenosine. Post-ischemic function is improved with both vasodilators however, and may result from either enhanced washout of metabolic byproducts such as lactate or improved utilization of substrates.

LIMITATIONS OF THE STUDY

A major criticism of this work is the lack of specifity of the vasodilator responses of either adenosine or ATP in regards to the endothelium. Although ATP relaxes preconstricted arteries via the release of EDRF in vitro, its mechanism of vasodilation in the in tact animal may be more nonspecific. Likewise, adenosine has been considered to be an endothelial-independent vasodilator but recent work suggests that its receptors may also be linked to guanylate cyclase (28). We feel however, that the inability to attenuate the vasodilator response of either agent in this model of myocardial stunning suggests that the endothelium remains functionally in tact.

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Chapter 8

Pressure-maximal coronary flow relationship in regionally stunned porcine myocardium.

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submitted for publication

Abstract

Pressure-maximal coronary flow relationship in regionally stunned porcine myocardium. Variable results have been reported on maximal coronary flow in stunned myocardium. In view of the dependency of maximal coronary flow on perfusion pressure, we wished to study the maximal coronary conductance, defined by the slope of the pressure-maximal coronary flow relationship, in regionally stunned myocardium. Myocardial stunning was induced in 12 anesthetized swine by 15 min of coronary perfusion at 20 mm Hg perfusion pressure, resulting in low flow (10-20% of baseline) myocardial ischemia, followed by 30 min of reperfusion. Pressure-maximal coronary flow relationships were obtained with intracoronary adenosine (20 μg/kg/0.2 ml) at 6 different coronary perfusion pressures within the autoregulatory range. Subendocardial systolic segment length shortening was 19 ± 5% at baseline and 7 ± 6% at 30 min of reperfusion ($P<0.01$), respectively. Post-systolic shortening was -4 ± 5% at baseline and 4 ± 6% at 30 min of reperfusion ($P<0.01$). The slope of the pressure-maximal coronary flow line increased from 3.34 ± 1.03 to 3.89 ± 1.33 ml/min/mm Hg ($P<0.01$), while calculated maximal coronary flow at 40 mm Hg perfusion pressure was 46 ± 21 ml/min at baseline and 40 ± 15 ml/min following stunning (NS). Because myocardial stunning per se may have an effect on the maximal coronary conductance we additionally studied the effects of altering contractile function of stunned myocardium via selective chronotropic (atrial pacing, n = 6) or combined chronotropic and inotropic (intravenous dobutamine, n = 6) interventions. Atrial pacing at 40 beats/min above the intrinsic heart rate further reduced the fractional systolic segment shortening by 2 ± 2% (absolute change, $P<0.05$), while post-systolic shortening was not affected. In contrast 4 μg/kg/min dobutamine enhanced systolic shortening by 7 ± 4% ($P<0.05$), while post-systolic shortening was reduced by 7 ± 5% ($P<0.05$). Despite the diverse effects on contractile function, neither intervention significantly altered the pressure-maximal coronary flow relationship. In conclusion, severe myocardial stunning was associated with an increase in maximal coronary conductance in the autoregulatory range. With interventions which altered contractile function, we were unable to show that this increase in maximal conductance was related to the change in contractile function.

Introduction

Coronary flow reserve has consistently been reported to be reduced in myocardium subjected to periods of ischemia and reperfusion, that are long enough to induce myocardial necrosis (5, 13, 15, 19, 27). Mechanisms that have been implicated are mechanical obstruction possibly due to capillary damage (2), neutrophil plugging (7), myocardial tissue edema or ischemic contracture (11, 22) and increases in vasomotor tone (8). In contrast, reports on coronary flow reserve following brief periods of ischemia and reperfusion, which induce reversible loss of myocardial contractile function in the absence of myocardial necrosis, i.e. "myocardial stunning", show variable results. In a number of studies in conscious (16) and anesthetized dogs (12, 25) it has been shown that the coronary blood flow responses to maximal doses of vasodilators are unchanged in reperfused stunned myocardium following single or multiple brief periods (5-10 min) of myocardial ischemia. Similar observations were made in viable myocardium during reperfusion following longer periods (45-90 min) of ischemia (21, 27). In contrast with these findings, it has been reported in dogs that maximal coronary blood flow is reduced during reperfusion following 15 min of ischemia (4, 20).

Discrepancies may in part be related to the steepness of the pressure-flow relationship during maximal vasodilation, whereby even small changes in coronary perfusion pressure could lead to significant changes in coronary blood flow (14, 18). A more appropriate way to characterize the coronary circulation is to determine the slope of the coronary pressure-flow relationship during maximal vasodilation, i.e. determine the maximal coronary conductance. In the present study we therefore wished to investigate the effects of 15 min of severe myocardial ischemia, followed by 30 min of reperfusion, on the pressure-maximal coronary flow relationship in anesthetized swine.

Another factor to be considered, when studying the effects of a brief period of ischemia followed by reperfusion on the maximal coronary conductance, is the alteration in contractile function that is inherent to myocardial stunning. A change in the pattern of myocardial contraction may affect maximal coronary blood flow (17) and the pressure-maximal coronary flow relationship (14, 26). Alterations in the maximal coronary conductance that are due to ischemia and reperfusion-induced changes in the coronary vascular responsiveness to vasodilators could be modulated by the change in contractile function. To assess the influence of contractile function on the maximal coronary conductance in stunned myocardium, we additionally studied and compared the effects of selective chronotropic (atrial pacing) and combined inotropic and chronotropic (intravenous dobutamine) interventions on the pressure-maximal coronary flow relationship in regionally stunned porcine myocardium.

Methods

General

All experiments were performed in accordance with the Guiding Principles in the Care and Use of Animals as approved by the Council of the American Physiological Society (DHEW Publication No. (NIH) 80-23, 1980) and under the regulations of the Animal Care Committee of the Erasmus University Rotterdam, Rotterdam, The Netherlands.

After an overnight fast, cross-bred Landrace x Yorkshire pigs (HVC, Hedel, The Netherlands) of either sex (25-45 kg, n = 12) were sedated with intramuscular 20 mg/kg ketamine (AUV, Cuijk, The Netherlands), anesthetized with intravenous 5 mg/kg metomidate (Janssen Pharmaceutica, Beerse, Belgium), intubated and connected to a ventilator for intermittent positive pressure ventilation with a mixture of O_2 and N_2 (1:2, v/v). Respiratory rate and tidal volume were set to keep arterial blood gases within the normal range: 7.35 < pH < 7.45; 35 mm Hg < PCO_2 < 45 mm Hg and 100 mm Hg < PO_2 < 160 mm Hg. Catheters (7F) were placed in the superior caval vein for administration of (i) 100 mg/kg α-chloralose (Merck, Darmstadt, Germany) followed by an infusion of 10 mg/kg/h α-chloralose and an infusion of 3-4 mg/kg/h sodium pentobarbitone (Sanofi, Paris, France), (ii) 4 mg of the muscle relaxant pancuronium bromide (Organon Teknika B.V., Boxtel, The Netherlands) prior to thoracotomy and (iii) haemaccel (Behringwerke A.G., Marburg, Germany). Catheters were also positioned in the descending aorta for withdrawal of blood samples and measurement of central aortic blood pressure. A 7F Sensodyn micromanometer-tipped catheter (B.Braun Medical B.V., Uden, The Netherlands) inserted via the left carotid artery, was used to measure left ventricular pressure and its first derivative (LVdP/dt). After thoracotomy an electromagnetic flow probe (2.5 or 3.0 mm diameter; Skalar, Delft, The Netherlands) and a hydraulic occluder (R.E. Jones, Silver Spring, Maryland, USA) were positioned around the most proximal part of the left anterior descending coronary artery (LAD). The balloon was connected to a 1 ml syringe (Hamilton Bonaduz, Bonaduz, Switzerland) driven by a micrometer (Hamilton Co., Reno, Nevada, USA). Meticulous attention was paid to the positioning and stability of the flow probe and zero flows were checked regularly throughout the experiments. Immediately distal to the flow probe and occluder, a small cannula (0.8 mm outer diameter) was inserted into the LAD for administration of adenosine and for measurement of coronary perfusion pressure. Reactive hyperemia following a 15 s occlusion was performed prior to and after insertion of the cannula into the LAD to ensure that no attenuation of maximal coronary blood flow occurred due to the presence of the cannula. Regional myocardial segment length shortening was measured by sonomicrometry (Triton Technology Inc., San Diego, Ca, USA) using two pairs of ultrasonic crystals (Sonotek Corporation, Del Mar, Ca, USA) placed in the subendocardial and subepicardial layers of the LAD perfused area approximately 10 mm apart (24). Pacings leads were attached to the left atrial appendage and connected to a pacing stimulator.

Experimental Protocol

Following completion of the surgical procedures, a 30 minute stabilization period was allowed before baseline recordings were made of central aortic blood pressure, left ventricular blood pressure (LVP) and its first derivative LVdP/dt, coronary perfusion pressure, LAD blood flow and subendocardial and subepicardial segment length (Figs. 1a and 1b). Subsequently, LAD blood flow and perfusion pressure were assessed

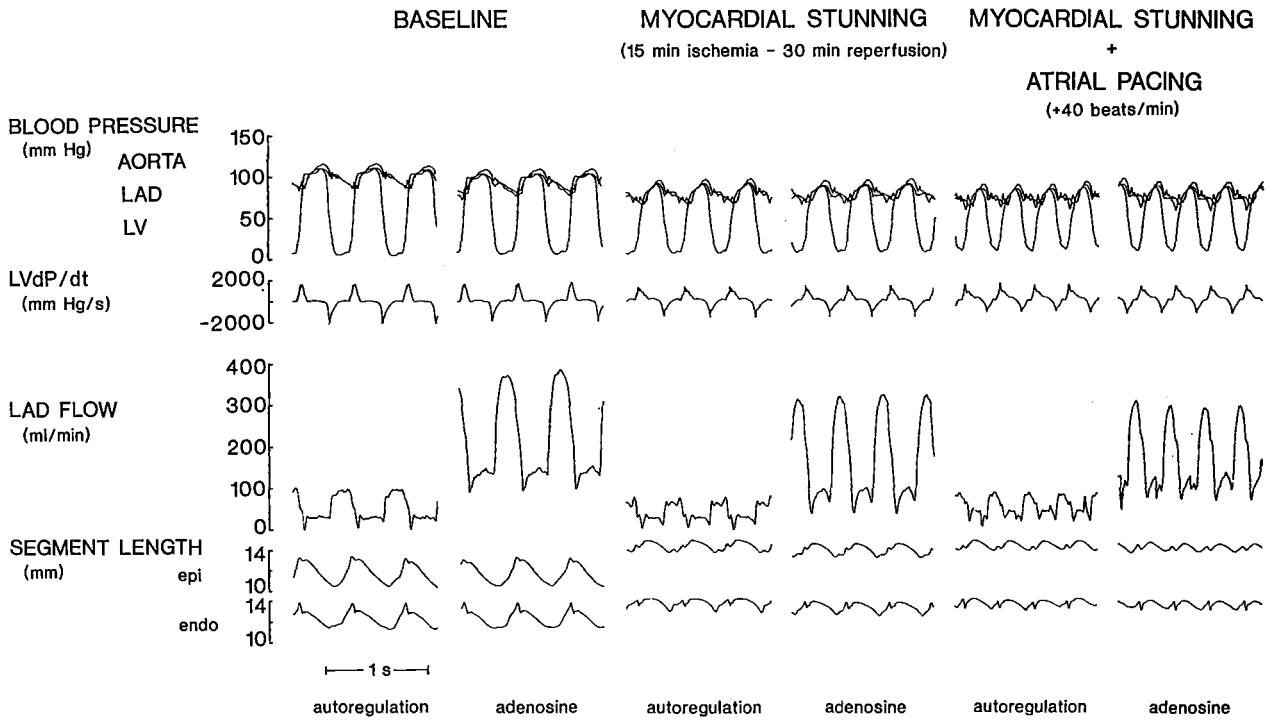

Figure 1a: *An example of a tracing from an experiment in which the animal was subjected to atrial pacing, following myocardial stunning. LAD = left anterior descending coronary artery; LV = left ventricle.*

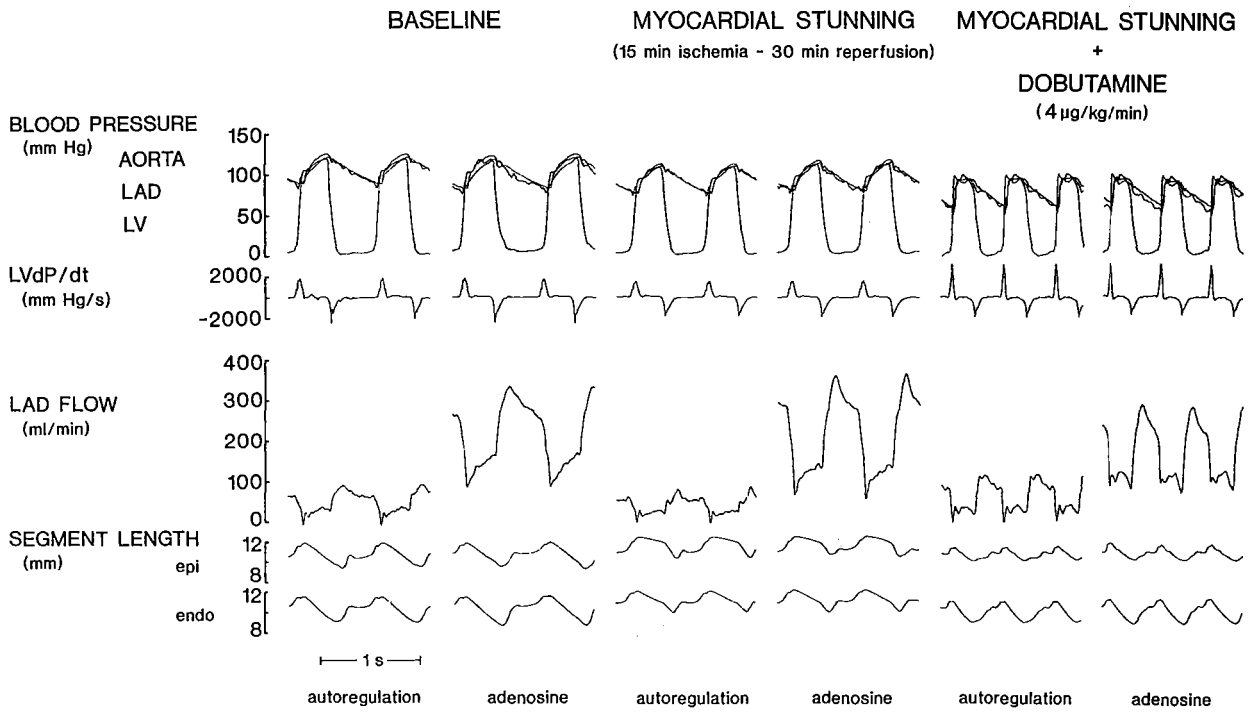

Figure 1b: *An example of a tracing from an experiment in which the animal received an intravenous dobutamine infusion, following myocardial stunning. LAD = left anterior descending coronary artery; LV = left ventricle.*

following maximal vasodilation with 20 μg/kg intracoronary adenosine (0.2 ml), which was slowly injected over 5 seconds. This dose of adenosine was compared to 30-40 μg/kg adenosine in each experiment and was found to give maximal vasodilation in each animal. Furthermore it always exceeded the peak reactive hyperemia following a 15 s occlusion. Subsequently, the balloon of the hydraulic occluder was inflated to create up to six different levels of coronary perfusion pressure. The perfusion pressures varied widely but were always within the autoregulatory range, to avoid changes in contractile function. Coronary blood flow and coronary perfusion pressure during maximal vasodilation with adenosine were recorded at each level of perfusion pressure.

Following these baseline recordings, myocardial ischemia was induced by inflation of the hydraulic occluder so that coronary perfusion pressure was 20 mm Hg, resulting in a 80-90% reduction of coronary blood flow. We chose this regimen because it induces myocardial stunning, with less ventricular fibrillation compared to complete occlusions. After 15 min, the hydraulic occluder was released and the heart was reperfused for 30 minutes, at which time all recordings were repeated.

Following all measurements at 30 min of reperfusion the 12 pigs were randomly divided into two groups. In six animals the left atrium was paced at 40 beats/min above the intrinsic heart rate level at 30 minutes of reperfusion, while the other six animals received an intravenous infusion of 4 μg/kg/min dobutamine. The 40 beats/min increase was chosen because this corresponded with the increment in heart rate induced by 4 μg/kg/min dobutamine in 3 pilot experiments. Ten minutes after the onset of atrial pacing or start of the dobutamine infusion, recordings were repeated. We chose to study the effects of dobutamine and atrial pacing in parallel experiments, rather than sequentially in each experiment, to exclude any possible reperfusion duration-dependent effects on myocardial contractile function and hence the pressure-maximal coronary flow relationship.

Data analysis and presentation

From each of the three experimental periods (baseline, myocardial stunning, atrial pacing or dobutamine) we obtained coronary blood flow under basal conditions (autoregulation) and during maximal vasodilation with adenosine. Coronary flow reserve ratio was calculated as the ratio of maximal coronary blood flow divided by coronary blood flow during autoregulation (9). Pressure-maximal coronary flow lines were generated from the 6 points collected during each sampling period, and correlations (r^2) and regressions were determined by first order curve fitting. In all 12 animals the r^2 of the pressure-maximal coronary flow line exceeded 0.98 at all time points.

From the tracings the segment lengths of the subendocardial and subepicardial layers were assessed at end-diastole, end-systole and post-systole. End-diastolic length (EDL) was measured at the onset of positive LVdP/dt, while end-systolic length (ESL) was measured at peak negative LVdP/dt (1, 10). Post-systolic length (PSL) was measured at the point where negative LVdP/dt had returned to zero. This point corresponded with maximal post-systolic shortening following stunning in 11 of the 12 animals. Fractional systolic shortening (SS) was calculated as

$$SS = \frac{EDL - ESL}{EDL} \times 100\ (\%)$$

and fractional post-systolic shortening (PSS) was calculated as

$$PSS = \frac{PSL - ESL}{EDL} \times 100\ (\%)$$

All results have been presented as arithmetic means ± SD. Data at baseline and following myocardial stunning have been presented as absolute values; data during either atrial pacing or dobutamine have been presented as absolute changes from myocardial stunning values. Statistical analysis was performed using the paired t-test with Bonferroni correction. The effects of dobutamine were compared to atrial pacing with the unpaired t-test.

Drugs

Adenosine (E. Merck, Darmstadt, Germany) was dissolved in saline at a concentration of 100 μg/kg/ml for intracoronary administration of a bolus infusion of 0.2 ml (20 μg/kg). Dobutamine (Eli Lilly Nederland, Amsterdam, The Netherlands) was dissolved in saline at a concentration of 4 μg/kg/ml and infused intravenously at a rate of 1 ml/min.

Results

During ischemia one animal encountered ventricular fibrillation, while two animals fibrillated during reperfusion. Sinus rhythm could be restored in each animal with a single DC-countershock and these animals have therefore been included in the analysis.

Systemic hemodynamics

Fifteen min of myocardial ischemia and 30 min of reperfusion resulted in a decrease in mean arterial blood pressure of 13±7% ($P<0.01$), and an increase in heart rate of 15±15% ($P<0.05$). $LVdP/dt_{max}$ was reduced by 23±13% ($P<0.01$), while left ventricular end-diastolic pressure was not affected (Table 1).

Mean arterial blood pressure did not change during either atrial pacing at 40 beats/min above the intrinsic heart rate or the intravenous infusion of 4 μg/kg/min dobutamine (Table 1). Dobutamine increased heart rate by 32±15 beats/min, which was not significantly different from the heart rate response in the atrial pacing group. $LVdP/dt_{max}$ increased by 72±25% during dobutamine, while this parameter was not affected by atrial pacing. Finally, left ventricular end-diastolic pressure was lowered by dobutamine but not by atrial pacing.

Myocardial contractile function

At 15 min of ischemia, subendocardial and subepicardial fractional systolic segment shortening were -1.9 ± 2.3% and -2.1 ± 2.0%, respectively. Post-systolic segment shortening was 9.4 ± 5.1% and 9.5 ± 3.1% in the subendo- and subepicardial layers, respectively. At 30 min of reperfusion myocardial stunning was present in both myocardial layers, as systolic shortening of the post-ischemic segment was approximately one-third of baseline value, while post-systolic shortening was still present (Table 2).

Atrial pacing did not affect end-systolic segment length but end-diastolic length

Table 1: *Systemic hemodynamics in 12 swine before and after myocardial stunning (15 min ischemia and 30 min reperfusion) and following subsequent stimulation with either atrial pacing (n = 6) or intravenous dobutamine (n = 6).*

	Baseline	Myocardial stunning	Absolute change from myocardial stunning	
			Atrial pacing	Dobutamine
Mean arterial blood pressure (mmHg)	93 ± 16	80 ± 12**	-1 ± 2	-5 ± 7
Heart rate (beats/min)	101 ± 20	115 ± 21*	40^{+}	$32 \pm 15^{+}$
$LVdP/dt_{max}$ (mmHg/s)	1740 ± 250	1330 ± 260**	70 ± 100	$1020 \pm 400^{+o}$
LV end diastolic pressure (mmHg)	9.3 ± 2.0	9.7 ± 2.1	-1.3 ± 1.5	$-2.1 \pm 0.4^{+}$

The left atrium was paced at 40 beats/min above the heart rate recorded following myocardial stunning; dobutamine was infused intravenously at a rate of 4 μg/kg/min. LV = left ventricular. Data at baseline and myocardial stunning have been expressed as absolute values. The data for atrial pacing and dobutamine have been expressed as absolute changes from the myocardial stunning values. All data have been presented as mean ± SD. $^{*}P<0.05$ myocardial stunning vs baseline; $^{**}P<0.01$ myocardial stunning vs baseline; $^{+}P<0.05$ vs myocardial stunning; $^{o}P<0.05$ dobutamine versus atrial pacing.

Table 2: *Normalized myocardial segment length and fractional shortening in 12 swine before and after myocardial stunning (15 min ischemia and 30 min reperfusion) and following subsequent stimulation with either atrial pacing (n = 6) or intravenous dobutamine (n = 6).*

	Baseline	Myocardial stunning	Absolute change from myocardial stunning	
			Atrial pacing	Dobutamine
Subendocardium				
Segment length (mm)				
end-diastole	10.0	10.3 ± 0.7	-0.2 ± 0.3	-0.6 ± 0.2^{+}
end-systole	8.1 ± 0.5	9.6 ± 1.1**	0.1 ± 0.4	-1.2 ± 0.4^{+o}
post-systole	8.5 ± 0.6	9.2 ± 0.8**	0.2 ± 0.2	-0.4 ± 0.2^{+o}
Fractional segment shortening (%)				
systole	19.4 ± 5.0	7.3 ± 6.2**	-2.2 ± 1.7^{+}	6.6 ± 3.7^{+o}
post-systole	-4.0 ± 4.5	3.9 ± 5.6**	-1.0 ± 2.8	-7.0 ± 4.7^{+}
Subepicardium				
Segment length (mm)				
end-diastole	10.0	10.6 ± 0.5**	-0.2 ± 0.2	-0.4 ± 0.3^{+}
end-systole	8.6 ± 0.4	10.1 ± 0.8**	0.1 ± 0.2	-0.9 ± 0.6^{+o}
post-systole	8.7 ± 0.4	9.5 ± 0.8**	0.1 ± 0.4	-0.5 ± 0.2^{+o}
Fractional segment shortening (%)				
systole	14.2 ± 3.9	4.6 ± 4.9**	-2.6 ± 1.6^{+}	4.7 ± 4.3^{+o}
post-systole	-0.7 ± 3.3	5.5 ± 5.0**	-0.3 ± 3.9	-3.8 ± 5.6

The left atrium was paced at 40 beats/min above the heart rate recorded following myocardial stunning; dobutamine was infused intravenously at a rate of 4 μg/kg/min. LV = left ventricular. Data at baseline and myocardial stunning have been expressed as absolute values. The data for atrial pacing and dobutamine have been expressed as absolute changes from the myocardial stunning values. All data have been presented as mean ± SD. $^{*}P<0.05$ myocardial stunning vs baseline; $^{**}P<0.01$ myocardial stunning vs baseline; $^{+}P<0.05$ vs myocardial stunning; $^{o}P<0.05$ dobutamine versus atrial pacing.

Table 3: *Coronary hemodynamics and the pressure-maximal coronary flow relationship in 12 swine before and after myocardial stunning (15 min ischemia and 30 min reperfusion) and following subsequent atrial pacing (n = 6) or intravenous dobutamine (n = 6).*

	Baseline	Myocardial stunning	Absolute change from myocardial stunning	
			Atrial pacing	Dobutamine
Coronary blood flow (ml/min)				
autoregulation	44 ± 12	37 ± 13**	4 ± 4	12 ± 9^{+}
during maximal vasodilation	185 ± 61	167 ± 62	-18 ± 16	-14 ± 23
Coronary perfusion pressure (mmHg)				
autoregulation	93 ± 16	80 ± 12**	-1 ± 2	-5 ± 7
during maximal vasodilation	82 ± 16	72 ± 12**	0 ± 2	-7 ± 9
Coronary flow reserve ratio	4.2 ± 1.0	4.7 ± 1.3*	-0.8 ± 0.5^{+}	-1.3 ± 0.6^{+}
Pressure-maximal coronary flow relationship				
slope (ml/min/mmHg)	3.34 ± 1.03	3.89 ± 1.33**	-0.18 ± 0.60	-0.04 ± 0.30
calculated flow at 40 mmHg (ml/min)	46 ± 21	40 ± 15	-6 ± 8	7 ± 6^{o}
calculated flow at 80 mmHg (ml/min)	179 ± 57	195 ± 65*	-13 ± 19	5 ± 12

The left atrium was paced at 40 beats/min above the heart rate recorded following myocardial stunning; dobutamine was infused intravenously at a rate of 4 μg/kg/min. Data at baseline and myocardial stunning have been expressed as absolute values. The data for atrial pacing and dobutamine have been expressed as absolute changes from the myocardial stunning values. All data have been presented as mean ± SD. $^{*}P<0.05$ myocardial stunning; $^{**}P<0.01$ myocardial stunning vs baseline; $^{+}P<0.05$ vs myocardial stunning; $^{o}P<0.05$ dobutamine versus atrial pacing.

was slightly smaller (Table 2), resulting in a reduction in systolic shortening in both myocardial layers. Post-systolic segment length and fractional post-systolic shortening were not affected by atrial pacing. In contrast, dobutamine doubled systolic shortening to 70% of baseline value in both myocardial layers. Furthermore, in the presence of dobutamine post-systolic shortening was absent.

Coronary blood flows and pressure-maximal coronary flow relationship

Myocardial stunning decreased coronary blood flow during autoregulation by 20 ± 13% ($P<0.01$; Table 3). In the presence of adenosine coronary blood flow in stunned myocardium was not significantly different from baseline, despite the 10 ± 9 mm Hg ($P<0.01$) drop in coronary perfusion pressure. Consequently, the calculated coronary flow reserve ratio increased from 4.2 ± 1.0 to 4.7 ± 1.3 ($P<0.05$). Fig. 2 shows representative examples of the pressure-maximal coronary flow lines from an animal in each intervention group. Myocardial stunning increased the slope of the pressure-maximal

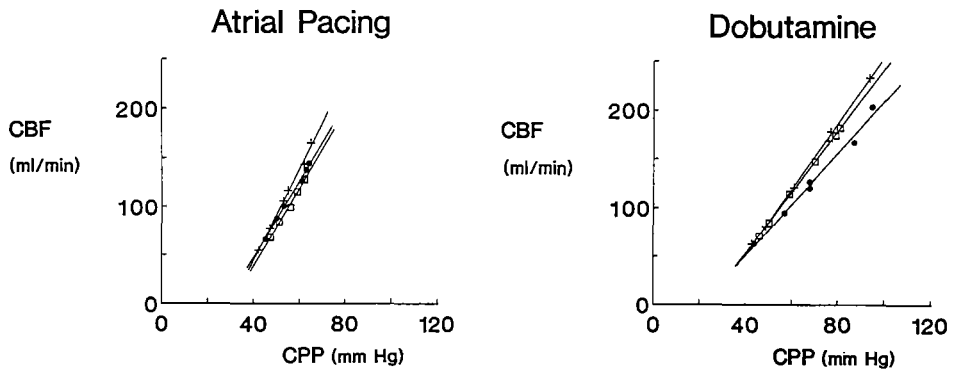

Figure 2: *Representative examples from two animals of pressure-maximal coronary flow relationships in the autoregulatory range. Presented are pressure-flow lines at baseline (●), following myocardial stunning (+), and during either atrial pacing at 40 beats/min above the intrinsic heart rate (□ , left panel) or intravenous infusion of 4 μg/kg/min dobutamine infusion (□, right panel). CPP = mean coronary perfusion pressure, CBF = mean coronary blood flow.*

coronary flow relationship from 3.34 ± 1.03 to 3.89 ± 1.33 ml/min/mm Hg ($P<0.01$, Table 3). The upward rotation of the pressure-flow line is also illustrated by calculating blood flows at two perfusion pressures: at 40 mm Hg coronary blood flows were 46 ± 21 ml/min and 40 ± 15 ml/min (NS) before and after stunning, respectively and at 80 mm Hg flows were 179 ± 57 and 195 ± 65 ml/min, respectively ($P<0.05$). There was a poor correlation ($r = 0.22$; $p = 0.5$) between the percent change in subendocardial fractional systolic shortening and the percent change in the slope of the pressure-maximal coronary flow relationship (not shown).

Although atrial pacing did not significantly increase coronary blood flow during autoregulation or reduce maximal coronary blood flow, the coronary flow reserve ratio was reduced. More important, the slope of the pressure-maximal coronary flow relationship was not affected by atrial pacing (Table 3). Dobutamine enhanced coronary blood flow during autoregulation by 30 %, while maximal coronary flow was unchanged. Consequently, the coronary flow reserve ratio decreased. As with atrial pacing, the slope of the pressure-maximal coronary flow relationship was unchanged during dobutamine.

Discussion

The main finding in the present study was that severe myocardial stunning, induced by 15 min of low flow ischemia and 30 min of reperfusion was associated with an increase in the maximal coronary conductance (defined by the slope of the pressure-maximal coronary flow relationship) in the autoregulatory range. This increase in maximal coronary conductance could not be shown to be related to a change in contractile function, as it was affected neither by selective chronotropic (atrial pacing) nor combined inotropic and chronotropic (intravenous dobutamine) interventions.

Other studies on coronary flow reserve measurements following brief periods of ischemia and reperfusion have shown variable results. In a number of studies in conscious (16) and anesthetized dogs (12, 25) it has been shown that the coronary blood flow responses to maximal doses of vasodilators are unchanged in reperfused "stunned" myocardium following single or multiple brief periods (5-10 min) of myocardial ischemia. Stahl et al. (25) found no change in coronary vasodilator response to intravenous dipyridamole and papaverine 90 min after 12 intermittent 5 min coronary artery occlusions in open-chest dogs. Jeremy et al. (12) using intracoronary adenosine in open-chest dogs reported no change in flow reserve at 60 min of reperfusion following 10 min of ischemia. Similar observations were made with intravenous adenosine in conscious dogs by Laxson et al. (16) at 60 min of reperfusion following 3 intermittent 10 min coronary artery occlusions, separated by 30 min of reperfusion. In contrast with these findings, maximal coronary blood flow was reduced during reperfusion following 15 min of ischemia in open-chest dogs (4, 20). Nicklas and Gips (20), using intracoronary adenosine, found an attenuation of maximal coronary flow, at 1 hour of reperfusion. At 4 hours of reperfusion, Bolli et al. (4), using intravenous adenosine and papaverine, also observed an attenuated maximal coronary flow. The latter authors suggested that the longer duration of coronary artery occlusion, i.e. 15 min versus 10 min, could be responsible for the different observations. However, in a recent study in open-chest dogs, 45 min of ischemia followed by 60 min reperfusion did not attenuate the vasodilator response to intracoronary nitroprusside (21). Also following 90 min of coronary artery occlusion and 150 min of reperfusion, maximal coronary flow assessed with intracoronary adenosine was maintained in post-ischemic viable myocardium (27). On inspection of

these studies, the length of reperfusion time does not seem to correlate with changes in coronary flow reserve. Furthermore, in all but two experimental protocols (21, 25) adenosine was used and therefore the discrepancies between the studies can not be ascribed to the use of different vasodilators.

In the present study we found an increase in maximal coronary conductance following stunning, which seems in contrast with all previous studies. Laxson et al. (16) observed a small, yet non-significant, increase in maximal coronary blood flow from 63 ml/min to 70 ml/min, following myocardial stunning, while arterial perfusion pressure decreased by 3 mm Hg, which was also not significant. As the slope of the pressure-coronary flow line during maximal vasodilation is very steep (14, 18), the slight reduction in arterial perfusion pressure may have, at least in part, offset the increase in maximal flow. Nicklas and Gips (20) reported following stunning a non-significant reduction in diastolic arterial blood pressure of 5 mm Hg, while maximal coronary blood flow decreased from 179 ml/min to 136 ml/min. In our study a 5 mm Hg drop in perfusion pressure would result in a 15-20 ml/min drop in maximal coronary blood flow, which would account for almost half of the observed reduction in maximal coronary blood flow in their study (20). Although the small mean changes in perfusion pressure do not fully explain the findings of the different studies or their discrepancies, they do, however, illustrate the importance of determining the pressure-flow relationship when studying coronary flow reserve (14, 18).

An explanation for the observed increase in maximal coronary conductance could be the loss of contractile function that occurs following ischemia and reperfusion. With the change in contractile function, extravascular compression may be altered which affects the pressure-maximal coronary flow relationship (14). To delineate between the effects on contractile function and the coronary vasculature, we studied and compared the effects of atrial pacing and dobutamine on the maximal coronary conductance in stunned myocardium. Atrial pacing at 40 beats/min above intrinsic heart rate did not have an effect on the pressure-maximal coronary flow relationship. Bache and Cobb (3), have shown that atrial pacing in conscious dogs from 100 beats/min to 150 beats/min slightly reduced maximal subendocardial flow but had no significant effect on total transmural myocardial blood flow, and that only at heart rates of 200 beats/min and 250 beats/min total transmural blood flow was reduced. The 40 beats/min (30%) increase in heart rate in our study, decreased the slope of the pressure-maximal coronary flow relationship in only two of the six animals and was apparently not large enough to consistently attenuate total transmural myocardial blood flow. Dobutamine, although not completely normalizing the contractile function, significantly improved contractile function with absolutely no effect on the maximal coronary conductance. This observation, taken together with the poor correlation between the degree of myocardial stunning and the change in maximal coronary conductance, strongly suggests that the increase in maximal coronary conductance is not related to the change in contractile function. The lack of effect of a change in contractile function on the pressure-maximal flow relationship seems in contradiction with an earlier study that showed an increase in maximal transmural blood flow when regional myocardial contractile function was abolished with lidocaine (17). However, other studies have shown the effects of changes in regional contractile function to be primarily on the transmural distribution of maximal coronary blood flow and minimal on maximal total transmural blood flow (6, 26). Several reasons can be forwarded to explain the lack of effect of a change in the contractile state of the myocardium on the pressure-maximal coronary flow relationship. Firstly, Doucette

et al. (6) showed that a loss of active contraction renders the myocardium more vulnerable to the compressive stress of left ventricular systolic pressure. A reduction in compressive stress due to a loss of active contraction may thus be partially offset by the increase in compressive stress due to the left ventricular systolic pressure generated by the normally contracting myocardium. A second explanation why an increase in contractility may not result in a reduction of maximal coronary conductance may be the duration of diastole. In our study, atrial pacing decreased the duration of diastole from 42% of the cardiac cycle to 31%. In contrast, dobutamine had no effect on the duration of diastole which was 45% and 46%, respectively, before and during dobutamine. Thus, relative to atrial pacing, dobutamine increased the duration of diastole, an action that is due to an increase in velocity of contraction and relaxation (23). It could thus be that the effects of dobutamine on systolic compression are counteracted by the increase diastolic perfusion time. Thirdly, in our study dobutamine not only enhanced systolic function, it also enhanced early diastolic relaxation, as indicated by an almost complete reversion of post-systolic shortening. This may result in a reduction of diastolic compressive stress on the vasculature, thereby balancing the systolic compressive effects of dobutamine on maximal coronary conductance.

In summary, severe myocardial stunning, induced by 15 min of low flow ischemia and 30 min of reperfusion, was associated with an increase in the maximal coronary conductance in the autoregulatory range. The increase in maximal coronary conductance seems not related to the change in contractile function, as it was affected neither by selective chronotropic nor combined inotropic and chronotropic interventions.

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Chapter 9

The relationship between myocardial blood flow, metabolism and contractile function in regionally stunned myocardium during chronotropic and inotropic stimulation

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in preparation

Abstract

Although stunned myocardium has been shown to retain normal functional reserve, the presence of coronary blood flow and metabolic reserve during increased myocardial demand following reperfusion has not been established. In 12 anesthetized swine, we occluded the left anterior descending coronary artery (LADCA) for 10 minutes and reperfused for 30 minutes for two successive cycles. The model has been previously shown to induce severe functional abnormalities in the absence of necrosis. At baseline, coronary blood flows in the control and LADCA regions were 1.49±0.43 and 1.48±0.42 ml/min/g, respectively. Thirty minutes following the second reperfusion period, control myocardial blood flow was 1.27±0.52 ml/min/g (NS) while LADCA flow was reduced to 0.94±0.27 ml/min/g ($p<0.05$). Regional function was determined from segment length shortening (SLS) and the integral of the LV pressure-segment length loops (indices of work). At baseline, SLS in the control and LADCA regions were 13.2±3.7% and 18.3±5.2%, respectively and following reperfusion were 12.9±3.8% (NS) and 6.6±4.6% ($p<0.05$), respectively. Compared with baseline values, indices of work following reperfusion were reduced to 75±10% (NS) in the control region and 50±14% in the LADCA region ($p<0.05$). Chronotropic and inotropic stimulation were then instituted following reperfusion. During atrial pacing and dobutamine, SLS was 10.2±3.5% in the control area and 14.0±5.0% in the LADCA region (each 77% of baseline values). Although this suggested that post-ischemic function was fully recruited, index of work remained higher in the control region (116±48% vs 97±27%). Blood flow during the same intervention was 1.76±0.60 ml/min/g in control and 1.50±0.44 ml/min/g in the LADCA region, thus parallelling the small differences in recruitable work. Oxygen extraction was 75% both at baseline and following reperfusion and increased to 79±11% ($p<0.05$) during pacing. Lactate extraction was 40±14% and 8±11% ($p<0.05$) at baseline and following reperfusion respectively and increased to 16±8% ($p<0.05$) with pacing and 19±9% ($p<0.05$) with dobutamine. In conclusion, coronary blood flow and metabolism are reduced with the functional abnormalities in this model of myocardial stunning. However, sufficient capacity to increase exists during augmented demands following reperfusion, thus emphasizing that neither are limiting factors for the contractile abnormalities.. The close relationship between changes in blood flow and recruitable work following reperfusion suggest that the two remain coupled.

Introduction

In models of myocardial stunning induced by an ischemic period too brief to induce necrosis, the relationships between coronary blood flow, metabolism and contractile function have not been well characterized. Although the capacity to maximally vasodilate in response to exogenous vasodilators remains intact following stunning (1), basal coronary blood flow may be either lower (2-5) or unchanged (6-9) compared with pre-ischemic values. In support of the latter, myocardial oxygen consumption may be higher than expected based on the severe reductions in post-ischemic contractile function. A number of mechanisms including mitochondrial uncoupling, altered efficiency of energy utilization and an increased energy use for maintenance of cellular homeostasis (7) have been proposed to explain this apparent oxygen wastage. In support of the observation that blood flow is lower during myocardial stunning is the importance of the contractile state of the myocardium as a determinant of myocardial oxygen consumption. The latter suggests that blood flow would be metabolically down-regulated with reductions in post-ischemic function.

We hypothesized that changes in blood flow in a model of myocardial stunning are metabolically related to changes in post-ischemic function and are not limited by the capacity to endogenously vasodilate. The design of these experiments was to assess the relationship between myocardial blood flow, metabolism and contractile function in regionally stunned myocardium during chronotropic (atrial pacing) and inotropic (dobutamine infusion during atrial pacing) stimulation. A model of stunning was used that has been shown to induce severe wall motion abnormalities in the absence of necrosis (10).

Materials

All experiments were performed in accordance with the Guiding Principles in the Care and Use of Animals as approved by the Council of the American Physiological Society (DHEW Publication No. (NIH) 80-23, 1980) and under the regulations of the Animal Care Committee of the Erasmus University Rotterdam, Rotterdam, The Netherlands.

Preparation

On the day of the experiment, animals were sedated with an intramuscular injection of ketamine (20 mg/kg; AUV, Cuijk, The Netherlands), anesthetized with an intravenous injection of metomidate (5 mg/kg; Janssen Pharmaceutica, Beerse, Belgium), intubated and connected to a ventilator for intermittent positive pressure ventilation with a mixture of oxygen and nitrogen (1:2). Respiratory rate and tidal volume were set to keep arterial blood gases, measured with an ABL3 (Radiometer, Copenhagen, Denmark) within the normal range: $7.35 < pH < 7.45$; $35 \text{ mmHg} < PCO_2 < 45 \text{ mmHg}$ and $100 \text{ mmHg} < PO_2 < 160$ mmHg. Catheters (7F) were placed in the superior caval vein for infusion of 10-15 mg/kg/h sodium pentobarbitone (Apharma, Arnhem, The Netherlands), haemaccel (Behringwerke A.G., Marburg, Germany) to replace blood withdrawn during sampling, and for infusion of dobutamine. Both femoral arteries were cannulated with 7F catheters, which were positioned in the descending aorta for measurement of central aortic blood pressure and for withdrawal of blood samples for blood gas analysis and calibration of the radioactive

microsphere method. A 7F Sensodyn micromanometer-tipped catheter (B.Braun Medical B.V., Uden, The Netherlands) inserted via the left carotid artery, was used to measure left ventricular pressure and its first derivative (LVdP/dt). Rectal temperature was monitored throughout the experiment and maintained near 37 °C with external heating pads. Pancuronium bromide (4 mg; Organon Teknika B.V., Boxtel, The Netherlands) was given intravenously, and following a midline thoracotomy, the heart was suspended in a pericardial cradle. The left mammary vessels were ligated and the second left rib was removed for ease of further instrumentation. The adventitia surrounding the aorta was dissected free and an electromagnetic flow probe (15 mm; Skalar, Delft, The Netherlands) was placed for measurement of ascending aortic blood flow. On the proximal third of the left anterior descending coronary artery (LADCA), a small segment was dissected free of its adventitia for subsequent positioning of the arterial clamp.

Regional Myocardial Contractile Function

To measure regional segment length changes in the area perfused by the LADCA, a pair of ultrasonic crystals (Triton Technology Inc., San Diego, CA, USA) was placed into the subendocardium, approximately 10 mm apart. Another pair of crystals was placed in the subendocardium of the area perfused by the left circumflex coronary artery (non-LADCA), remote from the myocardium perfused by the LADCA. From the tracings the lengths at end-diastole (EDL; time of onset of positive LVdP/dt) and end-systole (ESL; time of peak negative LVdP/dt, see ref. 11) were obtained and systolic segment length shortening (SLS) calculated as:

$$\text{SLS (\%)} \quad \frac{\text{EDL - ESL}}{\text{EDL}} \times 100$$

Regional Myocardial Blood Flows

To determine regional myocardial blood flows the left atrial appendage was catheterized for the injection of a batch of $1\text{-}2.10^6$ radioactive microspheres, 15 ± 1 (SD) μm in diameter (NEN Company, Dreieich, F.R.G.), labelled with either ^{95}Nb, ^{103}Ru, ^{113}Sn, ^{46}Sc or ^{141}Ce. Reference arterial blood samples were withdrawn from the descending aorta at a rate of 10 ml/min, starting 15 seconds before and lasting until 1 minute after injection of the microspheres. At the conclusion of each experiment, the myocardium perfused by the LADCA was identified by injecting patent violet dye into the left atrium during LADCA occlusion and the heart was excised. Hearts were fixed in 10% formalin for 48 hours, and separated into LADCA and non-LADCA regions. Each region was then divided into 3 layers of equal thickness (subendocardium, mesocardium and subepicardium). Vials containing either myocardial tissue or reference blood samples were then placed in a multichannel analyzer (Gamma Counter-5000; Packard Instrument Inc.; USA) and the radioactivity was counted for the determination of regional myocardial blood flows. Full details of the procedures and the calculation of flow data using the reference sample technique have been reported earlier (12).

Determination of Oxygen Contents and Lactate Concentrations
Blood samples were collected from the central aorta and from the cannulated vein accompanying the LADCA for the determination of oxygen and lactate contents. Oxygen saturation and hemoglobin concentrations were measured by an OSM_2 system (Radiometer; Copenhagen, Denmark). These data together with oxygen tensions were used to calculate oxygen contents. Lactate contents were determined from aliquots of one ml of whole blood that were transferred into iced-plastic tubes containing 2 ml of 0.6 M $HClO_4$ and promptly vortexed. At the conclusion of each experiment, samples were centrifuged for later analysis by the enzymatic technique (13). Lactate extraction was calculated by the arterial-coronary venous differences divided by arterial levels X 100. Myocardial oxygen and lactate consumptions were calculated for the myocardium perfused by the LADCA from the product of regional transmural blood flow and the arterial coronary venous differences in oxygen and lactate, respectively.

Experimental Protocol
Following a 30-45 minute stabilization period, baseline recordings were made of aortic and left ventricular pressures, LVdP/dt, ascending aortic blood flow and myocardial segment length changes in the area's perfused by the LADCA and the non-LADCA. Arterial and coronary venous blood samples were obtained simultaneously for determination of oxygen content and lactate concentrations and a batch of radioactive microspheres was injected into the left atrium for regional myocardial blood flow measurements. Using a small clamp, the LADCA was then completely occluded for 10 minutes (occlusion-1) and reperfused for 30 minutes (reperfusion-1). This sequence was then repeated (occlusion-2 and reperfusion-2). Throughout the two sequences of occlusion and reperfusion recordings were made of systemic hemodynamics and myocardial segment length changes. At the end of reperfusion-2, all measurements (systemic hemodynamics, segment length changes, arterial and coronary venous blood sampling and injection of radioactive microspheres) were repeated. Subsequently, the heart rate was increased by 50 beats/min via left atrial pacing and 5 minutes later all measurements were repeated. While atrial pacing was continued, an intravenous infusion of 2 μg/kg/min dobutamine was started and after 10 min measurements were again obtained. In none of the experiments this dose of dobutamine caused an increase in heart rate exceeding the heart rate set by atrial pacing. All recordings were made at 50 mm/s (Figure 1).

Statistics
Results are expressed as arithmetic means $\pm$ standard deviations. Changes were tested for significance at the $p<0.05$ level by analysis of variance with repeated measurements (Fischer's PLSD plus F-test). In addition, changes in regional myocardial blood flows in the LADCA and non-LADCA regions were compared by unpaired student's t-test.

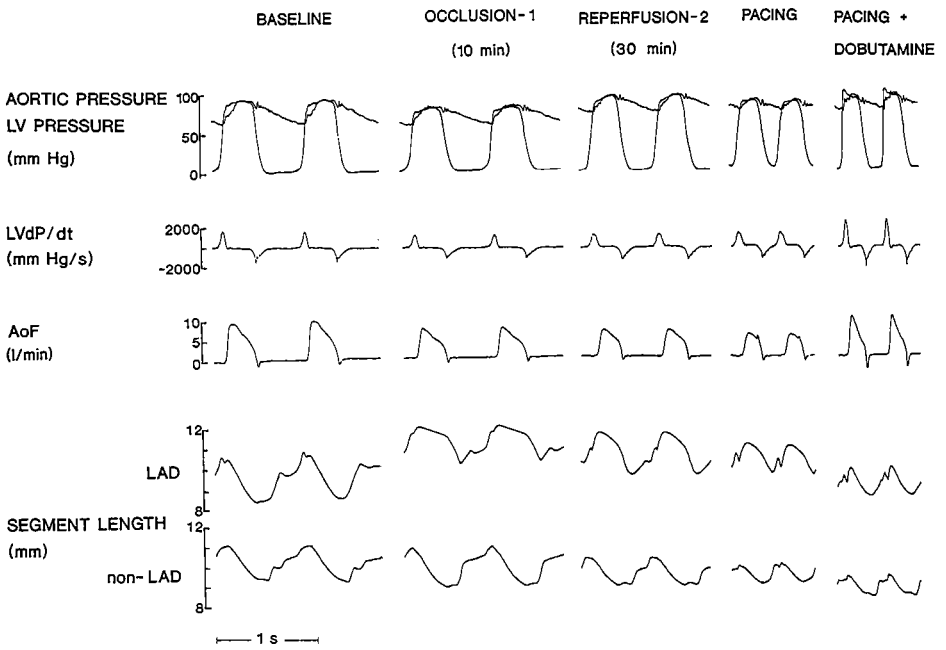

Figure 1: *Representative tracing from an animal at baseline, during the first occlusion (10 min), reperfusion-2 (30 min) and during chronotropic and inotropic stimulation following stunning.*

Results

Systemic hemodynamics

During the first coronary artery occlusion mean arterial blood pressure fell from 89 ± 13 mmHg to 74 ± 20 mmHg ($p < 0.05$), because the increase in systemic vascular resistance was insufficient to compensate for the decrease in cardiac output from 2.90 ± 0.77 l/min to 2.24 ± 0.77 l/min (Table 1). The latter was due to a decrease in stroke volume as heart rate did not change. In view of the drop in arterial blood pressure and the increase in left ventricular end-diastolic pressure from 7 ± 2 mmHg to 12 ± 5 mmHg, the 20% decrease in $LVdP/dt_{max}$ must have been responsible for the reduction in stroke volume. During the first reperfusion period there was a partial recovery of cardiac output and left ventricular end-diastolic pressure, but the other systemic hemodynamic parameters were not significantly affected by the restoration of myocardial blood flow. During the second coronary artery occlusion cardiac output again decreased, but did not recover during the subsequent reperfusion period. The other parameters were only minimally affected by the second cycle of occlusion and reperfusion (Table 1).

Table 1: *Systemic hemodynamics during chronotropic and inotropic stimulation of stunned porcine myocardium. Stunning was induced by two sequences of complete occlusion (10 min) and reperfusion (30 min) of the left anterior descending coronary artery in 12 anesthetized swine*

	Baseline	Occlusion-1	Reperfusion-1	Occlusion-2	Reperfusion-2	Atrial pacing	Atrial pacing + Dobutamine
Mean arterial blood pressure (mmHg)	89 ± 13	74 ± 20*	76 ± 14*	77 ± 11*	80 ± 11*	83 ± 10*	85 ± 10
Cardiac output (l/min)	2.90 ± 0.77	2.24 ± 0.77*	2.66 ± 0.67*	2.25 ± 0.59*	2.28 ± 0.68*	2.36 ± 0.72*	2.88 ± 0.93^{+o}
Heart rate (beats/min)	104 ± 13	103 ± 15	106 ± 19	102 ± 12	103 ± 15	153 ± 16*$^{+}$	153 ± 16*$^{+}$
LVdP/dtmax (mmHg/s)	2020 ± 440	1560 ± 400*	1630 ± 250*	1600 ± 310*	1550 ± 330*	1620 ± 300*	3320 ± 700*$^{+o}$
LV-end diastolic pressure (mmHg)	7 ± 2	12 ± 5*	9 ± 4*	9 ± 3*	7 ± 2	7 ± 3	5 ± 3*$^{+o}$
Systemic vascular resistance (mmHg.min/l)	32.1 ± 7.7	35.4 ± 9.2*	37.0 ± 9.4*	36.1 ± 10.1*	38.0 ± 11.3*	37.6 ± 10.7*	31.7 ± 8.2^{+o}
Stroke volume (ml)	27.4 ± 6.2	23.3 ± 5.4*	23.3 ± 5.1*	21.5 ± 5.5*	22.1 ± 5.7*	15.3 ± 4.2*$^{+}$	18.7 ± 5.6*$^{+o}$

Hearts were paced at 50 beats/min above the heart rate measured at the end of Reperfusion-2. Dobutamine was infused intravenously at a rate of 2 μg/kg/min. LV = left ventricular. Data have been expressed as means ± sd; * $P<0.05$ vs Baseline; $^{+}$ $P<0.05$ vs Reperfusion-2; ^{o} $P<0.05$ vs Atrial pacing

Atrial pacing had no effect on any of the systemic hemodynamic parameters, except for a decrease in stroke volume from 22.1 ± 5.7 ml to 15.3 ± 4.2 ml ($p<0.05$). During the additional intravenous infusion of dobutamine, stroke volume partially recovered to 18.7 ± 5.6 ml ($p<0.05$) due to a doubling of $LVdP/dt_{max}$ and a 15% reduction in systemic vascular resistance (Table 1).

Regional myocardial contractile function and work

During the first coronary artery occlusion, the segment perfused by the LADCA became dyskinetic as systolic segment length shortening decreased from 18.3 ± 5.2% to -3.7 ± 2.5% (Table 2), while the non-LADCA perfused segment showed enhanced shortening during occlusion (from 13.2 ± 3.7% to 15.4 ± 6.2%, $p<0.05$). At 30 min of reperfusion, contractile function of the LADCA perfused area had only partially recovered (7.2 ± 5.0%, $p<0.05$ vs baseline). During the second cycle of occlusion and reperfusion similar values for systolic segment length shortening were observed, indicating little additional effect of the second cycle on contractile function.

Atrial pacing reduced end-diastolic length in both the LADCA and the non-LADCA perfused areas, while the end-systolic lengths were minimally affected. As a result systolic segment length shortening decreased in both areas. Dobutamine increased systolic shortening from 4.3 ± 3.7% to 14.0 ± 5.0% ($p<0.05$) in the LADCA perfused area, but in the non-LADCA area the increase in systolic segment length shortening was only minor.

Figure 2 shows an example of the left ventricular pressure-segment length relationship for both the LADCA and non-LADCA perfused areas. During the first occlusion the myocardium perfused by the LADCA did not perform any work (Table 3). By the end of the second period of reperfusion, there was a return to 50 ± 14% of baseline. At the same time, the work performed by the myocardium remote from the LADCA perfused area, was reduced to 75 ± 10% of baseline. Atrial pacing reduced work per minute, performed by both the LADCA and non-LADCA perfused myocardium by 15-30%, compared to the measurements obtained at the end of reperfusion-2. The additional infusion of dobutamine restored work in both segments to values measured at baseline (Table 3).

Myocardial blood flow

Two cycles of coronary artery occlusion and reperfusion, reduced transmural myocardial blood flow in the LADCA perfused area from 1.48 ± 0.42 ml/min/g to 0.94 ± 0.27 ml/min/g ($p<0.05$), which was equally distributed over all layers (Table 4). In contrast, in the non-LADCA perfused area blood flow was reduced only in the subendocardial layers, as a result of which the subendocardial-subepicardial perfusion ratio decreased from 1.08 ± 0.13 to 0.94 ± 0.13 ($p<0.05$). The decrease in subendocardial perfusion of the LADCA area (0.51 ± 0.27 ml/min/g) was slightly larger than the decrease in subendocardial perfusion of the non-LADCA area (0.30 ± 0.43 ml/min/g). In contrast to the transmural myocardial perfusion, the response of the subendocardial perfusion in the LADCA area was not significantly different from that in the non-LADCA area ($p=0.08$).

Atrial pacing did neither affect transmural myocardial perfusion nor its distribution across the left ventricular wall. Dobutamine, infusion on the other hand, increased myocardial blood flow in both the LADCA and the non-LADCA perfused areas (Table 4).

Table 2: *Segment length changes during chronotropic and inotropic stimulation of stunned porcine myocardium. Stunning was induced by two sequences of complete occlusion (10 min) and reperfusion (30 min) of the left anterior descending coronary artery in 12 anesthetized swine*

	Baseline	Occlusion-1	Reperfusion-1	Occlusion-2	Reperfusion-2	Atrial pacing	Atrial pacing + Dobutamine
LADCA area							
EDL (mm)	12.2 ± 2.2	13.4 ± 2.3*	12.5 ± 2.3	13.3 ± 2.2*	12.6 ± 2.2	12.0 ± 2.0^{+}	11.7 ± 2.1*$^{+}$
ESL (mm)	9.8 ± 1.9	13.9 ± 2.6*	11.6 ± 2.4*	13.9 ± 2.6*	11.8 ± 2.4*	11.6 ± 2.2*	10.0 ± 2.0^{+o}
SLS (%)	18.3 ± 5.2	-3.7 ± 2.5*	7.2 ± 5.0*	-4.1 ± 2.6*	6.6 ± 4.6*	4.3 ± 3.7*	14.0 ± 5.0*$^{+o}$
non-LADCA area							
EDL (mm)	10.7 ± 1.1	11.1 ± 1.1*	10.9 ± 1.2	11.2 ± 1.2*	10.8 ± 1.1	10.1 ± 1.1^{+}	9.8 ± 1.2*$^{+}$
ESL (mm)	9.3 ± 1.1	9.4 ± 1.2	9.5 ± 1.1	9.6 ± 1.2	9.4 ± 1.0	9.3 ± 1.0	8.8 ± 1.0*$^{+o}$
SLS (%)	13.2 ± 3.7	15.4 ± 6.2*	13.2 ± 4.1	14.8 ± 4.9*	12.9 ± 3.8	8.2 ± 3.7*$^{+}$	10.2 ± 3.5*$^{+o}$

LADCA = left anterior descending coronary artery; EDL = end-diastolic length; ESL = end-systolic length; SLS = systolic segment length shortening. Hearts were paced at 50 beats/min above the heart rate measured at the end of Reperfusion-2. Dobutamine was infused intravenously at a rate of 2 μg/kg/min; Data have been expressed as means ± sd. * $P<0.05$ vs Baseline; $^{+}$ $P<0.05$ vs Reperfusion-2; ^{o} $P<0.05$ vs Atrial pacing.

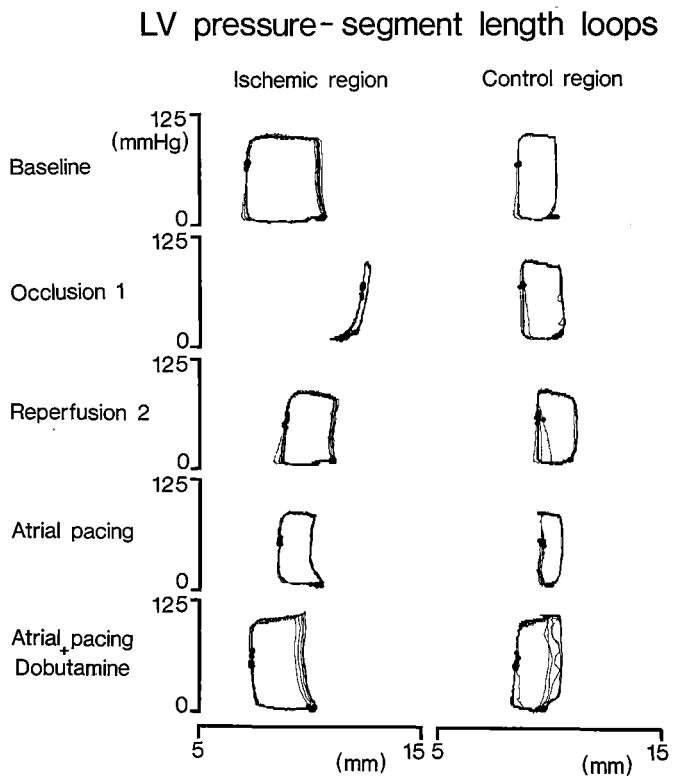

Figure 2: *Representative examples of LV pressure-segment length loops in both myocardial regions, at baseline, during occlusion-1, following reperfusion-2 and during atrial pacing and atrial pacing with dobutamine.*

Myocardial metabolism of the LADCA perfused area

At the end of reperfusion-2, myocardial oxygen consumption was 70%, whereas lactate consumption was only 15% of their respective baseline values (Table 4).

During atrial pacing myocardial oxygen consumption increased to 75% and lactate consumption to 30% of their respective baseline levels. During the additional dobutamine infusion, myocardial oxygen consumption returned to baseline but lactate consumption remained depressed at 40-45% of baseline (Figure 3).

Table 3: *Normalized work during chronotropic and inotropic stimulation of stunned porcine myocardium. Stunning was induced by two sequences of complete occlusion (10 min) and reperfusion (30 min) of the left anterior descending coronary artery in 12 anesthetized swine*

	Baseline	Occlusion-1	Reperfusion-2	Atrial pacing	Atrial pacing + Dobutamine
LADCA area					
Normalized work (mmHg.mm/min)	1959 ± 556	28 ± 153*	968 ± 322*	657 ± 508*$^{+}$	1926 ± 807^{+o}
non-LADCA area					
Normalized work (mmHg.mm/min)	1692 ± 563	1560 ± 626	1246 ± 365	1026 ± 393*	1881 ± 710^{+o}

LADCA = left anterior descending coronary artery. Hearts were paced at 50 beats/min above the heart rate measured at the end of Reperfusion-2. Dobutamine was infused intravenously at a rate of 2 μg/kg/min. Data have been expressed as means ± sd; * $P<0.05$ vs Baseline; $^{+}$ $P<0.05$ vs Reperfusion-2; ^{o} $P<0.05$ vs Atrial pacing.

Table 4: *Regional myocardial blood flows and metabolism during chronotropic and inotropic stimulation of stunned porcine myocardium. Stunning was induced by two sequences of complete occlusion (10 min) and reperfusion (30 min) of the left anterior descending coronary artery in 12 anesthetized swine*

	Baseline	Reperfusion-2	Atrial pacing	Atrial pacing + Dobutamine
Myocardial blood flow (ml/min/g)				
LADCA area				
transmural	1.48 ± 0.42	0.94 ± 0.27*	1.07 ± 0.35*	1.50 ± 0.44^{+o}
endocardium	1.44 ± 0.44	0.93 ± 0.28*	1.05 ± 0.36*	1.44 ± 0.42^{+o}
epicardium	1.52 ± 0.42	0.94 ± 0.27*	1.08 ± 0.34*$^{+}$	1.56 ± 0.42^{+o}
endo/epi	0.95 ± 0.13	0.99 ± 0.18	0.96 ± 0.12	0.92 ± 0.09
non-LADCA area				
transmural	1.49 ± 0.43	1.27 ± 0.52	1.47 ± 0.64	1.76 ± 0.60*$^{+o}$
endocardium	1.54 ± 0.47	1.23 ± 0.53*	1.37 ± 0.60	1.65 ± 0.57^{+o}
epicardium	1.42 ± 0.41	1.32 ± 0.53	1.55 ± 0.28^{+}	1.91 ± 0.66*$^{+o}$
endo/epi	1.08 ± 0.13	0.94 ± 0.13*	0.88 ± 0.07*	0.87 ± 0.10*
Myocardial metabolism				
LADCA area				
Oxygen content (µmol/ml)				
arterial	4.45 ± 0.80	4.72 ± 0.88*	4.70 ± 0.89	4.68 ± 0.92
arterial-venous difference	3.28 ± 0.47	3.48 ± 0.47*	3.62 ± 0.66*$^{+}$	3.20 ± 0.69^{+o}
Oxygen extraction (%)	75 ± 6	75 ± 10	79 ± 11*$^{+}$	68 ± 12*$^{+o}$
Oxygen consumption (µmol/min/g)	4.80 ± 1.40	3.24 ± 0.80*	3.84 ± 1.20*$^{+}$	4.70 ± 1.36^{+o}
Lactate content (µmol/ml)				
arterial	1.89 ± 0.92	1.91 ± 0.81	1.79 ± 0.68	1.60 ± 0.63*$^{+}$
arterial-venous difference	0.67 ± 0.22	0.16 ± 0.21*	0.27 ± 0.14*	0.30 ± 0.17*$^{+}$
Lactate extraction (%)	40.0 ± 14.3	7.5 ± 10.6*	15.7 ± 8.3*$^{+}$	19.0 ± 9.0*$^{+}$
Lactate consumption (µmol/min/g)	1.02 ± 0.46	0.16 ± 0.21*	0.29 ± 0.20*	0.44 ± 0.27*$^{+}$

LADCA = left anterior descending coronary artery. Hearts were paced at 50 beats/min above the heart rate measured at the end of Reperfusion-2. Dobutamine was infused intravenously at a rate of 2 µg/kg/min. endo/epi = ratio of the normalized endocardial and epicardial blood flows. Data have been expressed as means ± sd. * $P<0.05$ vs Baseline; $^{+}$ $P<0.05$ vs Reperfusion-2; ^{o} $P<0.05$ vs Atrial pacing; (see table 1)

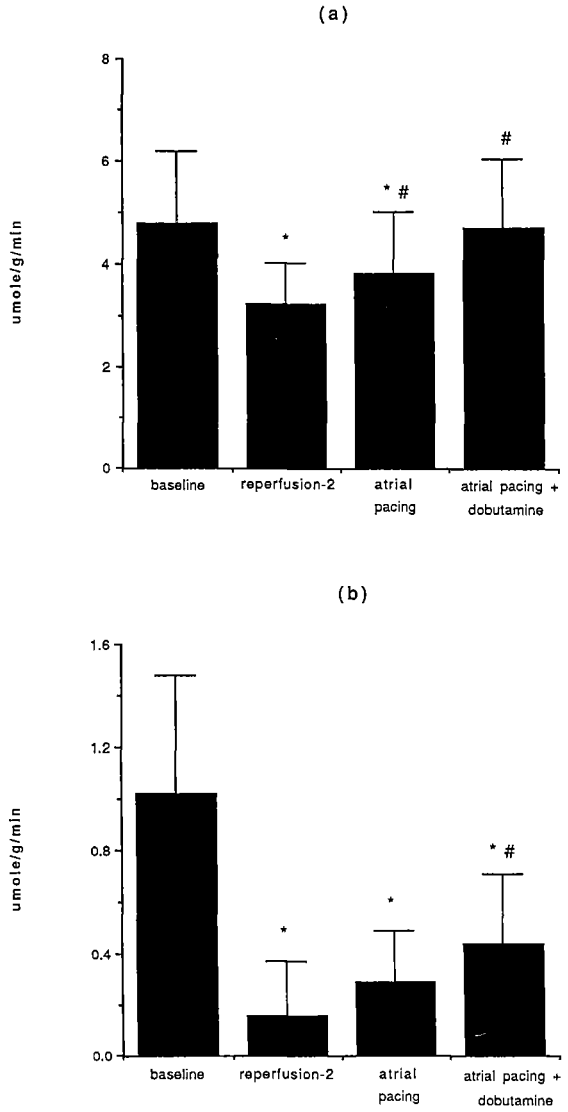

Figure 3: *(a) Oxygen and (b) lactate consumptions in the LADCA region at baseline, during reperfusion-2 (30 min) and with chronotropic and inotropic stimulation following stunning; data expressed as means+sd; n=12; *-p<0.05 vs baseline; #-p<0.05 vs reperfusion-2.*

Discussion

In this study, we have used a model of ischemia and reperfusion which induces severe changes in function following reperfusion without evidence of necrosis (10). We observed that at the end of reperfusion-2, myocardial blood flow was lower in the postischemic myocardium then in the control region. In view of the abundant evidence that the maximal vasodilatory capacity of the coronary vasculature is not or only minimally affected (5,8,9,14-16) in stunned myocardium, mechanical obstruction due to capillary plugging by neutrophils or edema formation, phenomena observed during prolonged periods of ischemia (17,18), can be excluded as the cause for the reduction in myocardial blood flow. Furthermore, our data demonstrate that coronary blood flow which is reduced at a time when postischemic function is severely depressed, can return to preischemic levels when the myocardium is stimulated by chronotropic and inotropic stimulation.

It has been suggested that the reduction in myocardial blood flow is metabolically regulated in accordance with the reduction in cardiac performance (19). That this reduction in blood flow is indeed the consequence, rather than the cause of the reduction in contractile function is also suggested by observations from other laboratories that contractile reserve is still present in stunned myocardium (20-22). However, in several studies using brief periods of ischemia and reperfusion myocardial blood flow has been shown to be similar to pre-ischemic values, despite significant reductions in contractile function (6-9). These observations suggest that myocardial oxygen consumption in regionally stunned myocardium is maintained due to either mitochondrial uncoupling, altered efficiency of energy utilization or an increased energy use for maintainance of cellular homeostasis (7). Our findings of a lower myocardial oxygen consumption are not necessarily inconsistent with these studies (6-9). The contractile state of the myocardium is an important determinant of myocardial oxygen consumption. With severe reductions in postischemic contractile function, the increase in oxygen consumption, due to mitochondrial uncoupling, altered efficiency or increased energy use for maintainance of cellular homeostasis may be masked by the attenuated oxygen demand due to the depression of contractile performance. Differences in studies may thus be explained by variable degrees of myocardial stunning. In the present study the changes in the index of myocardial work per minute parallelled the changes in blood flow following stunning, as well as following chronotropic and inotropic stimulation of the stunned myocardium with dobutamine. Another factor explaining the differences between our and previous studies (6-9) may be the response of systemic hemodynamics to ischemia and reperfusion. The index of myocardial work, used in the present study incorporates changes in heart rate and developed left ventricular pressure as well as changes in segment length shortening. This may explain the better prediction of blood flow and oxygen consumption by this index as compared to segment length shortening alone. During atrial pacing both systolic segment length shortening and the index of myocardial work decreased in both areas in the face of an increase in myocardial blood flow and oxygen consumption; an observation not readily explained. It could be that with atrial pacing activation of the myocardial contraction is not translated into myocardial work, because of an impaired filling of the left ventricle, which suggests that under these conditions a change in oxygen consumption is not solely governed by a change in myocardial work. Finally during

infusion of dobutamine myocardial blood flow and oxygen consumption returned to baseline levels however, myocardial substrate utilization was still altered, as reflected by a depressed lactate consumption. The mechanism leading to the altered substrate utilization can not be determined from the present study. In studies using longer periods of ischemia involvement of an increased production of lactate via enhanced uptake of substrates like glucose has been shown (23,24). In addition, a decreased oxidation of these substrates may play a role (25). From the data in Table 4, it becomes clear that the increase in oxygen consumption can not be explained by an increase in lactate consumption, suggesting an impairment in the lactate utilization capacity. Nevertheless, chronotropic and inotropic stimulation increased myocardial lactate consumption, indicating that this lactate utilization capacity was not exhausted.

Conclusion

In conclusion, we have shown in this model of myocardial stunning, that myocardial blood flow and myocardial oxygen consumption are reduced following reperfusion, parallel with a reduction in myocardial work. The observation that during chronotropic and inotropic stimulation with dobutamine blood flow and oxygen consumption increase, again in parallel with myocardial work, suggests that the lower blood flow in stunned myocardium is metabolically regulated and is not due to impairment of the vasodilatory capacity.]

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Chapter 10

Discussion

DISCUSSION

This book is divided into three sections. The first presents the concept of coronary flow reserve with a specific application to patient care; the second presents experimental data from anesthetized swine to help define the relationships between myocardial blood flow, metabolism and function once autoregulation of blood flow is lost; the final section also includes experimental data from swine and characterizes how brief periods of ischemia and reperfusion further alter the relationships defined in section 2.

Coronary flow reserve

Coronary blood flow reserve, the ratio of maximal to resting coronary flow, has become a widely used clinical tool. In selected patients, it can supply valuable information for the diagnosis and management of abnormal pathophysiological states (2-4). Many invasive tools have become availiable to measure coronary flow reserve in patients and have been well validated experimentally (5). These techniques are limited however, because they can not quantitate blood flow or measure regional differences in coronary blood flow. In addition, they measure absolute flow reserve, which is not specific to changes in the coronary vasculature (10).

Positron emission tomography (PET) is a non-invasive tool which measures the myocardial uptake of radioactive tracers, administered either intravenously or by inhalation. With rapid, high resolution scanning, tomographic slices of the entire heart can be acquired and time activity curves measured for the determination of regional myocardial blood flows. By comparing the maximal flow response between normal and abnormal regions, relative flow reserves can be determined which are a more independent measure of abnormal vasodilator capacity (10). In chapter 2, we showed how such measurements could be obtained in normal volunteers, and a group of patients with collateral-dependent myocardium. The results of the myocardial blood flows in both groups are consistent with flows acquired either from other techniques or from flows observed with radiolabeled microspheres in experimental animal preparations. The data show that coronary flow reserve in normal volunteers is greater than 4, which is also consistent with the findings of others (5). Myocardial blood flow in the distribution of completely collateralized myocardium in the patient group was normal at rest (0.8-1.1 ml/g/min) but did not increase normally following infusions of dipyridamole. The relative flow reserve values correlated well with the length of time and the workload performed on standard exercise tests. Thus, this technique quantitatively demonstrates why patients with well developed intramyocardial collateral flow continue to have angina during high work loads. It emphasizes that colleral blood formation in patients can provide normal perfusion at rest, but have a limited capacity to maximally vasodilate. Thus, PET can provide a better understanding of the pathophysiology of collateral blood flow in humans.

Chapter 3 addresses an important clinical question with the interpretation of flow reserve measurements in patients. That is, can the use of antiischemic therapies alter the results. In anesthetized swine, maximal coronary flow-pressure relationships were

characterized prior to and following 3 doses of either the selective beta-1 adrenoceptor antagonist metoprolol, the selective beta-1 partial agonist epanolol or the calcium antagonist nifedipine. Like relative flow reserve measurements, maximal flow-pressure lines accurately define changes in coronary vasculature and are minimally influenced by systemic hemodynamics (9). In this study, we showed that only nifedipine alters the maximal mean flow-pressure relationship. The data also support the hypothesis that calcium blockers are effective during ischemia by antagonizing endogenous adenosine and preventing redistribution of flow away from the subendocardium.

Reserve during ischemia

The purpose of the second section was to demonstrate how the myocardium adapts to acute ischemia. Autoregulation is the capacity of the myocardium to regulate resistance so that coronary blood flow remains constant over a wide range of perfusion pressures (11). It allows the myocardium to regulate its own flow according to myocardial oxygen demand, even in the presence of pathophysiological states such as coronary artery disease and ventricular hypertrophy. When perfusion pressure is reduced below a critical level, autoregulation is lost and ischemia develops. In chapter 4, we have shown that this "breakpoint" of the autoregulation curve in our model of anesthetized swine occurs at about 50 mm Hg. This is consistent with the findings of other studies, however the type of anesthesia and the animal species used may account for significant differences (14). In addition, hemodynamic changes such as heart rate may also alter the absolute level of coronary driving pressure at which ischemia develops (15).

Below this "breakpoint" of the autoregulatory curve, further reductions in perfusion pressure correlate in a near linear fashion with reductions in flow and transmural function and alterations in metabolism. Although these relationships have been well characterized in other models, the combination of all parameters over a wide range of ischemic pressures has not been previously performed. We see from chapter 4, that the reserve to maintain normal function during ischemia is limited. As has been shown elsewhere (19), oxygen extraction can increase during flow reductions, however this accounts for only a small increase in overall oxygen delivery. The degree of anaerobic glycolysis increased relative to the degree of flow reductions and may be responsible for a slight increaase in ATP production at a time when aerobic oxidation of substrates has ceased (65). The chapter emphasizes that once flow is reduced, the degree of metabolic reserve to supply and preserve function is minimal.

Because of differences in extravascular compression across the myocardial wall, the subendocardium is more vulnerable to reductions in perfusion pressure than the subepicardium (16). This probably accounts for differences in vasomotor tone across the wall, at a time when coronary blood flow is reduced. In chapter 4, we present a model of constant flow ischemia, with and without vasomotion intact. The data demonstrate that during moderate ischemia, vasomotor tone is greater in the subepicardial layers which when released with intracoronary adenosine, results in a reduction in subendocardial perfusion. This then causes a reduction in transmural function and an increase in the production of

lactate, at a time when overall oxygen consumption remains the same. This emphasizes another important component of myocardial reserve during ischemia which is the capacity to regulate vasomotor tone in different regions of the wall.

It is clear that the capacity to regulate coronary flow during ischemia is the most efficient means of the myocardium to preserve function. In chapter 5, we show that intracoronary infusions of adenosine during ischemia can increase transmural coronary blood flow during the increased demand of tachycardia. This has been demonstrated in other models during basal states of ischemia (28-30) and suggests that pharmacological vasodilation is greater than endogenous vasodilation during moderate ischemia. Interestingly, minimal improvement in either function or metabolism is observed during this extra vasodilation, which implies that factors other than flow may be important regulators of function during ischemia. Some of these factors may include the intracellular accumulation of lactate, hydrogen ion or intracellular calcium (66).

Reserve following ischemia and reperfusion

In the third section, we have presented a model of myocardial stunning induced by brief ischemia (5-15 minutes) and reperfusion. This is an important model because such short periods of ischemic time induce substantial reductions in function at a time when necrosis would not be expected (37). Chapter 6 introduces stunning in swine induced by partial coronary occlusions and reperfusion. It confirms that post-ischemic reductions in function are related to the degree of flow reduction during ischemia, and shows that the functional losses are additive following sequential ischemic periods. It also shows that the functional abnormalities occur at a time when the distribution of coronary blood flow as measured by microspheres has returned to normal.

The capacity to vasodilate in regionally stunned myocardium may be abnormally low (44). The mechanisms that could account for this include capillary plugging by neutrophils or edema formation as well as altered endothelial cell function (37-41). Many of these studies have been performed in models using extended ischemic times, when necrosis would also be expected. In chapters 7 and 8, we determined if the capacity to vasodilate was altered, in models of pure stunning. In the first study, we showed that the response of ATP, an endothelial-dependent vasodilator was not altered at a time when function was reduced following reperfusion. In the second study, we showed that slopes of the maximal coronary pressure-flow relationship with maximal intracoronary infusions of adenosine were not depressed, but actually were greater than preischemic baseline values. This enhanced flow reserve response supports the hypothesis that myocardial mechanical performance can be depressed following reperfusion, at a time when functional changes in the vasculature are normal. Because maximal flow was actually higher following reperfusion, it suggests that other factors may be responsible for the capacity to maximally vasodilate such as regional contractility.

In chapter 9, we show that total coronary blood flow and oxygen consumption in the distribution of regionally stunned myocardium is lower than the non-ischemic regions. Other studies have indicated that coronary blood flow in the stunned myocardial region is not

lower following reperfusion (44,45). This discrepancy may be a result of differences between animal species (eg. the presence of collaterals in dogs), the variable degree of stunning between models or changes in hemodynamics during the experiment, making it difficult to interpret changes in post-ischemic flow and function. We have shown that with the recruitment of function following reperfusion, blood flow and oxygen consumption increase to similar degrees. This suggests that the reduced blood flow following stunning is metabolically regulated to the reductions in work performed, and not to an altered capacity to vasodilate. We have also shown that oxygen and lactate utilization are abnormal following reperfusion, but can increase during the higher demands, showing the presence of metabolic reserve. These findings emphasize the fact that blood flow and metabolic alterations may coexist with functional alterations, but are not the primary cause of the contractile abnormalities.

Future perspectives

The theme of these chapters has been myocardial reserve. In the first section, positron emission tomogrophy measurements of relative flow reserve provided both important clinical information as well as an understanding of human collateral blood flow. The tool offers the researcher an important means to understand coronary blood flow regulation in humans, and could be applied to a number of pathophysiological conditions such as hypertrophic cardiomyopathy and Syndrome X. In addition to coronary blood flow, it has the capacity to quantitate regional differences in myocardial metabolism and thus can be used to identify viable myocardium in patients considered for interventions.

The second and third sections characterize myocardial reserve in swine during acute ischemia and following reperfusion. In the future, the most important impact in basic cardiovascular research will come from understanding the adaptive processes which occur as a result of chronic exposure to blood flow reductions and reperfusion. For instance, it is apparent that the myocardium has the potential to down-regulate its needs during severe ischemia, and prevent cellular death. This has been termed "hibernation" clinically (67), and involves different processes than those observed during the acute onset of ischemia outlined in the first section. Likewise, "stunning" may not be just a non-specific injury response which reduces oxygen consumption following reperfusion but rather an adaptation to maintain cellular homeostasis during stress. Recent evidence shows that the stunned myocardium undergoes a "preconditioned" state which protects it from subsequent prolonged periods of ischemia (68). These adaptations include changes in bioenergetics which improve energy supply (69) or may involve expression of different genes and the elaboration of new "stress" proteins which increase cellular resistance (70). The induction of these proteins may be protective to the heart, either by altering the metabolism or the vasculature. It is conceivable that they may explain some of our observations in the stunned myocardium, such as the improved coronary flow reserve measurements following reperfusion. A greater understanding of those mechanisms that allow the myocardium to adapt should provide the clinician with more therapies to improve function or maintain cellular viability with the ischemic syndromes.

SAMENVATTING

Dit proefschrift is opgebouwd uit drie delen, betrekking hebbend op "myocardiale reserve". Het eerste deel gaat over het concept van "coronaire flow reserve" en de specifieke toepassing daarvan in de patiëntenzorg. Het tweede deel behandelt de relaties tussen doorbloeding, metabolisme en functie van het myocard, in situaties waarin autoregulatie van de doorbloeding ontbreekt. Het derde deel bespreekt hoe deze relaties veranderd zijn tijdens reperfusie volgend op een korte periode van ischemie.

Coronary flow reserve

De coronaire flow reserve, gedefineerd als de verhouding tussen de maximale doorbloeding van de kransslagaders en de doorbloeding in rust, is in de kliniek een veel gebruikt hulpmiddel geworden, waarmee kwantitatieve informatie wordt verkregen over de ernst van een kransslagadervernauwing. Voor het meten van de coronaire flow reserve in patiënten staan diverse invasieve meettechnieken ter beschikking, waaronder de intracoronaire Doppler-bloedstroom snelheidsmetingen. Een beperking van deze technieken is dat ze niet in staat zijn om de doorbloeding te kwantificeren of de regionale verschillen in doorbloeding te meten. Een ander nadeel van de coronaire flow reserve metingen is dat een hogere doorbloeding in rust, een lagere flow reserve oplevert zonder dat de maximale flow capaciteit is veranderd.

Een non-invasieve meettechniek is de Positron Emission Tomography (PET). Met deze methode meet men de opname door het myocard van radioactieve tracers na toediening via intraveneuze injectie of via inhalatie. Verder kan men tomografische coupes van het hele hart verkrijgen, alsmede de "time-activity curve" waaruit men de regionale myocard doorbloeding kan bepalen. Door de normale en de abnormale geperfundeerde gebieden van het myocard met elkaar te vergelijken, kan men de relatieve flow reserve (de verhouding tussen maximale doorbloeding van myocard geperfundeerd door een stenotische coronair arterie en de maximale doorbloeding van myocard geperfundeerd door een normale coronair arterie) vaststellen. Het voordeel van de relatieve flow reserve is dat deze niet wordt beïnvloed door systemische hemodynamische veranderingen en niet afhankelijk is van de doorbloeding in rust. In het tweede hoofdstuk hebben we laten zien, op welke wijze men dergelijke metingen kan verkrijgen bij normale vrijwilligers en bij een groep patiënten, wier myocard voor de bloedvoorziening afhankelijk is van collateralen. Het bleek dat bij de normale vrijwilligers de doorbloeding van het myocard na toediening van dipyridamole kan verviervoudigen, hetgeen in overeenstemming is met coronaire flow reserve waarden die via andere technieken verkregen waarden. Bij patiënten waarvan in rust de myocard doorbloeding in rust afhankelijk van het collaterale vaatbed, vindt geen toename in doorbloeding plaats na toediening van dipyridamole. Er bestaat een goede relatie tussen de waarden van de relatieve flow reserve en de hoeveelheid en duur van arbeid tijdens standaard belastingsproeven. Deze non-invasieve techniek is daarom zeer geschikt om potentiëel ischemisch myocard te identificeren.

Een probleem is dat de invloed van anti-ischemische medicamenten op de coronaire flow reserve, niet goed gedocumenteerd is. In hoofdstuk 3 zijn de coronaire druk-

stroom relaties beschreven tijdens maximale vaatverwijding, geïnduceerd door adenosine in geanaestheseerde varkens van 3 anti-ischemische stoffen: de selectieve β_1-blokker metoprolol, de selectieve β_1 partieële agonist epanolol en de calcium antagonist nifedipine. Net als de metingen van de relatieve flow reserve zijn de maximale druk-stroom curves onafhankelijk van de basale doorbloeding en onafhankelijk van de coronaire perfusiedruk. In dit onderzoek hebben we aangetoond, dat alleen nifedipine de verhoudingen tussen maximale bloedstroom en druk verandert.

Reserve tijdens ischemie

Het myocard bezit het vermogen om de perfusie te autoreguleren, d.w.z. om de perfusie over een wijd bereik van de perfusiedruk hetzelfde te houden, bij een constant metabolisme. Valt de perfusiedruk beneden een kritisch niveau, dan gaat de autoregulatie verloren en treedt er ischemie op. Beneden het kritische punt van de autoregulatie curve neemt de perfusie met het dalen van de perfusiedruk bijna lineair af. Hierbij treden tevens een vermindering in de contractiele functie en een verandering in het metabolisme op. In hoofdstuk 4 hebben we aangetoond, dat het vermogen om tijdens ischemie een normale functie te behouden beperkt is. Hoewel het hart via toename zuurstofextractie en m.b.v. anaerobe glycolyse de ATP-productie tracht te handhaven blijkt dat er slechts een zeer geringe metabole reserve bestaat om de functie te behouden wanneer er een vermindering in de doorbloeding optreedt.

Vanwege de transmurale verschillen in extravasculaire compressie van het myocard, is het subendocard kwetsbaarder voor een vermindering in de coronaire perfusie dan het subepicard. In hoofdstuk 4 tonen we aan dat tijdens matige ischemie de vaattonus in de subepicardiale lagen groter is dan in de subendocardiale lagen. Vermindert men de vaattonus in het subepicard met behulp van een intracoronaire infusie van adenosine, dan leidt dit tot een afname van de subendocardiale perfusie met een vermindering van de transmurale functie en een toename in lactaatproduktie. Dit benadrukt een andere belangrijke eigenschap van het myocard tijdens ischemie, namelijk het vermogen om de vaattonus te reguleren in de verschillende lagen van de wand.

In hoofdstuk 5 beschrijven we, dat zelfs na verhoging van de zuurstofbehoefte, de transmurale doorbloeding van ernstig ischemisch myocard kan stijgen door een intracoronaire toediening van adenosine. Dit betekent dat op een moment dat de endogene "vasodilator reserve" is uitgeput, het nog mogelijk is om pharmacologisch het vaatbed nog verder te verwijden. Interessant is echter, dat recrutering van de vasodilator reserve niet resulteert in een onmiddellijke verbetering van de contractiele functie en metabolisme van het myocard. Het is dus waarschijnlijk, dat naast de doorbloeding er nog andere factoren zijn, die een rol spelen bij de regulatie van de functie tijdens ischemie. Hierbij kunnen we denken aan intracellulaire accumulatie van lactaat, protonen en calciumionen.

Reserve na ischemie en reperfusie

In het derde deel hebben we aspecten bestudeerd van "myocardiale stunning" (= verdoving) in genarcotiseerde varkens. Stunning werd geïnduceerd door een korte periode (5-15 min) van ischemie, gevolgd door reperfusie. Dergelijke korte perioden van ischemie resulteren in een substantiële afname in contractiele functie zonder dat necrose optreedt. In hoofdstuk 6 hebben we aangetoond, dat na 25 minuten reperfusie, op een moment dat de coronaire bloedstroom weer genormaliseerd is, de contractiele functie nog niet is hersteld. Op dat moment is er sprake van een verminderde coronaire bloedstroom naar en vertraaagde zuurstof consumptie door het "gestunde" myocard in vergelijking met het controle myocard. Dit wordt niet altijd gevonden. Proefdier gebonden eigenschappen (aanwezigheid collateralen), en mogelijk daarmee samenhangend, de mate van "stunning" en de veranderingen in systemisch hemodynamische parameters bemoeilijken echter het interpreteren van de veranderingen in doorbloeding en contractie, functie en zuurstofconsumptie. Wat we wel zien is dat tijdens reperfusie, de bloedstroom en de zuurstof consumptie in dezelfde mate toenemen, als het hart wordt gestimuleerd door de hartfrequentie te verhogen en door toediening van dobutamine suggereert (hoofdstuk 7), dat de gevonden daling in doorbloeding tijdens "stunning" op metabole wijze gereguleerd wordt. Reductie in de verrichte arbeid en niet een veranderd vermogen tot vaatverwijden, lijken ten grondslag te liggen aan de gedaalde doorbloeding. Tevens blijkt uit hoofdstuk 7 dat het gestunde myocard zowel voldoende functionele metabole als endogene vaatverwijdingsreserve bezit.

Sommige onderzoekers hebben gesuggereerd, dat na korte periode van ischemie gevold door het vermogen tot maximaal verwijden van de vaten verminderd is. In de hoofdstukken 7 en 8 tonen we echter aan dat tijdens "myocardiale stunning", het vermogen tot vasodilatatie in deze gebieden echter nog intact is. In het "gestunde" gebied treedt vasodilatatie op na toediening van zowel adenosine als de endotheel- afhankelijke vaatverwijder ATP. Met deze experimenten hebben we een onderscheid kunnen maken tussen post-ischemische veranderingen in de contractie functie van het myocard en de vaatresponsies.

Vooruitzichten in de toekomst

In dit proefschrift heeft het onderwerp functionele en flow reserve van het myocard centraal gestaan. In het eerste deel is beschreven hoe de coronaire flow reserve in patiënten is gemeten met behulp van de Positron Emissie Tomographie (PET). Met deze techniek is het mogelijk de relatieve flow reserve te meten. Het toepassingsgebied van dit apparaat lijkt niet beperkt tot het kunnen identificeren van potentiëel ischemisch myocard in patiënten. Het vermogen om verschillen in de regionale myocard perfusie op microcirculatoir niveau te meten, biedt de mogelijkheid informatie te verstrekken over zeldzame ischemische syndromen, zoals syndroom X. De vraag is of er ook informatie verschaft kan worden over regionale verschillen in myocardiaal metabolisme. De mogelijkheid om levend van dood myocard onderscheiden, is van belang bij het selecteren van patiënten die voor een operatie in aanmerking komen.

In het tweede deel van dit proefschrift lag de nadruk op de acute adaptieve processen in het myocard na een vermindering in perfusie. In de toekomst zal het accent waarschijnlijk meer verschuiven in de richting van chronische aanpassingen van het myocard. Er zijn recent aanwijzingen gevonden dat het myocard zijn zuurstofbehoefte kan verlagen zodat ondanks langdurige vermindering in perfusie de myocyten overleven. Dit proces wordt "hibernation" (= winterslaap) genoemd en is wezenlijk verschillend van processen die optreden tijdens acute ischemie. In deel 3 van dit proefschrift hebben we via interventies de functionele en flow reserve van "gestunned" myocard bestudeerd. In de nabije toekomst zal het onderzoek zich waarschijnlijk meer richten naar de processen die dit "stunnen" veroorzaken, maar om inzicht te krijgen waarom het myocard verminderd functioneert, terwijl het wel een normale functie reserve bezit. Men moet "stunning" misschien niet zien als een aspecifieke reactie op beschadiging ten gevolge van ischemie, die de zuurstof consumptie verlaagt gedurende de reperfusie, maar meer als een aanpassing om de homeostase van de cel onder stess te garanderen. Recent is aangetoond bewijs geleverd, dat "stunning" het myocard beter bestand maakt tegen een volgende periode van ischemie. Dit proces ("preconditioning") wordt mogelijk verklaard door een geringere zuurstofbehoefte van het myocard en/of geringere productie van lactaat, H^+ etcetera. Mogelijk speelt ook de expressie van specifieke genen, die de vorming van "stress" eiwitten reguleren, zoals het "heat-shock proteïne", een rol. Deze eiwitten zouden het hart dan beschermen via een overigens nog onbekend mechanisme. In de toekomst zullen we met het beter begrijpen van processen als "hibernation" en "stunning" therapieën voor ischemische syndromen kunnen ontwikkelen die meer in overeenstemming zijn met de beschermingsmechanismen die het hart zelf hanteert.

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