Coronary Perfusion and Ischaemia in the Isolated Heart

by

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ABSTRACT

The rabbit heart perfused by the non-recirculating Langendorff technique has been used to study the relationship between perfusion pressure and diastolic coronary flow and the relationship between these perfusion parameters and tension in the isolated heart. The effect of vasodilators (e.g. adenosine, dipyridamole, nitroglycerin) on coronary resistance has been investigated and the effect of ischaemia and hypoxia on coronary resistance and mechanical function determined. In this preparation there is an almost linear relationship between perfusion pressure and coronary flow. The vasculature is almost maximally dilated and tension is dependent on perfusion parameters. Mechanical function is impaired by ischaemia. The degree of post-ischaemic recovery is influenced by the method of reperfusion. Increasing periods of ischaemia cause a progressive increase in post-ischaemic coronary resistance.

It has been suggested that the increase in resistance to flow after ischaemia is at least partly due to an increase in extravascular compression (Harris, 1975). Rapid pacing (30 Hz) and perfusion with carbonyl cyanide p-trifluoromethoxyphenylhydrazone have been used to increase extravascular forces without affecting vascular tone and the changes in resistance produced compared with those produced by elevation of the K^+ concentration of the perfusate (which increases coronary tone but has no effect on resting tension). The maximal increase in resistance to flow produced by myocardial contracture was less than that produced by prolonged ischaemia. Myocardial contracture could not, therefore, be the sole cause of the

increase in resistance after prolonged ischaemia. The post-ischaemic increase in resistance could be partly prevented or reversed by vasodilators after short periods (15 min) of ischaemia. After prolonged ischaemia (60 min) these agents were ineffective. The increase in coronary resistance after short periods of ischaemia is thus partly due to vasoconstriction. After longer periods of ischaemia neither vasoconstriction nor increased myocardial compression can fully account for the increase in coronary resistance.

TABLE OF CONTENTS

	Page
Abstract	2
Table of Contents	4
List ofTables	20
List of Figures	22
Acknowledgements	26
Animals and Materials	27
Chapter 1 INTRODUCTION	28
Chapter 2 REVIEW OF LITERATURE	31
2.1 Coronary Anatomy and Perfusion	31
2.1.1 Coronary Anatomy - History	31
2.1.2 Coronary Perfusion - History	34
2.2 Factors Controlling Coronary Perfusion	35
2.2.1 Perfusion PRessure and Extravascular Compression	37

Page

2.2.2 Haematocrit 38

2.2.3 Neurohumoral Factors 39

2.2.3.1 Innervation 39

A. Sympathetic Innervation

B. Parasympathetic Innervation

2.2.3.2 Response to Neurohumoral Stimulation 41

A. Sympathetic Stimulation

B. Parasympathetic Stimulation

2.2.4 Myocardial Oxygen Demand 44

2.2.4.1 Oxygen 45

2.2.4.2 Carbon Dioxide 46

2.2.4.3 Adenosine Triphosphate 47

2.2.4.4 Capillary Recruitment 47

2.3 Chemical Mediators Controlling Coronary Perfusion 48

Page

2.3.1 Adenosine 49

2.3.1.1 Synthesis by the Heart 49

2.3.1.2 Mechanism ofAction 50

2.3.1.3 Effects of Exogenous Adenosine on Coronary Flow 50

2.3.1.4 Production of Adenosine in Response to Stimuli Increasing Coronary Flow 51

2.3.1.5 Effects of Modulators of Adenosine Action on Coronary Flow 52

2.3.1.6 Points Against Adenosine as the Chemical Mediator Controlling Coronary Flow 54

2.3.1.7 Interaction of Adenosine with Other Mediators 54

2.3.2 Potassium 55

2.3.3 Prostaglandins 57

2.3.4 Bradykinin 60

2.3.5 Histamine 61

Page

2.3.6 Osmolarity 61

2.3.7 Others 62

2.3.8 Summary 62

2.4 Myocardial Ischaemia 63

2.4.1 Athersclerosis and Myocardial Ischaemia 63

2.4.2 Coronary Spasm and Myocardial Ischaemia 66

2.4.3 Myocardial Contracture and Myocardial Ischaemia 67

Chapter 3 THE EXPERIMENTAL PREPARATION 68

3.1 Introduction 68

3.2 The Modified Langendorff Preparation 69

3.2.1 Animals 69

3.2.2 Perfusion of Hearts 71

3.2.3 Control of Heart-Rate 71

3.2.4 Control of Temperature 73

3.2.5 Method of Perfusion 75

3.2.6 Measurement of Perfusion Pressure 75

3.2.7 Measurement of Flow 76

3.2.8 Use of End-Diastolic Values 80

3.2.9 Measurement of Resting and Developed Force 80

3.3 Measurement of Nutrient Flow 82

3.3.1 Introduction 82

3.3.2 Experimental 83

3.3.3 Results 84

3.4 Discussion 84

3.4.1 Perfusion Pressure 85

3.4.2 Aortic Flow 85

3.4.3 Control of Perfusion Parameters 86

3.4.4 Temperature 87

3.4.5 Nutritive Flow 87

3.4.6 Conclusions 88

Chapter 4 PERFUSION PRESSURE AND CORONARY FLOW IN THE ISOLATED PERFUSED HEART 89

4.1 Introduction 89

4.1.1 Flow in Tubes 89

4.1.2 Control of Autoregulation 91

4.1.3 Minimum Perfusion Pressure Required for Flow 93

4.1.4 Calculation of Coronary Resistance 93

4.2 Experimental 96

4.2.1 Determination ofthe Relationship Between Perfusion Pressure and Flow 96

4.2.2 Determination of the Effect of Length of Perfusion on the Relationship Between Pressure and Flow in the Rabbit Heart 96

4.2.3 Determination of the Effect of Vasodilators on the Relationship Between Pressure and Flow 96

4.2.4 Treatment of Data 98

4.2.5 Materials 98

4.3 Results 101

4.3.1 Pressure and Flow in the Rabbit Heart 101

4.3.2 Effect of Length of Perfusion 101

4.3.3 Effect of Vasodilators 106

4.3.4 Pressure and Flow in the Guinea-Pig Heart 106

4.3.5 Effect of Adenosine onthe Pressure-Flow Relationship of the Guinea-Pig Heart 108

4.4 Discussion 108

4.4.1 Autoregulation 109

4.4.2 Applicability of Linear Regression 110

4.4.3 Prolonged Perfusion 110

4.4.4 Vasodilators 111

4.4.5 Conclusions 113

Chapter 5 PERFUSION PARAMETERS AND MYOCARDIAL CONTRACTION 114

5.1 Introduction 114

5.1.1 Influence of Coronary Flow 114

5.1.2 Influence of Perfusion Pressure 115

5.2 Experimental 116

5.2.1 Determination of the Relationship Between Pressure, Flow and Tension 116

5.2.2 Determination of the Effect of Prolonged Perfusion on Tension Development 116

5.3 Results 120

5.3.1 Effect of Perfusion Parameters on Tension 120

5.3.2 Effect of Prolonged Perfusion on Tension 120

5.4 Discussion 122

5.4.1 Effects on Resting Tension 122

5.4.2 Effects on Developed Tension 122

5.4.3 Conclusions 123

Chapter 6 EFFECTS OF HYPOXIA AND ISCHAEMIA ON MYOCARDIAL CONTRACTION AND CORONARY RESISTANCE 125

6.1 Introduction 125

6.2 Experimental 127

6.2.1 Determination of the Effect of Hypoxia and Rexygenation 127

6.2.2 Determination of the Effect of Low Flow Ischaemia 127

6.2.3 Determination of the Effect of No Flow Ischaemia 130

6.3 Results 130

6.3.1 Effect of Hypoxia 130

6.3.2 Effect of Low Flow Ischaemia 132

6.3.3 Effect of No Flow Ischaemia 135

6.3.3.1 Effect of Reperfusion on Resting Tension 141

6.3.3.2 Effect of Reperfusion on Developed Tension 141

6.3.3.3 Effect of Reperfusion on Perfusion Pressure 143

6.3.3.4 Effect of Reperfusion on Flow 145

6.3.3.5 Effect of Reperfusion on Resistance 145

6.4 Discussion 146

6.4.1 Criticism of Methods 146

6.4.1.1 Hypoxia 146

6.4.1.2 Pressure and Flow Measurement 147

6.4.2 Effect of Ischaemia on Mechanical Function 147

6.4.2.1 Decline of Contraction During Ischaemia 147

6.4.2.2 Developement of Contracture During Ischaemia 150

6.4.2.3 Mechanical Recovery on Reperfusion 151

6.4.3 Coronary Resistance During and After Ischaemia 153

Chapter 7 EFFECT OF DRUGS ON POST-ISCHAEMIC CORONARY RESISTANCE 156

7.1 Introduction 156

7.2 Experimental 158

7.2.1 Determination of the Effect of Adenosine, Nifedipine and Indomethacin on the Changes in Coroary Resistance and Mechanical Function Produced by Global Ischaemia 158

7.2.2 Materials and Methods 158

7.3 Results 159

7.3.1 Effects of Drugs on Control Values 159

7.3.1.1 Adenosine 159

7.3.1.2 Nifedipine 159

7.3.1.3 Indomethacin 162

7.3.2 Effects of Drugs after 15 min Ischaemia 162

7.3.2.1 Adenosine 162

7.3.2.2 Nifedipine 166

7.3.2.3 Indomethacin 168

7.3.2.4 Comparison with Results Obtained in the Absence of Drugs 169

7.3.3 Effects of Drugs After 60 min Ischaemia 171

7.3.3.1 Adenosine 171

7.3.3.2 Nifedipine 171

7.3.3.3 Comparison with Results Obtained in the Absence of Drugs 176

7.4 Discussion 176

7.4.1 Effect of Adenosine on Coronary Resistance After Global Ischaemia 176

7.4.2 Effect of Nifedipine on Coronary Resistance After Global Ischaemia 178

7.4.3 Effect of Indomethacin on Coronary Resistance After Global Ischaemia 180

7.4.4 Recovery of Mechanical Function After Ischaemia 180

7.4.5 Conclusions 181

Chapter 8 EXTRAVASCULAR FACTORS AND CORONARY RESISTANCE 183

8.1 Introduction 183

8.1.1 Extravascular Forces in Systole 183

8.1.2 Extravascular Forces in Diastole 185

8.1.3 Vascular Waterfalls 186

8.1.4 Arguements Against the Operation of a Vascular Waterfall 188

8.1.5 Physiological Contribution of Extravascular Forces 188

8.1.5.1 Oedema 189

8.1.5.2 Myocardial Contracture 190

8.1.5.3 Exhaustion of Vasodilatory Reserve 190

8.2 Experimental 191

8.2.1 Determination of the Effect of Intraventricular Pressure 191

8.2.2 Determination of the Effect of Increased Stimulation Rate 192

8.2.3 Determination of the Effect of FCCP 192

8.2.4 Materials 192

8.3 Results 194

8.3.1 Effect of Increased Left Ventricular Pressure 194

8.3.2 Effect of Increased Stimulation Rate 194

8.3.3 Effect of FCCP 194

8.4 Discussion 198

8.4.1 Increased Left Ventricular Pressure 198

8.4.2 Stimulation Rate 200

8.4.3 FCCP 201

8.4.4 Conclusions 203

Chapter 9 VASOCONSTRICTION AND CORONARY RESISTANCE 205

9.1 Introduction 205

9.1.1 Coronary "Spasm" 205

9.1.2 The No Reflow Phenomenon 207

9.2 Experimental 208

9.2.1 Determination of the Effect of Vasopressin 208

9.2.2 Determination of the Effect of Increased Perfusate K^+ Concentration 208

9.2.3 Determination of Extracellular K^+ Concentration During Total Global Ischaemia and Hypoxia 209

9.2.4 Materials 214

9.3 Results 214

9.3.1 Effect of Vasopressin 214

9.3.2 Effect of Increased Perfusate K^+ Concentration 214

9.3.3 Extracellular K^+ Concentration 218

9.4 Discussion 223

9.4.1 Vasopressin and Coronary Constriction 223

9.4.2 K^+ and Coronary Constriction 224

9.4.3 Extracellular K^+ in Ischaemia 225

9.4.4 Vasoconstriction and Coronary Resistance 226

Chapter 10 GENERAL DISCUSSION 227

10.1 The Experimental Preparation 227

10.2 Perfusion Parameters and Tension 229

10.3 Coronary Resistance and the Minimum Pressure Required for Flow 230

10.4 Ischaemia and Coronary Perfusion 234

10.5 Coronary Perfusion and Myocardial Function After Ischaemia 237

Chapter 11 CONCLUSIONS 238

Appendix 1 RESPONSES OF EPICARDIAL CORONARY ARTERY SEGMENTS TO K^+ AND FCCP 241

References 246

LIST OF TABLES

		Page
4.1	Effect of perfusion on resistance to flow in the rabbit heart	100
4.2	Effect of vasodilators on resistance to flow in the rabbit heart	105
5.1	Effect of perfusion on tension	121
6.1	Effect of myocardial cellular hypoxia on diastolic resistance to flow in the rabbit heart	131
6.2	Effect of total global ischaemia and method of reperfusion on resting tension in the rabbit heart	140
6.3	Effect of total global ischaemia and method of reperfusion on diastolic perfusion pressure in the rabbit heart	142
6.4	Effect of total global ischaemia and method of reperfusion on diastolic coronary flow in the rabbit heart	144
7.1	Effect of drugs on the increase in resistance to flow after 15 min total global ischaemia in the rabbit heart	170
7.2	Effect of drugs on the increase in resistance to flow after 60 min total global ischaemia in the rabbit heart	177

		Page
8.1	Effect of parameters which increase extravascular forces on resistance to coronary flow	199
9.1	Effect of perfusion with a 'high potassium' solution on the diastolic resistance to flow in the rabbit heart	217
9.2	Effect of total global ischaemia on extracellular K^+ concentration in the rabbit heart	222

LIST OF FIGURES

		Page
2.1	The main blood vessels of the heart	32
2.2	Interrelationship of factors regulating coronary blood flow	36
2.3	Synthesis of prostaglandins	58
3.1	The perfusion apparatus	70
3.2	Response of thermistor to temperature	72
3.3	Assessment of pressure loss within the perfusion apparatus	74
3.4	Response of the electromagnetic flow meter	77
3.5	Speed of response of the electromagnetic flow meter	78
3.6	Perfusion pressure and flow during prolonged diastole	79
3.7	Response of the tension transducer	81
4.1	Relationship between pressure and flow	90
4.2	Errors inherent in estimating coronary resistance	94
4.3	Examples of the changes in perfusion pressure, tension and flow resulting from alteration of hydrostatic pressure	97
4.4	Examples of the relationship between perfusion pressure and flow	99
4.5	Example of the effect of adenosine on the relationship between perfusion pressure and flow	102
4.6	Example of the effect of nitroglycerin on the relationship between perfusion pressure and flow	103

		Page
4.7	Example of the effect of dipyridamole on the relationship between perfusion pressure and flow	104
4.8	Example of the relationship between perfusion pressure and flow in the guinea-pig heart	107
5.1	Examples of the changes in perfusion pressure, tension and flow resulting from alteration of hydrostatic pressure	117
5.2	Effect of perfusion pressure on tension	118
5.3	Example of the relationship between perfusion pressure and tension	119
6.1	Effect of 25 min hypoxia	128
6.2	Relationship between the increase in resting tension and the increase in coronary resistance during the first 15 min of hypoxia	129
6.3	Example of the effect of reducing perfusion pressure	133
6.4	Effect of 90 min 'low flow' ischaemia on coronary resistance and resting tension	134
6.5	Effect of 15 min total global ischaemia on perfusion pressure, flow and tension	136
6.6	Effect of 60 min total global ischaemia on perfusion pressure, flow and tension	138
6.7	Effect of total global ischaemia on the relationship between perfusion pressure and flow	154

		Page
7.1	Examples of the effect of adenosine on the changes in perfusion pressure, tension and flow produced by 15 min total global ischaemia	160
7.2	Examples of the effect of nifedipine on the changes in perfusion pressure, tension and flow produced by 15 min total global ischaemia	164
7.3	Example of the effect of indomethacin on the changes in perfusion pressure, tension and flow produced by 15 min total global ischaemia	167
7.4	Example of the effect of adenosine on the changes in perfusion pressure, tension and flow produced by 60 min total global ischaemia	172
7.5	Examples of the effect of nifedipine on the changes in perfusion pressure, tension and flow produced by 60 min total global ischaemia	174
8.1	Example of the effect of increasing left ventricular pressure on the relationship between perfusion pressure and flow	193
8.2	Example of the effect of increasing stimulation rate on the relationship between perfusion pressure and coronary flow	195
8.3	Example of the effect of FCCP on perfusion, pressure, tension and flow	196

		Page
8.4	Example of the effect of FCCP on the relationship between perfusion pressure and flow	197
9.1	Construction of the K^+ electrode	210
9.2	Example of the effect of vasopressin on perfusion pressure, tension and flow	212
9.3	Example of the effect of vasopressin on the relationship between perfusion pressure and flow	213
9.4	Example of the effect of increasing the perfusate K^+ concentration on perfusion pressure, tension and flow	215
9.5	Example of the effect of increasing the perfusate K^+ concentration on the relationship between perfusion pressure and flow	216
9.6	Example of the effect of 15 min total global ischaemia on extracellular K^+ concentration	219
9.7	Example of the effect of 60 min total global ischaemia on extracellular K^+ concentration	220
9.8	Example of the effect of hypoxia on extracellular K^+ concentration	221
10.1	Interventions and minimum pressure required for flow	231
10.2	Interventions and diastolic coronary resistance	233
10.3	Effect of interventions on resting tension and end-diastolic coronary resistance	235

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SOURCE OF ANIMALS AND MATERIALS

New Zealand White rabbits were obtained from Hylyne, Cheshire, England and Dunkin Hartley guinea-pigs from Shamrock Farms Ltd., Sussex, England.

Standard laboratory chemicals of analar grade were purchased from BDH Ltd., Poole, Dorset, England. The following drugs were obtained as indicated.

Drug	Molecular weight	Obtained from
adenosine	267	BDH Ltd., Poole, Dorset
dipyridamole	504	Sigma Chemical Co., London
FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone)	254	Sigma Chemical Co., London
indomethacin	357	Sigma Chemical Co., London
nifedipine	346	Bayer A.G., W. Germany
nitroglycerin	227	Arnar-Stone Labs. Inc., Illinois
vasopressin (pitressin)	773	Parke Davis and Co., Pontypool, Gwent

CHAPTER 1

INTRODUCTION

The circulation delivers oxygen and substrates to the tissues of the body in quantities sufficient to meet metabolic requirements. The circulation also removes the metabolic products. If the energy requirement of a tissue increases either the substrate supply to that tissue is modified or anaerobic metabolism can occur. Substrate availability is initially raised by increasing extraction from the blood and subsequently by increasing blood flow. In the myocardium the oxygen extracted at rest is almost maximal (77.9%) (Bergman 1980) and increased oxygen requirements are met by increased coronary flow rather than by enhanced extraction.

Myocardial ischaemia can be defined as a decrease in blood flow which is severe enough to have metabolic consequences (Jennings, 1970). Because the fractional uptake of oxygen by the heart is much greater than the fractional uptake of other substrates (e.g. glucose 2.6%, FFA 18.2%, lactate 15.7% (Bergman, 1980)) flow becomes rate-limiting for oxygen before it is rate-limiting for other substrates. Myocardial ischaemia is thus primarily a consequence of insufficient oxygen supply. The removal of hydrogen ion and heat may be limiting at the same time. The decrease in coronary blood flow sufficient to impair oxygen supply is dependent on the metabolic state of the myocardium. Thus the higher the work load the lower the

decrease in blood flow required before ischaemia results. In man, short periods of myocardial ischaemia are manifest as angina, whilst severe and prolonged ischaemia leads to myocardial infarction characterised by irreversible damage and tissue necrosis. Death from acute myocardial infarction and ischaemic heart disease now accounts for over 1/4 of the total annual mortality in England and Wales. In 1980 there were 581,385 deaths, of which 107,123 were due to acute myocardial infarction, whilst 47,248 were attributed to other ischaemic heart disease (World Health Organisation, 1982). Ischaemic heart disease is thus one of the most common serious health problems of contemporary society. Investigation of the factors which control coronary perfusion before, during and after ischaemia is an important topic in cardiological research.

Much attention has been focused on the mechanism by which coronary perfusion is controlled under physiological conditions (Chapter 2). The aim of the experiments described in this thesis was to develop a simple preparation in which the effects of ischaemia on the coronary circulation could be studied. The isolated perfused rabbit heart was chosen because, as discussed in Chapter 3, perfusion conditions could be rigorously controlled and cardiac and coronary parameters could be measured in the absence of extrinsic influences. The equipment used facilitated measurement of perfusion pressure, diastolic coronary flow and tension and hence permitted investigation of the relationship between perfusion pressure and coronary flow (Chapter 4) and that between these perfusion parameters and tension (Chapter 5).

In this preparation a period of ischaemia caused an increase in the resistance of the coronary circulation (Chapter 6). It has been

suggested that increases in post-ischaemic resistance in man are at least partly due to an increase in extravascular compression rather than an increase in vascular smooth muscle tone (Harris, 1975). Harris also argued that this increase in extravascular compression might result in further ischaemia, hence giving rise to a vicious circle where ischaemic damage to the myocardium results in an increase in extravascular compression which in turn promotes more ischaemia thus exacerbating an anginal attack to myocardial infarction. The effect of both myocardial contracture and vascular smooth muscle contraction on the coronary resistance of the isolated rabbit heart were investigated and their contribution to the increase in coronary resistance after ischaemia assessed (Chapters 8 and 9). On the basis of the results of these experiments interventions were made in an attempt to prevent the increase in resistance after ischaemia and hence improve post-ischaemic perfusion and mechanical function(Chapter 7).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Coronary Anatomy and Perfusion

2.1.1 Coronary Anatomy - History

The first comprehensive theory to explain the nourishment of the body and the heart by the blood was proposed by Galen (131-201 A.D.), physician to the Roman Emperor Marcus Aurelius. He proposed that body function proceeds from:

1. Coction of food in the stomach which is prepared for absorption by ducts from the intestines and transferred to the liver.

2. Here made into blood containing "nutritive spirits" necessary for nourishing all parts of the body and distributed thereto by veins.

3. Some of the nutritive spirits passing through pores in the septum of the heart are combined in the left chamber with air coming from the lungs to form the "vital spirits" necessary for life and heat in all parts of the body to which they are distributed by boiling over of the heart, by arteries and

4. some of these vital spirits permeating the cribriform plate are converted in the brain into "animal spirits" necessary for motion in every part of the body to which they are distributed by nerves.

(translated by Singer and Rabin,1946).

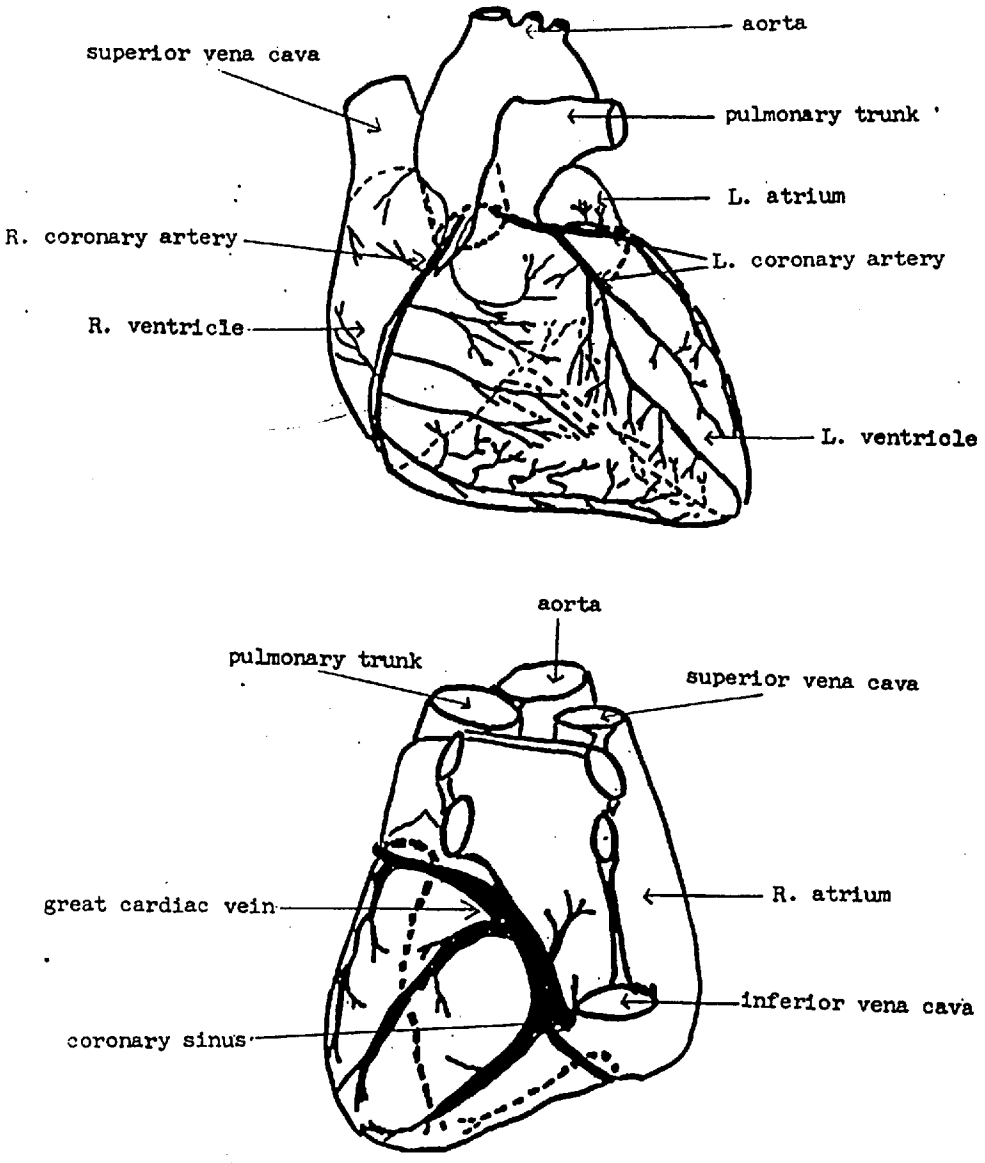


Figure 2.1 The main blood vessels of the heart. The upper panel shows the arterial vessels and the lower one the venous drainage. Dotted lines are used to represent vessels which traverse the opposite side of the heart.

This theory remained in popular use throughout the Middle Ages and was only finally dispelled by the description of the anatomy and function of the "third circulation" by William Harvey in 1649. In 1704 Ruysch used a corrosion technique to visualise the ventricular branches of the coronary arteries and two years later Vieussens (1706) injected saffron into the coronary arteries and showed connections between them and the cardiac cavities. In 1708 Thebesius showed connections between the veins and the cardiac chambers. The function of the capillary system, however, was not clarified until late in the nineteenth century (Krogh,1929).

In the twentieth century radiographic techniques have been developed which enable visualisation of the coronary circulation without surgical intervention. Selective angiography was first used to opacify the coronary tree by Rousthoi (1933) and the technique was modified and developed by Sones (1959) enabling the changes in coronary anatomy which occur in disease to be observed.

The course and distribution of the major coronary arteries is similar in all mammalian species (Grant and Regnier, 1926). The heart is supplied by two main coronary arteries which have their origin just above the aortic valve. Both transverse the epicardial surface of the heart (Figure 2.1,upper panel) giving rise to surface and transmural branches. Most of the intramural branches of the left coronary artery are short and divide to supply the outer two-thirds of the ventricular wall (Fulton, 1969). The inner one-third is supplied by large caliber vessels which pass through the outer layers without branching. In the innermost layers they form small branches to supply the myocardial cells in the immediate vicinity. The venous system of

the heart can be divided into two parts; the superficial and the deep circuits. The superficial circuit, shown in Figure 2.1(lower panel), comprises the great cardiac vein and coronary sinus which drain the left ventricle and the anterior cardiac veins which drain the right ventricle. Both the coronary sinus and the anterior cardiac veins empty into the right atrium (Gregg, 1950). The deeper venous circuit has communications with both atrial and ventricular cavities via Thebesian and sinusoidal channels (Gregg, 1950).

Acute ligation of the left anterior descending artery in the dog or monkey reduces perfusion of the coronary bed of this artery by only 50% (Grayson and Lapin, 1966; Rees and Redding, 1967) suggesting that there are functional connections between capillary beds primarily supplied by different arteries. Connections between epicardial arteries were described by Lower (1670), Thebesius (1708) and more recently by Grayson and co-workers (1974). Geary and co-workers (1981) found anastomoses between epicardial arteries in the dog but not in the pig or the baboon suggesting that species differences occur. Spalteholz (1907) suggested that functional anastomoses existed in all layers of the heart and between all sizes of coronary vessels. This latter conclusion has been disputed; Brown (1965) found no anastomoses between coronary vessels less than 100 μm in diameter, and others have suggested that the coronary capillary bed of both man and dog form end capillary loops with discrete perfusion fields (Okun et al., 1979; Factor et al., 1982).

2.1.2 Coronary Perfusion - History

In 1687 J. Batista Scaramucci suggested that the deep coronary

vessels might be squeezed empty during systole. In 1898 Porter suggested that the beating of the heart propelled blood through the coronary arteries by a milking action. Support for both views was forthcoming; in 1927 Anrep reported that there was little or no flow during systole, whilst Hochrein (1930) and Wiggers and Cotton (1933) reported that systolic flow was considerable. All these studies suffered from an inability to measure phasic blood flow accurately. The development of the "orifice plate" flow meter (Gregg and Green, 1940) enabled accurate measurement of phasic flow and revealed that coronary flow was greatly reduced during systole but did not stop completely. The "massage" theory was briefly revived by Wiggers (1954) but was finally dismissed by Sabiston and Gregg (1957). In a classic experiment they perfused a heart from an elevated reservoir and showed that vagal asystole or fibrillation evoked a transient increase in blood flow hence showing that systolic contraction inhibited coronary flow.

The idea that extravascular forces influence coronary flow has been expanded by Downey and Kirk (1975) resulting in the proposition of the existence of "vascular waterfalls" within the coronary circulation (see Chapter 8) .

2.2 Factors Controlling Coronary Perfusion

The most important determinant of the rate of blood flow through the heart is the tone of the coronary vessel walls. Coronary flow is, however, also influenced by:

a) perfusion pressure,

b) extravascular forces and

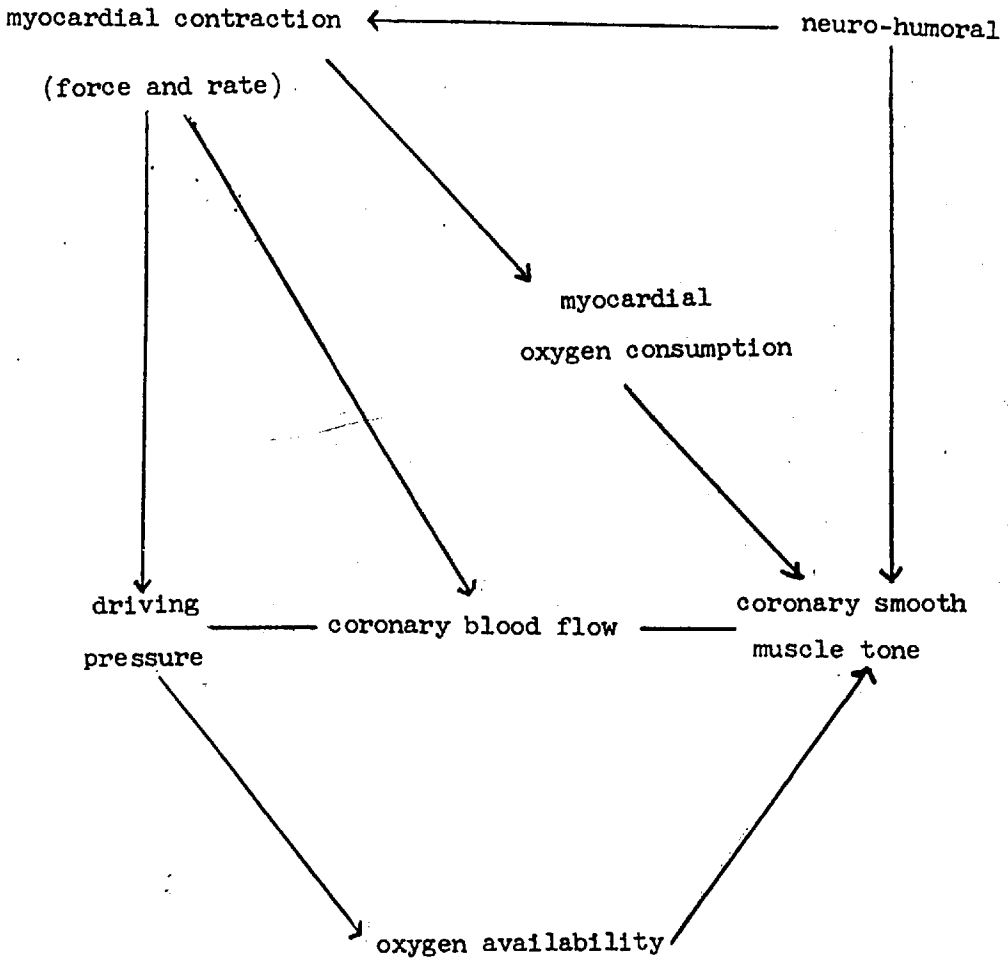


Figure 2.2 Interrelationship of factors regulating coronary blood flow.

c) haematocrit.

Coronary vascular smooth muscle tone is regulated by:

a) the oxygen requirements of the myocardium,

b) neural stimulation and

c) humoral agents e.g. adrenaline.

These determinants of coronary blood flow are interrelated as shown in Figure 2.2 and stimulation of one invariably leads to alteration of others. For example, sympathetic stimulation produces a decrease in coronary flow by direct constriction of the coronary vessels. However, this is rapidly followed by vasodilation in response to increased myocardial oxygen demand caused by the positive inotropic and chronotropic effects of sympathetic stimulation on the myocardium. The interdependence of the factors makes it difficult to determine the effect of one factor alone. Attempts have, however, been made to do this and the results of these studies are discussed below.

2.2.1 Perfusion Pressure and Extravascular Compression

Some vascular beds operate as a system of distensible tubes and flow through them increases with increased perfusion pressure. In the coronary circulation a different relationship exists (see Chapter 4). Mean flow varies with perfusion pressure as expected at pressures below 70 mmHg and above 140 mmHg. Between these pressures "autoregulation" occurs with large changes in perfusion pressure resulting in little change in mean flow (Mosher et al., 1964; Weisfelt and Shock, 1970). The flow at which autoregulation occurs is regulated by the oxygen requirements of the myocardium (Mosher et al., 1964). Autoregulation can be abolished by vasodilating drugs or

hypoxia (Weisfelt and Shock, 1970) and the relationship between perfusion pressure and flow becomes linear or curvilinear (Klocke et al., 1981).

As the heart contracts it compresses intramural coronary vessels and may also distort them (see Chapter 8). This results in an increased resistance to flow. The relationship between perfusion pressure and coronary flow is thus influenced by both the force of myocardial contraction and heart rate as the duration of diastole is preferentially reduced as heart rate is increased.

In diastole intramyocardial pressure is greater than coronary venous pressure (Stein et al., 1980; see Chapter 8) and together with arterial pressure determines the driving pressure for coronary perfusion. Diastolic intramyocardial pressure is thus a determinant of the relationship between perfusion pressure and coronary flow.

2.2.2 Haematocrit

The myocardial effects of mild anaemia are the consequence of insufficient oxygen supply to the heart. Coronary flow increases but there is no change in myocardial oxygen consumption or in systemic parameters. In more severe anaemia heart rate and cardiac output are increased, myocardial oxygen consumption increases and peripheral resistance decreases. Despite a tripling of coronary flow the consequent increase in myocardial oxygen consumption may result in a decrease in coronary venous oxygen content. The increase in coronary flow is primarily due to active vasodilation in response to myocardial hypoxia, which is exacerbated by the increase in myocardial work. A decrease in the viscosity of the blood may also contribute to the

increase in coronary flow. Ultimately when coronary vasodilation and oxygen extraction become maximal, and no further compensation for insufficient oxygen delivery can be made, myocardial failure occurs (Case et al., 1955).

In polycythemia vera where an increase in blood volume and an increase in erythrocyte concentration occurs there is a decrease in coronary flow, together with an increase in oxygen extraction, whilst oxygen consumption is unchanged (Regan et al., 1960). The decrease in coronary flow is probably a consequence of both the increased oxygen capacity of the blood and its increased viscosity.

2.2.3 Neurohumoral Factors

2.2.3.1 Innervation

Little was known about the innervation of the coronary arteries until the 1960s although morphological studies using the impregnation technique and staining with methylene blue had described nerve fibres within the arterial wall (Woolard, 1926; Hirsch and Borghard-Erdle, 1961). A fundamental advance came with the development of electron microscopy and histochemical techniques enabling visualisation of catecholamines (Falck, 1962) and specific acetyl cholinesterases (Koelle, 1950) within nerve fibres.

A. Sympathetic Innervation

The Falck-Hillarp histochemical fluorescence technique (Falck, 1962) shows that sympathetic fibres accompany all coronary vessels. In

the large conduit arteries monoaminergic bead like nerve terminals are found evenly distributed around the circumference of the adventitia outside the external elastic membrane (Dolezel, 1973; Denn and Stone, 1976). In intramural and subepicardial arteries the terminals are restricted to two sites on the circumference of the vessel in the adventitia or on the surface of the media (Dahlstrom et al., 1965; Ehinger et al., 1966; Angelakos et al., 1967; Denn and Stone, 1976) although single fibres may penetrate into the media (Schenk and El Badawi, 1968). In these arteries a large gap, of the order of 1 μm (Gerova, 1982), exists between the nerve terminals and the effector smooth muscle cells. In small arteries the distance between nerve terminal and smooth muscle cell is reduced (<5000 A, Malor et al., 1973) and in arterioles fusion may occur between the basement membrane of the nerve terminal and the smooth muscle cell (Malor et al., 1973). Sympathetic nerve terminals have also been found associated with the endothelial cells of the capillaries; (Malor et al., 1973; Thaemert, 1966) and it has been suggested that they might play a part in controlling capillary permeability.

B. Parasympathetic Innervation

In 1965 Navaratnam and Palkama showed the presence of acetyl cholinesterase in fibres accompanying large coronary arteries and small arterioles. In the large conduit arteries cholinergic fibres are found in two layers: in the perivascular connective tissue and in the adventitia between the elastic lamellae (Denn and Stone, 1976; Gerova et al., 1979a). In the smaller arteries the nerve terminals are restricted to two areas on the circumference of the vessel outside the

media i.e. similarly distributed to the sympathetic fibres. Electron microscopy studies show cholinergic nerve terminals close to arterioles and to the endothelium of the capillaries (Lever et al., 1965; Thaemert, 1966; Malor et al., 1973). Thus the cholinergic nerve fibres show a similar distribution to monoaminergic fibres and all of the arterial side of the coronary circulation is innervated by both sympathetic and parasympathetic fibres.

2.2.3.2 Response to Neurohumoral Stimulation

A. Sympathetic Stimulation

In vitro studies on coronary strips show that coronary arteries have a mixed population of α- and β-adrenoceptors. In large coronary arteries of man and dog α-adrenoceptors are predominant, but the contractile response seen can be enhanced by β-adrenoceptor blockade (Zuberbuhler and Bohr, 1965; Mekata and Niu, 1969; Anderson et al., 1972; Trinker, 1973; Ross, 1976; Golenhofen, 1978). In rabbit (de la Lande et al., 1974), kitten (Cornish et al., 1974), cow (Peiper and Schmidt, 1972) and pig (Takenaka, 1966; Peiper and Schmidt, 1972) the predominant response to noradrenaline is relaxation indicating the dominance of β-adrenoceptors. In small arteries of less than 0.5 mm diameter relaxation is also seen (Zuberbuhler and Bohr, 1965; Mekata and Niu, 1969). The β-receptors of coronary artery strips have been characterised as β_1 (Johansson, 1973; de la Lande et al., 1974) or β_2-receptors (Bayer et al., 1974). In vivo dilation to isoprenaline in the presence of β-blockade with practolol has been

taken as evidence that the vascular receptors are β_2 (McRaven et al., 1971; Mark et al., 1972) but other workers have suggested that both β_1 and β_2-adrenoceptors are present in the coronary bed (Singh et al., 1982).

In vivo the direct effect of noradrenaline and adrenaline on coronary vascular tone is more difficult to elucidate because of the effects of the catecholamines on contraction of the myocardium and systemic resistance. In the anaesthetised dog intravenous injection of the α-adrenergic agonist methoxamine decreases large coronary artery diameter and cross-sectional area despite the opposing influence of increased distending pressure (Kelley and Feigl, 1978; Vatner et al., 1980), whilst β-adrenergic agonists produce vasodilation (Klocke et al., 1965; Zuberbuhler and Bohr, 1965; Pitt et al., 1967; Parratt, 1980). Thus the coronary vasculature is responsive to both α and β-receptor stimulation. If arterial blood pressure is maintained and heart rate kept constant to prevent the secondary changes in coronary resistance caused by increased myocardial oxygen demand intravenous injection of noradrenaline produces a transient decrease followed by a sustained increase in coronary resistance in the conscious dog (Vatner et al., 1974) indicating that in vivo α-effects predominate. β-adrenoceptor blockade has been used to eliminate the chronotropic and inotropic effects of catecholamines on the myocardium and under these conditions electrical stimulation of the sympathetic nerves to the heart results in an increase in coronary resistance (Feigl, 1967; Ross and Mulder, 1969; McRaven et al., 1971; Nayler and Carson, 1973; Feigl, 1975) which is abolished by α-adrenoceptor blockade (Feigl, 1967; McRaven et al., 1971; Feigl, 1975). In the presence of both α and β-adrenergic blockade stimulation of the stellate ganglion

produces no change in coronary flow (Feigl, 1967) suggesting that the sympathetic supply to the coronary vessels does not contain sympathetic cholinergic fibres.

Under physiological conditions the direct effects of sympathetic stimulation of the coronary vasculature are obscured by secondary changes in response to alterations in systemic pressure and myocardial oxygen demand produced by sympathetic effects on the myocardium. In the anaesthetised dog α-adrenoceptor blockade magnifies the increase in coronary flow produced by noradrenaline, or carotid sinus hypotension (which produces a reflex increase in sympathetic outflow to the heart) by about 30% (Mohrman and Feigl, 1978) hence indicating that α- adrenergic stimulation plays a physiological role in modifying the vasodilative response to increased oxygen demand. β_2-adrenoceptor mediated vasodilation appears to be of little physiological significance: electrical stimulation of the sympathetic nerves to the heart in the presence of α - and β_1-adrenoceptor blockade produces little (Hamilton and Feigl, 1976) or no (McRaven et al., 1971) effect on coronary blood flow or large coronary artery diameter and perfusion pressure (Gerova et al., 1979b).

B. Parasympathetic Stimulation

Most in vitro studies show that acetylcholine induces contraction of isolated coronary arteries (Norton and Detar, 1972; Garland and Keatinge, 1982). Recent studies on aortic strips suggest that contraction occurs only in the absence of endothelium and that if the endothelium is intact acetylcholine produces relaxation (Furchgott and Zawadzki, 1980). In vivo intravenous injection of acetylcholine

produces a decrease in coronary vascular resistance (Gregg, 1950; Schreiner et al., 1957; Denison and Green, 1958; Levy and Zleske, 1969; Blesa and Ross, 1970; Murray and Sparks, 1978) and an increase in large vessel diameter (Gerova et al., 1979a; Cox et al., 1981) if myocardial activity is controlled. These effects are abolished by atropine. Stimulation of the vagus nerve in paced dog hearts reduces late diastolic coronary resistance (Feigl, 1969). In the presence of atropine a small transient increase in resistance is seen, probably caused by the few sympathetic fibres in the vagosympathetic trunk (Randall, 1967). While these results indicate that vagal stimulation can elicit coronary vasodilation the rate of stimulation used (30 Hz) is probably beyond the usual physiological range. Parasympathetically mediated vasodilation is seen as an early response to carotid chemoreceptor stimulation (Vatner and McRitchie, 1975) but the response is rapidly obscured by α-adrenoceptor mediated vasoconstriction and other physiological situations in which parasympathetically mediated coronary control play a part remain to be determined.

2.2.4 Myocardial Oxygen Demand

The close correlation between coronary blood flow and myocardial oxygen supply is well documented. The relationship has been studied by looking at the increase in flow produced by systemic hypoxia or ischaemia (e.g. Eckenhoff et al., 1948), increasing metabolic work (e.g. Shipley and Gregg, 1945) or by increasing the oxygen delivery to the heart (e.g. Berne, 1964). Although the relationship between coronary flow and myocardial oxygen supply is well established the

mechanism by which an increase in oxygen demand results in an increase in coronary flow is not as clear. Coronary vasodilation could be a direct consequence of decreased coronary PO_2 or the concomitant rise in PCO_2 or it could be due to an increase in ATP turnover within the myocardium. Alternatively increased myocardial demand could result in capillary recruitment or alteration in the amount of a vasoconstrictor or vasodilator produced by the myocardium.

2.2.4.1 Oxygen

A decrease in coronary PO_2 could be the stimulus for coronary vasodilation but whether an intermediary chemical mediator is involved is more difficult to determine. Hilton and Erchholtz (1925) suggested that as a low PO_2 in the coronary arteries of the canine heart-lung preparation produced vasodilation without myocardial hypoxia the reaction was occurring without the release of metabolites and must therefore be a direct effect of oxygen on the coronary vessels. A similar line of reasoning was used by Daniell and Bagwell (1968) and a study by Coburn (1977) on isolated aortic strips suggested that there was an oxygen sensitive system other than the electron-transport chain regulating vascular smooth muscle tone. Nishiki and co-workers (1979) took the increase in coronary flow seen in the isolated rat heart in the presence of amylobarbitone (which inhibits mitochondrial electron transfer whilst decreasing myocardial oxygen consumption) as evidence that coronary vascular tone changes in response to a metabolic product of oxygen utilisation, not to oxygen per se.

Evidence of direct interaction between oxygen and vascular smooth

muscle cells is scarce. Studies on isolated coronary strips suggest that PO_2 must be reduced below 5 mmHg before tone is affected (Gellai et al., 1973). This suggests that a decrease in coronary PO_2 triggers or inhibits the release of a chemical mediator, or increases the sensitivity of the smooth muscle to a mediator already present (Gellai et al., 1973) rather than having a direct effect on coronary tone.

2.2.4.2 Carbon Dioxide

The majority of studies show that increasing systemic PCO_2 results in an increase in coronary resistance (Markwalder and Starling, 1913; Hilton and Erchholtz, 1925; Feinberg et al., 1960; Kittle et al., 1965; Ledingham et al., 1970; Vance et al., 1979). An increase in systemic PCO_2 stimulates the carotid chemoreceptors and hence increases sympathetic outflow to the heart. This results in coronary constriction (see section 2.2.3.2A) which will obscure any direct vasodilatory effect of carbon dioxide in the coronary circulation. Increased arterial PCO_2 also shifts the oxyhaemoglobin dissociation curve to give a greater PO_2 for a given blood oxygen content, also tending to obscure vasodilation produced by carbon dioxide.

Increasing coronary PCO_2 without altering systemic values produces a mild (Daugherty et al., 1967; Rooke and Sparks, 1980) or dramatic (Case and Greenberg, 1976; Case et al., 1978) coronary vasodilation. The increase in coronary sinus PCO_2 after an increase in myocardial work is small (Rooke and Sparks, 1980) and occurs too slowly to participate in the initial adjustment of coronary

flow (Case, 1981) hence suggesting that change in PCO_2 is not an important factor in the metabolic regulation of blood flow.

2.2.4.3 Adenosine Triphosphate

Under aerobic conditions myocardial oxygen utilisation reflects turnover of ATP (Williamson et al., 1976). In cell suspensions oxidative phosphorylation is dependent on oxygen tension throughout the physiological range. This is reflected by a progressive decrease in cytosolic $[ATP]/[ADP][P_i]$ as oxygen tension is reduced (Wilson et al., 1979). Respiration and hence ATP production remain almost undiminished down to very low oxygen concentrations because of an increase in the reduction of cytochrome c (Wilson et al., 1979). In the isolated rat heart changes in coronary flow produced by amylobarbitone, hypoxia or increased work load are accompanied by parallel opposing changes in $[ATP]/[ADP][P_i]$ (Nuutinen et al., 1982). The decrease in $[ATP]/[ADP][P_i]$ ratio in the myocardium in the presence of reduced PO_2 might be paralleled by a similar decrease in the vascular smooth muscle cells producing relaxation. Alternatively, the build up of ADP and inorganic phosphate in the myocardium could result in the generation of secondary messengers (e.g. adenosine, see Chapter 2.3.1.1) hence producing vasodilation indirectly.

2.2.4.4 Capillary Recruitment

It has been suggested that the primary response to increased tissue oxygen demand is an increase in open capillary density, and

that vasodilation only occurs when this mechanism is exhausted (Granger et al., 1975). Several studies suggest that open capillary density is not maximal in the resting heart (e.g. Martini and Honig, 1969; L'Abbate et al., 1976; Duran et al., 1977) and that open capillary density is affected by PO_2 (Martini and Honig, 1969) and cardiac activity (Duran et al., 1977) although not by PCO_2 or pH (Bourdeau-Martini and Honig, 1973). The mechanism by which open capillary density is altered has not been elucidated and as in control of coronary diameter could involve direct stimulation or involvement of a chemical mediator.

2.3 Chemical Mediators Controlling Coronary Perfusion

Direct evidence for the involvement of a chemical mediator in the control of coronary tone comes from the work of Broadley (1976). When the metabolic work of an isolated guinea-pig heart was increased by atrial pacing, administration of adrenaline, isoprenaline or histamine and the coronary effluent reoxygenated and used to perfuse a second heart, a decrease in coronary resistance was seen in the recipient heart despite the presence of antagonists to the inotropic agent. A similar decrease in coronary resistance was seen if the donor heart was made hypoxic. Compounds which have been proposed as the chemical mediator involved are adenosine, potassium, prostaglandins, bradykinin and histamine.

2.3.1 Adenosine

2.3.1.1 Synthesis by the Heart

Adenosine is produced in the heart by the action of 5′-nucleotidase on AMP. The AMP is produced from breakdown of myocardial ATP (or possibly cAMP) (Olsson et al., 1973; Schrader and Gerlach, 1976; Manfredi and Sparks, 1982). The enzyme (5′-nucleotidase) is thought to be located at the extracellular borders of myocardial cells (Rostgaard and Behnke, 1965; Rubio et al.,1973) although it may exist within the cells (Schrader and Schutz, 1979). The majority of myocardial adenosine is extracellular (Schrader et al., 1972) but an intracellular pool has been postulated (Schrader and Gerlach, 1976; Olsson et al., 1978a; Fricke and Lowenstein, 1978) possibly in the form of 5-adenosylhomocysteine (Schrader and Schutz, 1979). There are four paths for removal of adenosine from the interstitial space. It can be taken up into the myocardial cells by facilitated diffusion (Olsson et al., 1972) and reconverted by adenosine kinase to AMP (Olsson et al., 1972; Schrader et al., 1972; Wiedmeier et al., 1972) or degraded by adenosine deaminase to inosine (Schrader et al., 1972). It can interact with adenosine receptors on the outside of vascular smooth muscle cells and produce relaxation of these cells (Olsson et al., 1976; Schrader et al., 1977a). It can be taken up by the endothelial cells and degraded to inosine and hence by the action of nucleoside phosphorylase to hypoxanthine or it can pass into the bloodstream where it is gradually degraded by adenosine deaminase present in the red blood cells.

2.3.1.2 Mechanism of Action

2.3.1.2 Mechanism of Action

The mechanism by which adenosine elicits relaxation of the coronary vascular smooth muscle is still unknown. It has been proposed that adenosine acts by increasing cAMP or by reducing uptake or interfering with utilisation of Ca^{2+}. The addition of a low concentration of adenosine causes cAMP accumulation in guinea-pig myocardial slices (Huang and Drummond, 1976) and increases cAMP in brain slices (Sattin and Rall, 1970). However, pharmacological concentrations of adenosine are needed to produce an increase in cAMP in hog and dog arterial strips (Herlihy et al., 1976) and adenosine decreases the adenylate cyclase activity of broken cell preparations (McKensie et al., 1977). In skeletal muscle adenosine inhibits caffeine induced rigor attributed to excess Ca^{2+} (De Gubareff and Sleator, 1965; Berne et al.,1976) whilst in guinea-pig atrium adenosine inhibits the slow Ca^{2+} current (Schrader et al., 1974) either by reducing the number of Ca^{2+} channels or their permeability (Belardinelli et al., 1979). Calcium flux studies in dog and hog arteries have, however, proved inconclusive (Berne, 1980). Thus no conclusive evidence for either mechanism of action is available.

2.3.1.3 Effect of Exogenous Adenosine on Coronary Flow

The isolated canine coronary artery contracted with noradrenaline or potassium chloride relaxes in response to 10^{-6} mol.litre^{-1} adenosine (Herlihy et al., 1976) and the isolated perfused heart shows a dose related relaxation in response to 10^{-7}-10^{-6} mol.litre^{-1} adenosine (Schrader et al., 1977b).In blood perfused hearts higher

concentrations of adenosine have to be used to elicit vasodilation because of its rapid inactivation by the cellular elements in the blood (see section 2.3.1.1). Dilation is,however, still seen at administered concentrations lower than those produced during ischaemia (Rubio et al., 1969).

2.3.1.4 Production of Adenosine in Response to Stimuli Increasing Coronary Flow

Myocardial adenosine content rises in the dog when myocardial metabolic activity is increased by aortic constriction (McKensie et al., 1980), stellate ganglion stimulation (Miller et al., 1979) or treadmill exercise (Watkinson et al., 1979). Aortic constriction also causes an increase in myocardial adenosine in the rat (Foley et al., 1978) and an increase in adenosine release is seen from the isolated saline perfused guinea-pig heart stimulated with catecholamines (Wiedmeier and Spell, 1977). However, if metabolic work is increased by increasing heart rate no increase in adenosine production is seen (Manfredi and Sparks, 1982). Hypoxia causes an increase in the adenosine content of the coronary effluent from isolated guinea-pig (Berne, 1963; Rubio et al., 1969; Kukovetz and Poch, 1971) or cat hearts (Katori and Berne, 1966) and in the open chested dog (Olsson, 1970; Berne et al., 1971). There is also evidence that adenosine is released during reactive hyperaemia - the reflex increase in flow which occurs after brief arterial occlusion (Rubio et al., 1969; Berne et al., 1971; Schrader et al., 1977b; Olsson et al., 1978b) although it has been suggested that adenosine is not involved in the reactive hyperaemia produced by occlusion lasting less than 10 heart beats

(Raberger et al., 1975; Schutz et al., 1977). Schrader and his coworkers (1977b) reported an increase in myocardial adenosine content and release of adenosine when coronary perfusion pressure was decreased in steps from 60 to 20 cmH_2O thus suggesting that adenosine is involved in autoregulation (see Chapter 4). Other workers have found no change in the amount of adenosine in the coronary effluent when perfusion pressure was increased (e.g. Frick and Lowenstein, 1976; Nuutinen et al., 1982). One study suggests that myocardial adenosine concentration alters on an intra-beat basis. Thompson and his coworkers (1980) slowed down guinea-pig hearts to forty beats per minute and froze them at various periods in the cardiac cycle. They found that the total myocardial adenosine, hypoxanthine and inosine concentration was higher during systole than diastole and suggested that increases in myocardial adenosine seen as a result of increased force of contraction were due to an extension of this intra-beat difference.

Although these studies show that the adenosine content of the myocardium and that of the coronary effluent increases in response to stimuli which result in vasodilation they give little indication of the adenosine concentration in the resistance arterioles, where coronary resistance is regulated. The increased adenosine concentration could cause the changes in coronary resistance or may just increase concurrently as a result of altered myocardial metabolism.

2.3.1.5 Effect of Modulators of Adenosine Action on Coronary Flow

Studies with antagonists or potentiators of adenosine have not produced as conclusive results as the infusion of adenosine, or

measurement of its release. Methylxanthines (theophylline and aminophylline) competitively antagonise the effects of exogenous adenosine (Schaumann et al., 1970; Alfonso, 1970) and were shown to attenuate the reactive hyperaemic response by Juhran and coworkers (1971) and Curnish and coworkers (1972) and to attenuate coronary vasodilation induced by atrial pacing by Lammerant and Becsei (1975). However other workers have found no or minimal attenuation of the reactive hyperaemic response (Alfonso, 1970; Juhran and Dietmann, 1970; Bittar and Pauly, 1971; Nuutinen et al., 1982) and inability of methylxanthines to prevent the vasodilative response to hypoxia and increased myocardial oxygen demand (e.g. Wadsworth, 1972). A similar pattern is seen in studies where dipyridamole was used to potentiate adenosine action. Jageneau and coworkers (1969) found dipyridamole potentiated the reactive hyperaemic response in conscious and anaesthetised dogs, anaesthetised cats and isolated hearts whilst other workers have found no potentiation of this response (Bittar and Pauly, 1970; Juhran et al., 1971) or the vasodilation produced by hypoxia (Parratt and Wadsworth, 1972a) or increased myocardial oxygen demand (Parratt and Wadsworth, 1972b). The methylxanthines and dipyridamole have a number of pharmacological actions and their mechanism of interaction with adenosine remains unclear. In one study dipyridamole was reported to inhibit endogenous adenosine release (Kurovetz and Poch, 1971).

The non specificity of these agents may account for the inconclusive results obtained in the above studies. There are, however, other points against adenosine as the sole chemical mediator of coronary vasodilation.

2.3.1.6 Points Against Adenosine as the Chemical Mediator Controlling Coronary Flow

As already mentioned not all methods of increasing cardiac work result in an increase in adenosine release(Manfredi and Sparks, 1982). In reactive hyperaemia the interstitial adenosine concentration estimated to be required to produce a given degree of vasodilation does not correspond to the amount of exogenous adenosine which must be applied (Moir and Downs, 1972) or to the amount of adenosine seen in the coronary venous effluent in the presence of the same degree of vasodilation. This can be partly accounted for by degradation of adenosine by the capillary endothelial cells and red blood cells (see section 2.3.1.1). It may reflect an increase in responsiveness to adenosine under hypoxic conditions (Olsson et al., 1978b) or adenosine may not be the sole mediator of the reactive hyperaemic response. One point against adenosine as a mediator of hypoxic vasodilation is that although the adenosine increases during hypoxia in the rat heart the increase in coronary flow occurs before the adenosine in the coronary effluent starts to rise and the maximal dilation produced by hypoxia is greater than that which can be elicited with adenosine (Nuutinen et al., 1982). The results from these studies together with those in which modulators of adenosine action were used suggest that adenosine can not be the sole chemical mediator controlling coronary tone.

2.3.1.7 Interaction of Adenosine with other Mediators

As already stated (section 2.2.4.1) a decrease in PO_2 has no effect on the tone of the isolated canine coronary artery until a very

low PO_2 is reached. However, in the presence of adenosine, the same preparation produces a stepwise relaxation in response to decreases in PO_2 between 40 and 10 mmHg (Gellai et al., 1973). A similar response is seen in the isolated heart (Moir and Jones, 1973). Decreases in pH cause a progressive increase in the degree of relaxation produced by a given dose of adenosine in the beef coronary artery (Mustafa and Ghai, 1981), in the isolated guinea-pig heart (Merrill et al., 1978) and in the open chested dog (Raberger et al., 1975). An increase in PCO_2 also increases the degree of vasodilation produced by adenosine in the dog (Case and Greenberg, 1976). Reduced oxygen tension, acidosis and elevation of pCO_2 all occur in myocardial ischaemia and the role of adenosine in this situation may be to increase the sensitivity of the vascular smooth muscle cells to these agents.

2.3.2 Potassium

Potassium has been shown to exert a vasodilator effect on coronary vessels both in vitro (Gellai and Detar, 1974) and in vivo (Driscol and Berne, 1957; Brace et al., 1974; Murray and Sparks, 1978) provided that the potassium concentration does not exceed 10 $mmol.litre^{-1}$. The coronary vasodilation is however transient (Brace et al., 1974; Gellai and Detar, 1974).

If myocardial work is increased by increasing heart rate or

contractility there is a transient increase in coronary venous potassium concentration (Gilmore et al., 1971; Sybers et al., 1971). It has been estimated that the increase in interstitial potassium concentration preceeds the increase in coronary flow and is of the right magnitude to account for about half the initial coronary vasodilation (Murray and Sparks, 1976). The coronary sinus potassium concentration increases during hypoxia (Sybers et al., 1971) and during ischaemia potassium accumulates in the extracellular space (Weiss and Shine, 1982). However, ouabain, which blocks the vasodilator activity of potassium (Bunger et al., 1976; Murray and Sparks, 1978), has little effect on hypoxic vasodilation in isolated guinea-pig hearts (Bunger et al., 1976). This could be because ouabain is blocking a myocardial cell ion pump, as well as the electrogenic pump in the vascular smooth muscle cells (Brace, 1974) and hypoxic potassium efflux from the myocardial cells is potentiated. In the dog coronary sinus potassium increases even after short coronary occlusions and although the time course of the decrease in potassium concentration after the occlusion is too slow for potassium to be a primary determinant of the reactive hyperaemic response it may play a role in the second half of this response (Murray et al., 1979). Studies with ouabain give support to this hypothesis; ouabain has no effect on the vasodilation produced by a 10 s occlusion in the isolated guinea-pig heart, but slightly attenuates that of a 40 s occlusion (Bunger et al., 1976). These results suggest that potassium is probably important in initiating the vasodilatory response to an increase in myocardial work, may play a part in hypoxic vasodilation, but has only a minor role in reactive hyperaemia.

2.3.3 Prostaglandins

The coronary vasculature is capable of synthesising prostaglandins (Needleman et al., 1975; Alexander et al., 1975; Moretti et al., 1976) and there is evidence that there is a basal release of prostaglandins from the isolated guinea-pig heart (Schror et al., 1976) although not from the isolated rabbit heart (Needleman et al., 1975).Prostaglandins are formed by the enzymatic oxidation of polyunsaturated fatty acids (Van Dorp et al., 1964). The fatty acid precursor arachidonic (5,8,11,14; eicosatetraenoic) acid or dihomo-γ-linolenic (8,11,14;eicosatrienoic) acid must be released from phospholipids or triglycerides by the action of lipases before prostaglandin synthesis can occur (Vogt et al., 1966). The pathway for the synthesis of different prostaglandins are shown in Figure 2.3. Prostaglandins are unstable and spontaneously breakdown so that an increase in the concentration of a prostaglandin in the coronary effluent can be taken as evidence of increased synthesis. The cyclo-oxygenase, and hence prostaglandin synthesis, can be inhibited by indomethacin or aspirin (Vane, 1971).

Administration of prostaglandin E (PGE) produces coronary vasodilation in the isolated guinea-pig heart (Logan and Wiedemeier, 1973; Krebs and Schror, 1975) and in the anaesthetised (Nakano and McCurdy, 1967) and conscious (Hintze and Kaley, 1977) dog. Prostacyclin (PGI_2) has also been shown to be a potent coronary vasodilator in the isolated rabbit heart (Needleman et al., 1978; Blass et al., 1980) and in the anaesthetised dog (Armstrong et al., 1977; Kaley et al., 1977) whilst prostaglandin $F_{2\alpha}$ has little effect on coronary tone (Hintze and Kaley, 1977).

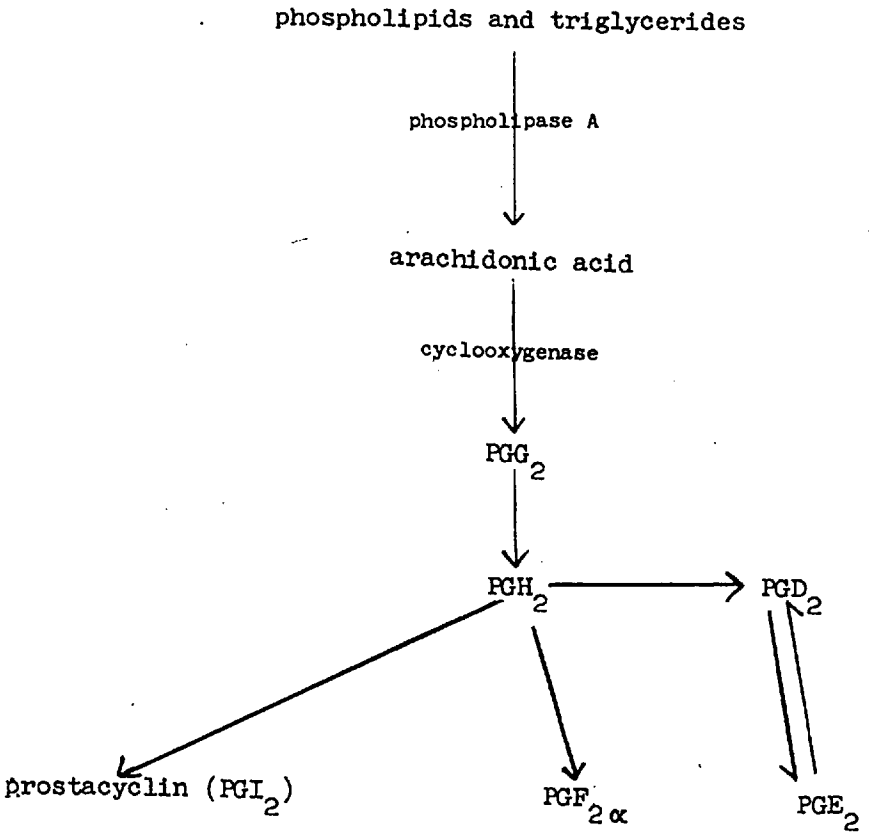


Figure 2.3 Synthesis of the prostaglandins.

Hypoxia causes an increase in the release of a PGE like substance into the coronary effluent. This release is transient (Needleman et al., 1975) and only occurs on reoxygenation or reperfusion (Wennmalm et al., 1974; Block et al., 1975; Needleman et al., 1975). Hypoxic vasodilation is not attenuated by inhibition of prostaglandin synthetase in the isolated rabbit heart (Block et al., 1975; Needleman et al., 1975) or in the in situ dog heart (Hintze and Kaley, 1977) although a small reduction in vasodilation was seen by Alfonso and coworkers (1974) in the intact dog.

Release of a PGE like compound is also seen when flow is restored after brief occlusions, i.e. during reactive hyperaemia (Minkes et al., 1973; Alexander et al., 1975; Needleman et al., 1975). Indomethacin reduces the reactive hyperaemic response in open-chested dogs (Alexander et al., 1975) but has no effect in intact dogs (Owen et al., 1975; Giles and Wilcken, 1977).

Moretti and coworkers have suggested that prostaglandins play a part in autoregulation in the rabbit heart (Moretti et al., 1976). This does not seem to be true of the isolated guinea-pig heart (Schrader et al., 1977b) or of the dog (Rubio and Berne, 1975). Indomethacin does not alter the increase in coronary flow produced by isoprenaline in the open-chested dog (Harlan et al., 1978) and it has been suggested that prostaglandins might act to limit the degree of vasodilation produced by increased myocardial oxygen demand: indomethacin increases the vasodilation produced in response to noradrenaline, Ca^{2+} and increased heart rate (Talesnik and Sunuhara, 1973; Sen et al., 1976; 1977) whilst infusion of PGE_2 reduces the vasodilatory response to these interventions in the isolated rat heart (Sen et al., 1976; 1977). Thus although

prostaglandins which have vasodilatory properties are produced when vasodilation is occurring, e.g. in reactive hyperaemia and in response to hypoxia, prostaglandins seem to play little part in mediating this vasodilation under physiological conditions.

2.3.4 Bradykinin

Bradykinin is a potent coronary vasodilator (Antonio and Rocha e Silva, 1962; Scholtholt et al., 1965; Lochner and Parratt, 1966) and it has been suggested that it is involved in the regulation of coronary flow (Lochner and Parratt, 1966). It has been proposed that bradykinin produces its vasodilatory action by stimulating deacylation of phospholipids and triglycerides and hence increasing the availability of arachidonic acid for prostaglandin synthesis (Nasjletti and Malik, 1979; Sivakoff et al., 1979). Prostaglandin synthetase inhibitors only partly inhibit the vasodilatory response to bradykinin in the isolated guinea-pig (Schror et al., 1979) and rabbit heart (Regoli et al., 1977) and in the open-chested dog (Hintze and Kaley, 1977) hence indicating a prostaglandin independent vasodilatory mechanism.

Alterations in bradykinin production are monitored by following changes in its precursor, plasma kininogen, because bradykinin itself is rapidly degraded in the plasma. The coronary sinus plasma kininogen content is equal to arterial content under resting conditions in the open-chested dog. However, in early ischaemia there is a 50% decrease in the coronary sinus content, thus indicating increased production of bradykinin (Marshall and Parratt, 1980). Changes in plasma kininogen during hypoxia, reactive hyperaemia and autoregulation have not been studied.

2.3.5 Histamine

The involvement of histamine in the regulation of coronary flow was first suggested by Marcou (1939). He linked an increase in myocardial work with an increase in histamine release. Both H_1- and H_2-receptors have been found in the coronary bed of the rabbit. H_1-receptor stimulation produces vasoconstriction whilst H_2-receptor stimulation produces vasodilation (Coruzzi et al., 1979). Intra-coronary administration of histamine produces vasodilation in monkeys and baboons (Parratt, 1969) which is unaffected by β-adrenoceptor or H_1-receptor blockade but which is inhibited by the H_2-receptor antagonist cimetidine (Parratt, 1982). The reactive hyperaemic response in the dog was not, however, altered by combined H_1- and H_2-receptor blockade (Giles et al., 1977). The effect of histamine receptor blockade on hypoxic and metabolic vasodilation and autoregulation has not been determined.

2.3.6 Osmolarity

Scott and Radaswski (1971) found an increase in the osmolarity of the coronary sinus blood after a 30 s occlusion in the dog. However, they found no change in the osmolarity after stellate ganglion stimulation. Brace and coworkers (1975) found only a poor correlation between the osmolarity of the blood and coronary vasoconstriction in the dog. These results suggest that osmolarity plays little part in the regulation of coronary tone.

2.3.7 Others

Lactate and hydrogen ions have been proposed as the mediators of coronary vasodilation resulting from coronary occlusions of greater than 10 s. Both accumulate in the myocardium during ischaemia and appear in the coronary sinus effluent on reperfusion (Olsson and Gregg, 1965; Scott and Radaswaski, 1971; Dunn et al., 1979). The reactive hyperaemic response in conscious dogs can be attenuated by procaine or propranolol (Juhran et al., 1971; Schwartz and Stone, 1977) suggesting that noradrenaline release is involved in reactive hyperaemic vasodilation. However, although Wollenberger and coworkers (1967) and Newman and coworkers (1971) found an increase in noradrenaline release during 15 s to 3 min occlusions, surgical denervation or catecholamine depletion with reserpine failed to alter the reactive hyperaemic response of the conscious dog (Bache et al., 1975). Control of coronary vascular tone might involve a vasoconstrictor as well as a vasodilator. Myocardial production of this constrictor would be inhibited when an increase in coronary blood flow was required e.g. at elevated myocardial work load, or in hypoxia. Constrictor production and tissue oxygen tension could be linked if O_2 was involved in the synthesis of the mediator.

2.3.8 Summary

The evidence suggests that a chemical mediator is involved in the regulation of coronary vascular smooth muscle tone. Much attention has been concentrated on adenosine as this mediator, whilst other possible candidates have been neglected. The evidence for the involvement of

adenosine in changes in coronary flow produced during hypoxia and reactive hyperaemia is overwhelming but the evidence for its involvement in metabolic vasodilation and autoregulation is less convincing. Adenosine is probably not the sole mediator controlling coronary tone. There is evidence that potassium ions play a role in initiating metabolic vasodilation and possibly prolong the reactive hyperaemic response. Osmolarity and the prostaglandins probably play no part in the physiological regulation of coronary blood flow whilst the contribution of bradykinin, histamine, hydrogen ions and lactate needs further study.

2.4 Myocardial Ischaemia

Myocardial ischaemia occurs when mechanisms for increasing coronary perfusion are no longer effective, that is coronary reserve is exhausted, without fulfilling myocardial oxygen requirements. In man short periods of ischaemia give rise to angina whilst severe and prolonged ischaemia leads to myocardial infarction.

In man myocardial ischaemia is usually regional and results from inadequate blood supply through one of the main coronary arteries.

2.4.1 Atherosclerosis and Myocardial Ischaemia

The most frequent cause of myocardial ischaemia is obstructive coronary artery disease secondary to atherosclerosis. Atherosclerosis has occurred in man for over 3000 years. Czermak (1852) obtained histological evidence of sclerosis of the aorta in Egyptian Mummies whilst Shattock (1909) described the aorta of King Merneptah as

"affected by severe atheromatous disease, large calcified patches being distinctly visible", and examination of a mummified 50 year old of the 21st dynasty (1000 B.C.) by Long (1931) showed marked fibrous thickening (chiefly of the intima) and calcification of the coronary artery.

Although coronary artery disease occurred in ancient Egypt, and Eber's Papyrus contains a description of symptoms that were interpreted by Grapow (1935) as angina pectoris, the cause of the disease was not understood by the ancient Egyptians. The first person to link the clinical symptoms of coronary artery disease and biological ideas concerning an impediment in blood flow was Hippocrates in "Regime in Acute Diseases" Appendix, Chapter 5 (Littre II, 407; Adams I, 136; translated by Jones, 1923):

"With this alteration in the blood, air (Spiritus Vitalis, Pneuma) cannot pervade the natural passages. This stasis produces a cooling down, obscuring of the sight, loss of speech, heaviness of head and convulsions as soon as stasis reaches the heart".

Leonardo da Vinci drew sclerotic and tortuous coronary vessels in "Anatomy of the Old Man" and stated :

"vessels in the elderly through the thickening of their tunnels restrict the transit of the blood"

and thus also linked atherosclerosis and its deleterious effects.

In 1560 Amatus Lusitanus described a sudden death due to obstruction of the heart but provided no anatomical evidence and in 1683 Bellini described a patient with calcified coronary vessels with occlusion and the appropriate clinical signs of ischaemia. In 1698 Chirac carried out the first coronary ligation experiment in the dog and showed that rapid failure of the heart-beat occurred after

ligation. However, it was not until the eighteenth century that a firm correlation was made between myocardial ischaemia and coronary artery disease.

Heberden in his book "Some Account of a Disorder of the Breast" gave the first organised and lucid clinical description of angina pectoris. Edward Jenner suggested that angina was caused by coronary ossification and was supported in this by his close friend Dr. Caleb Hillier Parry (Parry, 1799).About the same time Dr. Samuel Black came independently to the same conclusion. He wrote;

"The primary cause of the disorder is perhaps, in every instance, the ossification of the coronaries....the diseased state of the heart

(Black, 1805)

In 1809 Burns published a book :" Observations on some of the most frequent and important diseases of the heart ", in which he linked ossification of the coronary arteries with impairment in perfusion and failure of myocardial function.

The term "atherosclerosis" was coined in 1833 by J.F. Lobstein, and Virchow introduced the concepts of "thrombosis", "infarction" and "embolism" in his first paper on the pulmonary artery in 1846. The concepts of increased coagulability of the blood, leading to sluggishness of the circulation, and thrombotic lesions were not connected with ischaemic changes in the heart until 1880 when Weigert described the myocardial changes following thrombotic occlusion of a coronary artery.

In the nineteenth century the theory that angina was caused by calcification of the coronary arteries gained widespread recognition but it did not get complete acceptance. In 1812 Warren suggested that the calcification theory was "apt to simplify too much", and Laennec in

his "Traite de l'Auscltation Medicale" (1826) explained angina as "neuralgia of the heart originating in the sympathetic nervous system". Coronary spasm was also proposed as an alternative cause of angina (Latham,1876).

In the present century fixed obstruction due to atheroma has been widely accepted as the sole cause of myocardial ischaemia mainly as a result of pathological investigations where firm atherosclerotic lesions were almost universally found. As stated by Keefer and Resnik (1928): "it is difficult to understand how vasoconstriction could affect a coronary artery which was already altered by atherosclerosis".

2.4.2 Coronary Spasm and Myocardial Ischaemia

In a coronary vessel narrowed by atherosclerosis oxygen supply to the myocardium is sufficient under resting conditions and myocardial ischaemia (evidenced by angina) only occurs when myocardial oxygen requirements increase and the resultant increase in blood flow is insufficient to meet these requirements. Patients with variant angina experience periods of precordal pain lasting seconds or minutes which cannot be linked to an increase in myocardial work and can therefore not be attributed to atherosclerosis. The symptoms of this group were classified in detail by Prinzmetal and his associates (1959). They ascribed these cyclic attacks to "tonus", "spasm" or constriction of an otherwise patent coronary artery, or in other cases to a slight transient increase in tone in an artery already occluded by a large structural mass.

Development of non-invasive techniques such as coronary

angiography (first used by Roustoi, 1933) has led to direct observation of "spasm" of a major coronary vessel in patients with Prinzmetal angina (e.g. Oliva et al., 1973; Chahine et al., 1975). Coronary constriction has also been seen in patients with exercise angina (e.g. Yasue et al., 1979) and during myocardial infarction (e.g. Maseri et al., 1978). Thus although atheroslerosis can be pinpointed as the underlying cause of the majority of cases of myocardial ischaemia there is increasing evidence that coronary spasm may also be involved.

2.4.3 Myocardial Contracture and Myocardial Ischaemia

Myocardial ischaemia results primarily from a defect in perfusion. This is usually attributed to a defect in the vasculature, i.e. coronary atherosclerosis or coronary artery "spasm". Flow through the coronary circulation is, however, also dependent on the contractile state of the myocardium (Sabiston and Gregg, 1957) and on intramyocardial tissue pressure.It has been proposed that myocardial contracture may promote or exacerbate an anginal attack (Harris, 1975) and that the vunerability of the subendocardium to ischaemia is a result of high tissue pressure.(Hoffman et al.,1983).

CHAPTER 3

THE EXPERIMENTAL PREPARATION

3.1 Introduction

The relationship between perfusion pressure and coronary flow, in the mammalian heart, has been studied in several experimental models. The majority of investigations have used the in-situ dog heart (Mosher et al., 1964; Rouleau et al., 1979; Sherman et al., 1980; Eng et al., 1981; Kirkeede et al., 1981) although isolated rat (Weisfelt and Shock, 1970), guinea-pig (Arnold et al., 1968) and rabbit hearts (Day and Johnson, 1959) have also been studied. The in-situ dog heart has the advantage of being suitable for chronic studies, has normal innervation, and is perfused with blood. Coronary measurements are, however, altered by changes in systemic haemodynamics. The isolated perfused heart preparation permits rigorous control of extrinsic factors which influence cardiac perfusion and metabolism and, hence, provides a suitable preparation in which cardiac and coronary function can be studied in isolation.

The basic method for perfusion of an isolated, non-working, whole heart remains similar to that used by Langendorff in 1895. A physiological salt solution is delivered to the heart via a cannula tied into the aorta so that its tip is above the aortic valve and the coronary ostia. The position of the cannula allows perfusion of the coronary circulation, but prevents fluid from directly entering the

chambers of the heart. The perfusion fluid enters the right and left coronary arteries and after traversing the capillary bed drains into the right atrium via the coronary sinus, or into the left ventricle via the Thebesian veins. The rate of perfusion is controlled by the hydrostatic pressure of a column of fluid above the heart, or by a perfusion pump. Perfusion pressure, coronary flow and myocardial tension can easily be measured. The effluent dripping from the heart can be collected for the measurement of biochemical parameters whilst drugs can be administered as an infusion or as a bolus injected into the perfusate.

This preparation thus provides a simple model in which the effects of physiological, biochemical or pharmacological interventions on coronary vascular tone, mechanical performance or myocardial metabolism can be determined. In this chapter the modifications made to the basic Langendorff preparation are described and the proportion of perfusate passing through the capillary bed is estimated.

3.2 The Modified Langendorff Preparation

3.2.1 Animals

Adult male New Zealand White rabbits (2.5 - 3.0 Kg) or Dunkin Hartley guinea-pigs (0.3 - 0.4 Kg) were used in these experiments. The rabbits were heparinised and anaesthetised by injection of 2500 IU. heparin and 90 mg phenobarbitone sodium into the lateral ear vein. Guinea-pigs were killed by cervical dislocation.

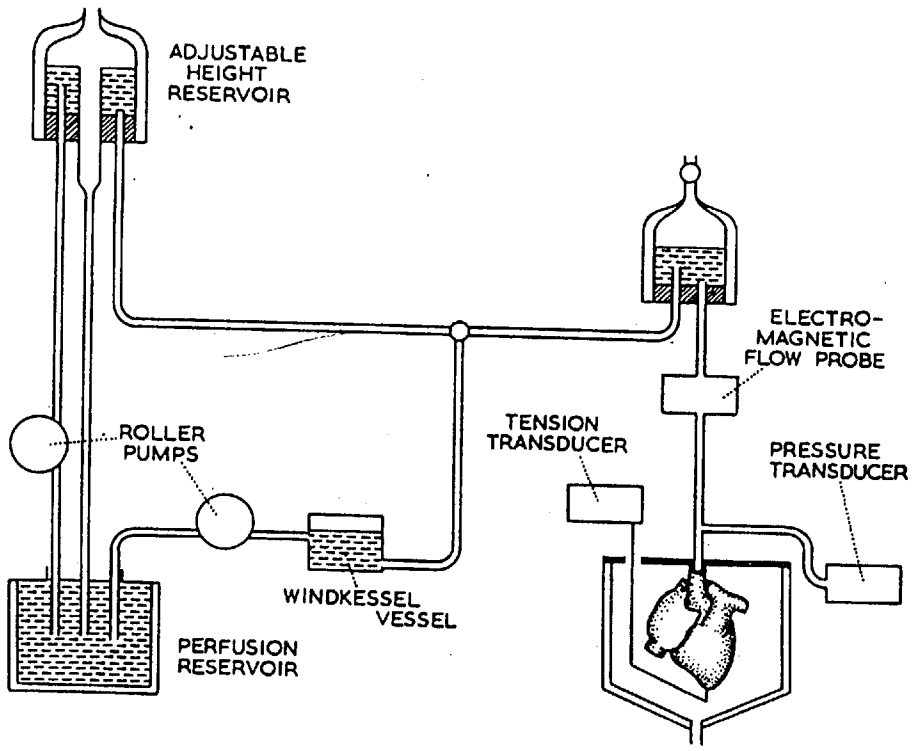


Figure 3.1 The perfusion apparatus. Either perfusion pressure or flow can be controlled. The pressure at which the coronary vessels are perfused is controlled by altering the height of the 'adjustable height reservoir'. The flow of perfusate is controlled by altering the speed of the roller pump.

3.2.2 Perfusion of the Hearts

The hearts were rapidly removed from the animal and immersed in ice-cold perfusate. After the extraneous fat and connective tissue had been removed the aorta was exposed and the heart suspended from a polythene cannula, 2 mm internal diameter for rabbit and 1.6 mm internal diameter for guinea-pig hearts(Fig.3.1). The hearts were perfused with a physiological salt solution containing in mmol.litre NaCl 118.40, KCl 4.75, $MgSO_4.7H_2O$ 1.19, KH_2PO_4 1.19, $NaHCO_3$ 25.0, $CaCl_2$ 1.80 and glucose 11.10. The solution was gassed in the perfusion reservoir with 95% O_2, 5% CO_2. After cannulation flow was gradually increased until systolic perfusion pressure reached about 50 mmHg. A small tube, 1 mm internal diameter, was inserted through the apex of the left ventricular wall to drain the left ventricular cavity. Pressure, flow, contraction and heart temperature were recorded (Recorder Model MX412, Ormed Engineering, Welwyn Garden City, England). A stabilisation period of 20 min was allowed after cannulation before any experimental intervention was made.

3.2.3 Control of Heart-Rate

After A-V dissociation had been achieved by cutting the interatrial septum at its junction with the ventricles, the hearts were paced at 3 Hz using suprathreshold rectangular pulses of 0.2 ms duration delivered from a Model 6032 stimulator (Scientific and Research Instruments, Croydon, England). The stimuli were delivered by a pair of platinum electrodes inserted into the left ventricular

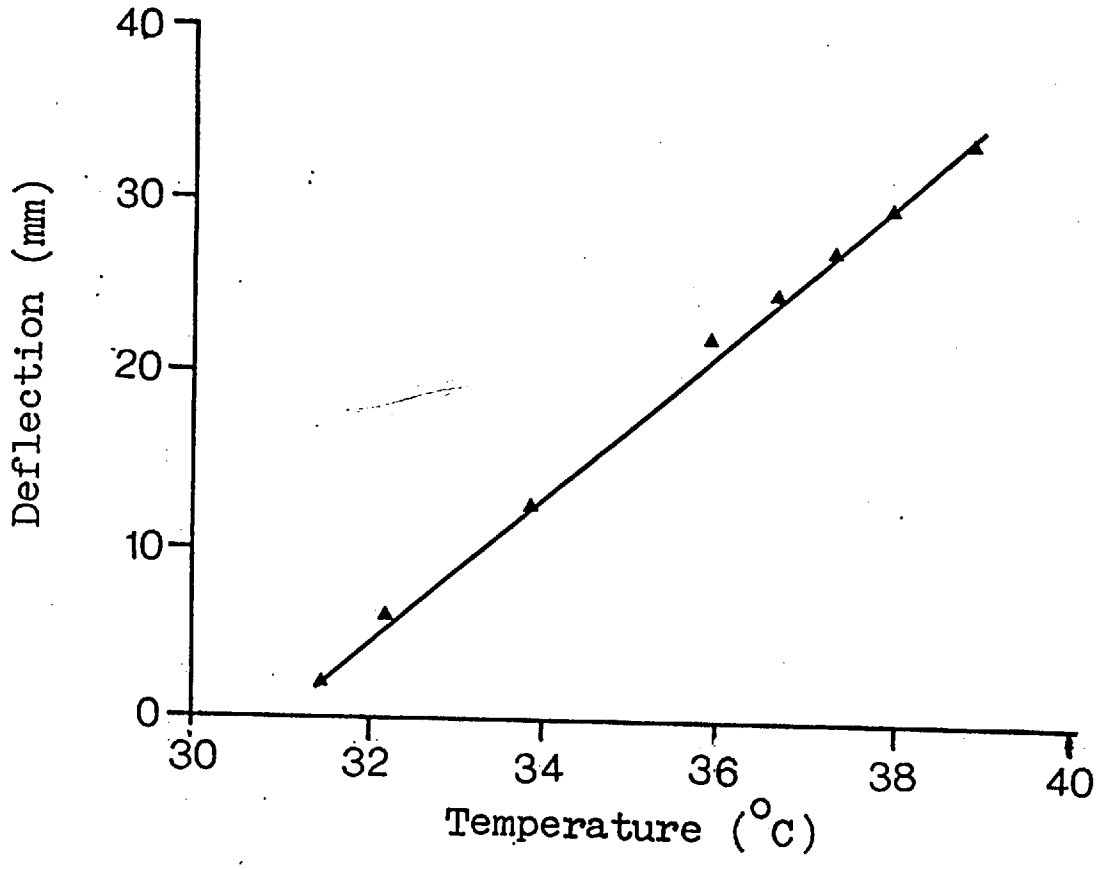


Figure 3.2 Response of thermistor to temperature. The response was measured by immersing the thermistor in beakers of water kept at different temperatures. Deflection on the chart recorder is plotted against temperature.

wall. The stimulus voltage was usually less than 10 V.

3.2.4 Control of Temperature

Temperature was measured with a small thermistor (GM 22W, ITT) the output from which changed linearly with changes in temperature between 30 and 40°C (Fig. 3.2).Perfusate was kept at 36°C. Heat loss from the perfusion system was minimised by surrounding the perfusion reservoir, the "adjustable height" reservoir, the tubing connecting them to the bubble trap and the bubble trap itself by a heated water jacket in which the circulating water was maintained at 37°C. Lagging the tubing connecting the bubble trap to the cannula was impractical so this distance was kept as short as possible. Preliminary experiments in which a thermistor was used to measure perfusate temperature at the cannula tip showed that these measures were sufficient to maintain perfusate temperature at 36 ± 0.5°C independent of flow rate.

Preliminary experiments showed that in the absence of an isothermic chamber myocardial temperature as measured by a thermistor inserted in the left ventricular wall was maintained as long as perfusion was continued. During no flow conditions, however, heart temperature declined so that after 15 min myocardial temperature was 32 ± 0.5°C. Enclosing the heart in a chamber heated with water supplied from a bath maintained at 39°C resulted in an air temperature within the chamber of 36°C and maintenance of myocardial temperature throughout global no flow ischaemia.

In all the experiments described in this thesis the heart was enclosed within the isothermic chamber and myocardial temperature was

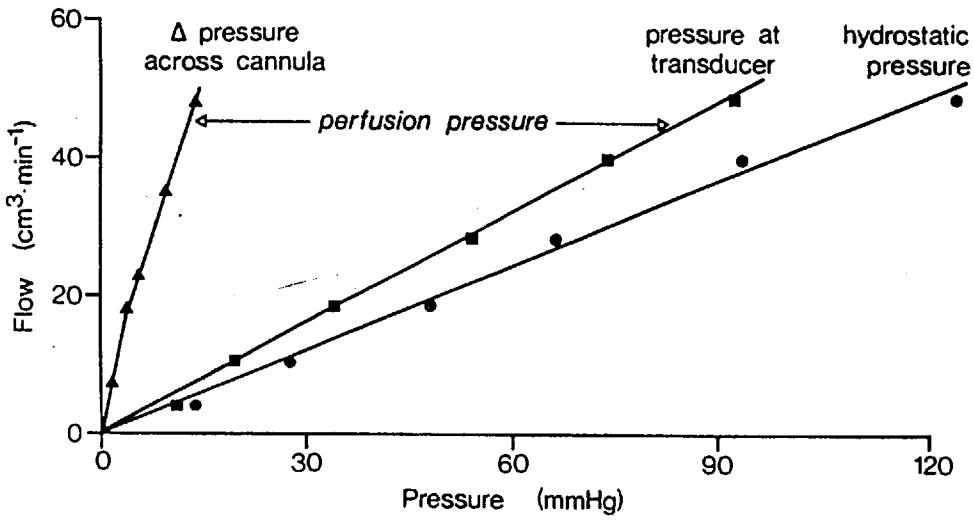


Figure 3.3 Assessment of pressure loss within the perfusion apparatus. Hydrostatic pressure and pressure recorded by a pressure transducer just above the aortic cannula were measured at a series of flows. The pressure drop across the cannula was also recorded. Note that the difference between the hydrostatic pressure and that recorded by the transducer increases with flow, as does the pressure drop across the cannula.

monitored by a thermistor inserted into the left ventricular wall. Temperature was maintained at 36 $\pm$ 0.5^{o}C.

3.2.5 Method of Perfusion

The two methods of perfusion used are illustrated in Figure 3.1. When perfusion pressure was controlled perfusate was continuously pumped to the "adjustable height" reservoir at a rate of 0.11 litre.min^{-1} by a roller pump (Model MHRE 200, Watson Marlow,Falmouth, England). This rate of flow was greater than aortic flow. The large overflow pipe in the upper reservoir ensured that hydrostatic pressure was kept constant and that perfusate was continuously recirculated from the perfusion reservoir. The perfusion pressure was altered by changing the height of the upper reservoir above the heart.

When flow was controlled and pressure allowed to vary a roller pump (Model MHRE 200, Watson Marlow, Falmouth, England) pumped perfusate to the bubble trap via a Windkessel vessel which eliminated oscillations in flow caused by the rotary mechanism of the pump. Flow rate was changed by altering the speed of the pump.

3.2.6 Measurement of Perfusion Pressure

The majority of workers measure only the hydrostatic pressure at which an isolated heart preparation is perfused. In this study a pressure transducer (Statham P23De), connected to a side arm of the aortic cannula below the flow probe, was also used (Fig.3.1). The pressure measured by this transducer was less than the hydrostatic pressure when the heart was perfused from the "adjustable height"

reservoir; at a flow of 0.040 litre.min^{-1} the difference was 11 mmHg (Fig. 3.3). The pressure loss occurs because the tubing in the perfusion apparatus is resistant to flow through it. Poiseuille's Law (Poiseuille, 1846) states that the pressure drop across a tube is a function of the resistance of the tube and the flow through it, thus the pressure drop in the perfusion system increases not only with the resistance of the tubing but also with an increase in flow (Fig. 3.3). A similar relationship exists in the tubing between the pressure transducer and the heart (Fig. 3.3). The pressure measured by the pressure transducer had to be corrected for this flow-dependent drop in pressure to obtain the true pressure at which the heart was being perfused. The pressure loss across the cannula was measured by perfusing at a series of flows in the absence of a heart. In this situation the pressure measured by the transducer was that due to the resistance of the cannula. At a flow of 0.040 litre.min^{-1} the pressure loss across the cannula was 10 mmHg. In all the experiments described in this thesis the pressure measured by the pressure transducer was corrected for the pressure loss across the cannula before pressure-flow relationships were constructed.

3.2.7 Measurement of Flow

An electromagnetic flow probe (Model EP 300A, Carolina Medical Electronics Inc., North Carolina, USA) was connected in series with the aortic cannula (Fig. 3.1). The output from the associated flow meter (Model 501D, Carolina Medical Electronics Inc., North Carolina, USA) was linearly related to flow between 0 and 0.1 litre.min^{-1} (Fig. 3.4). The flow meter responded to changes in flow (initiated by

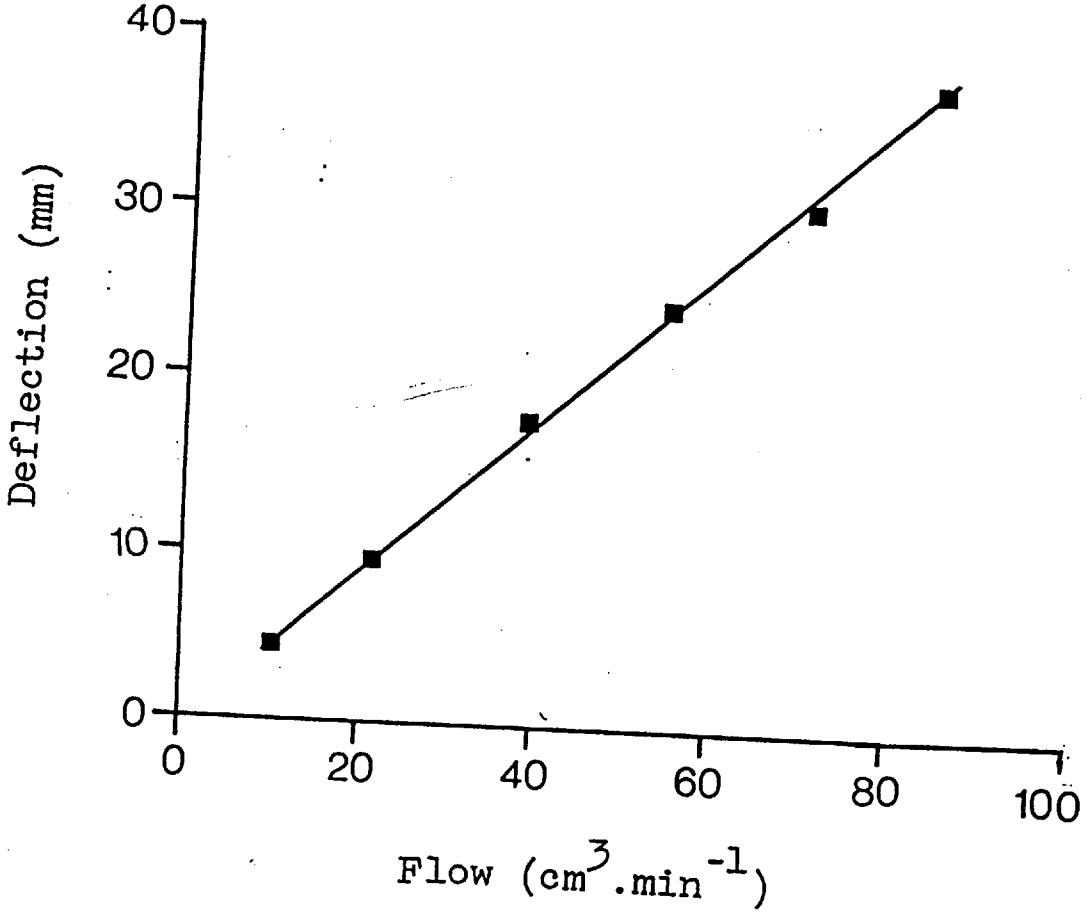


Figure 3.4 Response of the electromagnetic flow meter. Deflection on the chart recorder is plotted against flow. Note that the response increases linearly with flow.

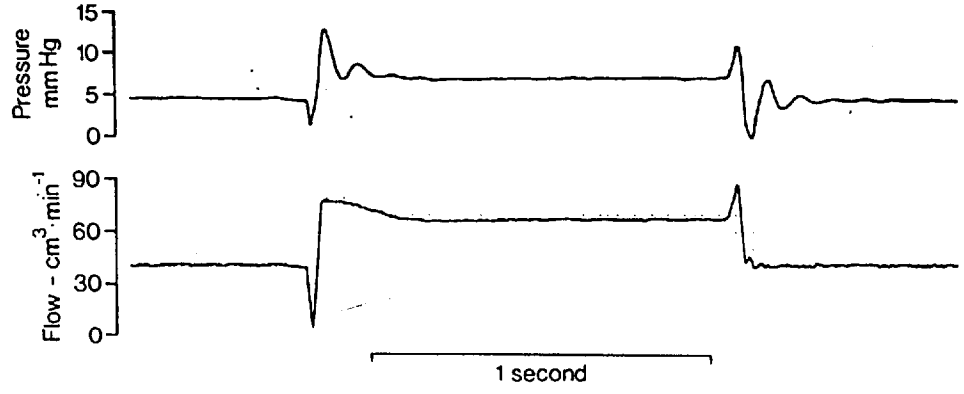


Figure 3.5 Speed of response of the electromagnetic flow meter. Flow through the perfusion apparatus was changed by rapidly altering the height of the 'adjustable height' reservoir. The trace shows the resultant changes in pressure (recorded by a Statham P23De pressure transducer) and flow recorded by the flowmeter. Secondary oscillations on the pressure trace are due to resonance. Note that the recorded flow change closely mimics the change in pressure and that the response is completed within 30 ms.

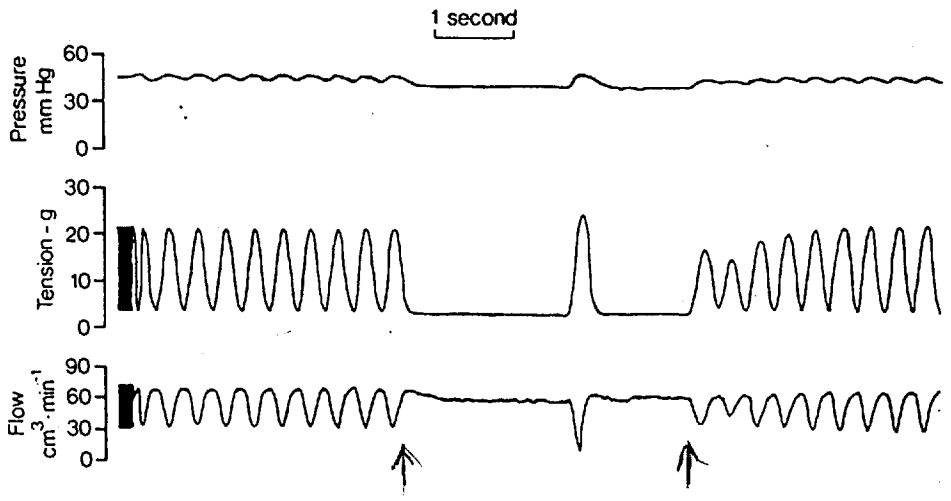


Figure 3.6 Perfusion pressure and flow during prolonged diastole. Electrical stimulation was stopped in the period between the arrows. Note that in the resultant prolonged diastole both flow and perfusion pressure remain constant.

changing hydrostatic pressure) within 30 ms (Fig. 3.5). Assuming that in an isolated heart stimulated at 3 Hz the proportion of time in systole and in diastole is equal, each phase lasts for approximately 170 ms, the speed of response of the flow meter was adequate to record systolic and diastolic flows as well as mean flow.

3.2.8 Use of End-Diastolic Values

As the myocardium contracts it occludes coronary vessels and reduces coronary inflow (Sabiston and Gregg, 1957). The magnitude of this effect depends on the force of contraction. Mean coronary flow values are therefore dependent on force of contraction. Mean coronary flow is also affected by heart rate because the proportion of time in diastole declines as heart rate is increased. In order to obtain measurements which were independent of contractile events end-diastolic values of flow and true perfusion pressure were used to construct pressure-flow relationships. This enabled changes in resistance caused by changes in vessel tone to be separated from those due to altered myocardial function. Both end-diastolic flow and pressure measurements were unchanged when diastole was prolonged (Fig. 3.6). This indicated that end-diastolic values measured at normal stimulation rates were free from the effects of contraction.

3.2.9 Measurement of Resting and Developed Force

Contraction was monitored with a force displacement transducer (Model UF1, Devices, Welwyn Garden City, England) the output from which was linear at loads up to 30 g (Fig. 3.7). Enclosing the heart

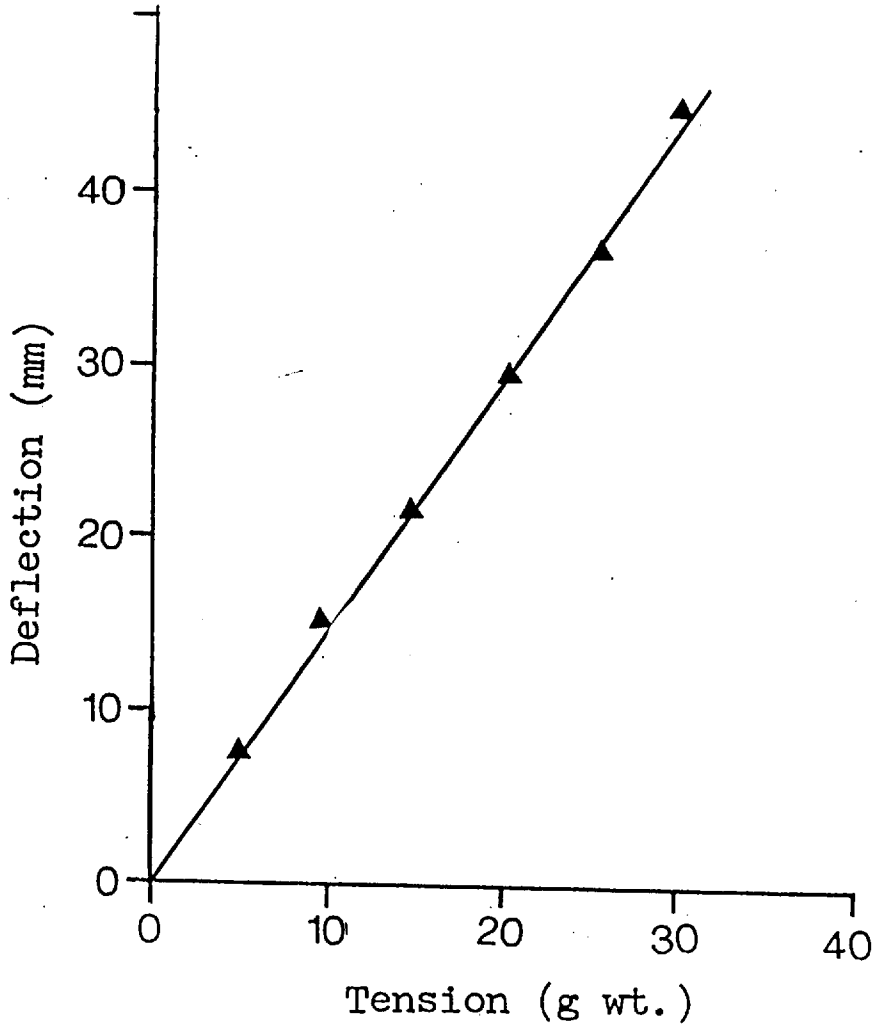


Figure 3.7 Response of the tension transducer. The transducer was calibrated with a series of known weights. Deflection on the chart recorder is plotted against weight. Note that the response is linear up to 30 g.

within an isothermic chamber prevented direct connection between the transducer and the heart. A steel rod 1 mm diameter was bolted to the tension transducer and inserted into the chamber through a hole 0.5 cm diameter. The steel rod was bent underneath the heart and attached to the apex with a ligature (Fig. 3.1). The force of myocardial contraction was relayed through the rigid steel rod to the transducer.

3.3 Measurement of Nutrient Flow

3.3.1 Introduction

Radioactively labelled microspheres were used to determine the proportion of the aortic flow which passed through the coronary bed. Radioactive microspheres have been shown to be distributed in proportion to flow in a system of branching tubes (Rudolph and Heymann, 1967). Measurements made with microspheres in vivo correlate well with other flow markers e.g. ^{86}Rb (Yipintsoi et al., 1973; Cohen, 1978) and antipyrine (Yipintsoi et al., 1973; Utley et al., 1974) and suggest that microspheres are distributed in proportion to flow in vivo. The microspheres have a larger diameter than the vessels of the capillary bed and become trapped in the precapillary vessels. The small proportion of spheres collected in the effluent reflects non-capillary flow. Sphere sizes of between 50 and 9 μm have been used to measure coronary blood flow. The larger spheres are preferentially deposited in regions of high flow whilst smaller spheres show a similar distribution to diffusible indicators (Yipintsoi et al., 1973). Large spheres become trapped towards the proximal end of the coronary bed and may occlude major arteries and cause ischaemia.

Spheres smaller than the capillary diameter will pass through the circulation and are of no use in estimating nutritive flow. In this study spheres of "9 μm" and "25 μm" diameter were used. Coronary capillary diameter has been estimated to be about 5 μm (Bassingthwaighte et al., 1974; Grayson et al., 1974) so the "9 μm" spheres should be trapped in the pre-capillary bed. Spheres collected in the effluent therefore reflect flow that has not passed through the capillary bed. The larger spheres (25 μm) are trapped more proximally in the coronary bed and detection of these in the effluent reflects shunts greater than 25 μm diameter.

3.3.2 Experimental

The polystyrene spheres, of nominal diameter 9 or 25 μm, were labelled with ^{141}Ce, ^{85}Sr (3M Company, Minnesota, USA) or ^{57}Co (N.E.N., Massachusetts, USA). The spheres were suspended in saline to which 0.05% Tween 80 had been added to prevent aggregation (Phibbs et al., 1967). The suspensions were shaken vigo rously before use. In some experiments two types of spheres were mixed before injection into the heart.

Rabbit hearts were perfused from the adjustable height reservoir. After the initial stabilisation period systolic pressure was adjusted to 50 or 80 mmHg and stabilisation allowed for 5 min. The microsphere suspension (0.4 cm^3, containing approximately 1.2 x 10^7 spheres) was injected directly into the aortic cannula. The effluent from the heart was collected in 5 s samples for 5 min. Both effluent and tissue samples were counted in a gamma-counter (Model 6500, ICN, Twickenham, England).

3.3.3 Results

The spheres of nominal diameter 9 μm labelled with ^{85}Sr had a mean diameter of 8.1 ± 1.4 μm (mean ± SD, estimated by 3M Company) whilst those labelled with ^{57}Co had a mean diameter of 11.5 ± 1.4 μm (estimated by N.E.N.). The spheres labelled with ^{141}Ce (nominal diameter 25 μm) had a mean diameter of 25.8 ± 2.2 μm (estimated by 3M Company). There was no significant difference in the percentage of the radioactivity from the ^{85}Sr and ^{57}Co labelled spheres collected in the coronary effluent (12.8 ± 3.3% vs. 10.4 ± 2.2%, n = 4). Results obtained from ^{85}Sr and ^{57}Co labelled spheres were therefore combined. The proportion of radioactivity from the "9 μm" spheres collected in the effluent from hearts perfused at a diastolic pressure of 45.3 ± 1.6 mmHg was 12.1 ± 2.1% (n = 7). At the higher diastolic perfusion pressure (71.6 ± 2.0 mmHg) the proportion was 16.1 ± 5.9% (n = 4). These values were not significantly different (unpaired t-test) from those at the lower perfusion pressure. The proportion of radioactivity collected in the effluent when "25 μm" spheres were used was 7.4 ± 1.8% at a diastolic pressure of 70.0 ± 1.6 mmHg (n = 3). A paired t-test showed a significant difference between the washout of the "9 μm" and the "25 μm" spheres ($p < 0.05$).

3.4 Discussion

The perfusion system used to perform the experiments described in this thesis facilitated measurement of aortic flow and true perfusion pressure as well as myocardial contraction. It also allowed rigorous

control of temperature.

3.4.1 Perfusion Pressure

In many previous studies where an isolated heart was perfused from an elevated reservoir and perfusion pressure was determined by the height of the reservoir only hydrostatic pressure was measured (e.g. Day and Johnson, 1959).The hydrostatic pressure is not, however, the pressure at which the heart is perfused. Pressure is lost as the perfusate flows through the system. The size of the pressure loss depends both on the resistance of the system and the rate of flow through it (Poiseuille, 1846). If the hydrostatic pressure is used to calculate the resistance of the coronary circulation an artificially high value is obtained. Overestimation of coronary resistance can also occur if the resistance of the tubing between the pressure transducer and the heart is not taken into account. In the experiments described in this thesis perfusion pressure was measured just above the heart and corrected for the pressure loss across the cannula. The pressure-flow relationships constructed from these values therefore reflected the relationship between the true perfusion pressure in the main coronary arteries and the flow through the heart.

3.4.2 Aortic Flow

The speed of response of the electromagnetic flow meter enabled differentiation between systolic and diastolic flows. Contraction of the myocardium occludes coronary vessels and decreases coronary flow (Sabiston and Gregg, 1957). The magnitude of this effect is dependent

on the force of contraction. Use of diastolic values, which were free of the effects of myocardial contraction (prolonging diastole had no effect on flow) enabled changes in coronary flow caused by changes in coronary resistance to be distinguished from those caused by alterations in force of contraction.

In the anaesthetised dog, prolongation of diastole by vagal stimulation results in a decline in flow with time (Bellamy, 1978). This is partly due to a decline in systemic blood pressure (and hence coronary perfusion pressure) but is also due to discharging of a capacitance within the coronary circulation (Dole and Bishop, 1982). Pressure-flow relationships constructed from prolonged diastoles in the dog do not take this capacitance into account and hence overestimate both coronary tone and the minimum pressure required for flow (Dole and Bishop, 1982). In the isolated rabbit heart perfused at constant pressure no decline in flow occurs when diastole is prolonged. Thus, pressure-flow relationships constructed at normal heart rates in this preparation are not affected by capacitance effects and reflect the true relationship between pressure and flow.

3.4.3 Control of Perfusion Parameters

When the preparation was perfused from the "adjustable height" reservoir a change in coronary resistance resulted in a change in flow. This change in flow altered the pressure loss in the tubing and hence true perfusion pressure was also altered. When the rotary pump was used to control perfusion a change in coronary resistance resulted in a change in perfusion pressure.At high pressures the efficiency of the pump was reduced and a decrease in flow occurred although pump speed

was unaltered. Thus when resistance changes were occurring within the heart neither perfusion pressure nor flow could be kept precisely constant.

3.4.4 Temperature

The preliminary experiments showed that enclosing the heart within an isothermic chamber was essential for the maintenance of myocardial temperature during global ischaemia. Rate of decline of contraction and development of contracture during ischaemia are dependent on temperature as is contractile recovery on reperfusion (Bing et al.,1975). A constant temperature was therefore essential to allow independent assessment of the effect of an intervention applied in conjunction with global ischaemia.

3.4.5 Nutritive Flow

Mean coronary flow in this preparation is approximately twice that in vivo (Neutze et al., 1968). Measurement of flow distribution with microspheres shows that although some flow does not pass through the capillary bed and some does not traverse vessels less than 25 μm in diameter almost 90% does pass through the capillary bed. The high flow is therefore not due to large shunts within the myocardium or incompetence of the aortic valve. The flow rate is similar to that found in other isolated heart preparations (Day and Johnson, 1959; Arnold et al., 1968; Weisfelt and Shock, 1970) and is probably due to the properties of the perfusate which was a physiological solution with a lower viscosity than blood. The low oxygen capacity of the

perfusate probably caused vasodilation which also contributed to the flow rate.

3.4.6 Conclusions

In the perfusion system used to perform the experiments described in this thesis perfusion parameters could be strictly controlled and changes produced by altering perfusion conditions could be accurately measured. The coronary flow in this preparation was about twice that measured in vivo but there was no evidence of large shunts within the myocardium or aortic incompetence.

CHAPTER 4

PERFUSION PRESSURE AND CORONARY FLOW IN THE ISOLATED PERFUSED HEART

4.1 Introduction

4.1.1 Flow in Tubes

The factors governing the flow of fluids through a system of rigid cylindrical tubes were first identified by the French physiologist Poiseuille. He accurately described the relationship between pressure-head, flow and tube dimensions (Poiseuille, 1846) although it was not until later that the contribution of viscosity was added giving Poiseuille's Law its present form:

$$Q = \frac{(P_1 - P_2)R^4}{3^{8}\,L}$$

where Q is the rate of flow of a liquid, η the viscosity of that liquid, R the radius and L the length of the tube and (P_1-P_2) the driving force.

If the fluid viscosity and the vessel dimensions are constant flow is a linear function of the driving pressure (Fig. 4.1, lefthand panel).

The relationship between driving pressure and flow through a

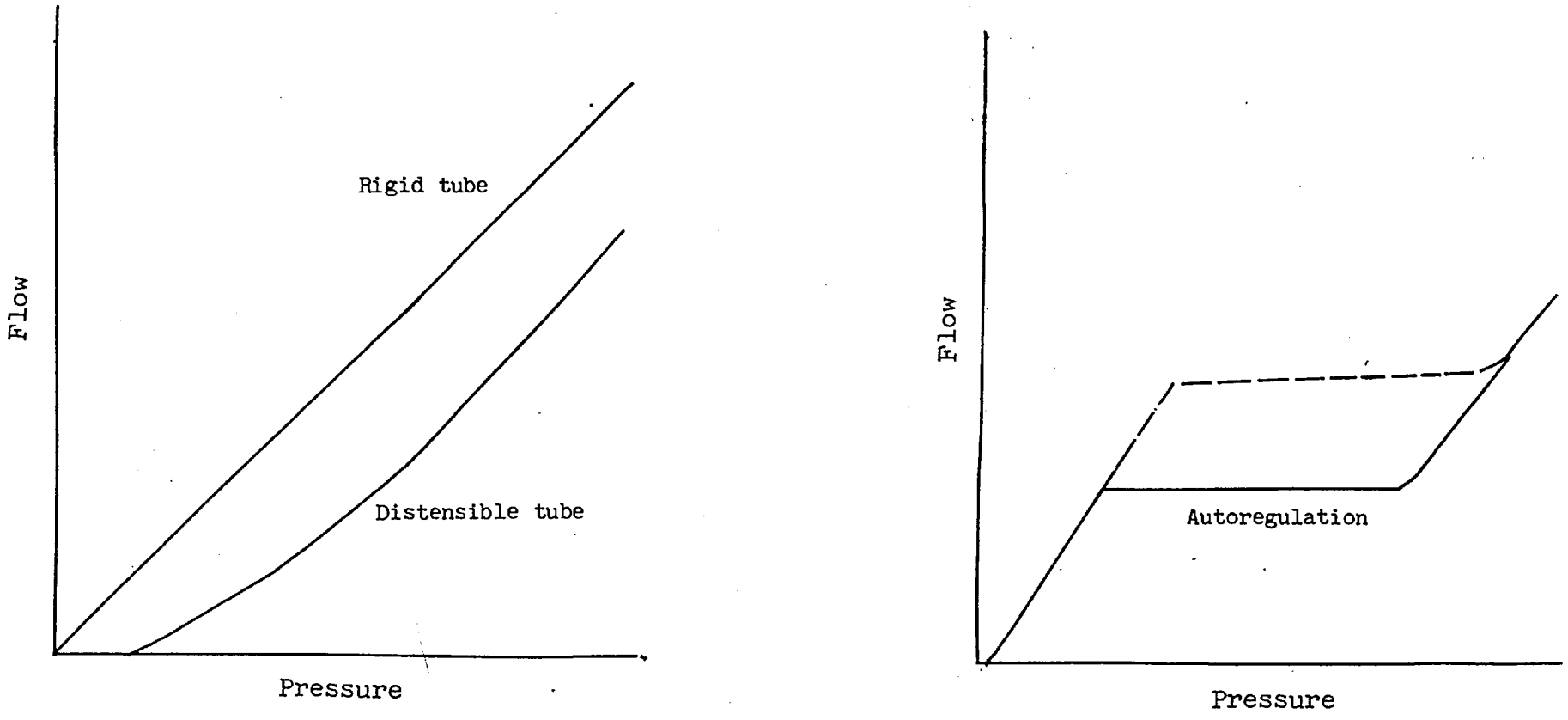


Figure 4.1 Relationship between pressure and flow in a rigid or distensible tube (lefthand panel) and in the coronary circulation (righthand panel); the relationship at a higher myocardial oxygen consumption is shown by the dotted line.

distensible tube is more complicated. In this case increasing inflow pressure increases transmural pressure and results in an increase in diameter as well as an increase in the driving pressure. The increment in flow for a given increment in pressure is greater than for a rigid tube and results in a curvilinear relationship (Fig. 4.1,lefthand panel). The relationship becomes linear once maximum distension is achieved.

Some vascular beds behave as a system of distensible tubes producing pressure-flow relationships similar to that seen for a single distensible tube (Fig. 4.1). In the coronary circulation, however, a different pressure-flow curve is seen. At pressures below 70 mmHg and above 140 mmHg mean flow varies with pressure as expected. Between these pressures "autoregulation" occurs with large changes in pressure resulting in little change in mean flow (Fig. 4.1,righthand panel) (Mosher et al., 1964; Weisfelt and Shock, 1970).

4.1.2 Control of Autoregulation

The maintenance of constant flow over a range of driving pressures requires controlled changes in vascular smooth muscle tone to give the required changes in coronary resistance. The mechanism by which these changes are brought about is unknown but metabolic mediators, increased tissue pressure and myogenic mechanisms have been postulated. Until recently a myogenic mechanism was favoured because it was thought that the compensatory flow change in response to an alteration in pressure occurred too fast during autoregulation for chemical mediators to be involved (Eikens and Wilcken, 1974). However, the observation of changes in myocardial adenosine concentration within a

single cardiac cycle by Thompson and his co-workers (1980) throws doubt on this assumption. The chemical mediators involved in the control of coronary flow and the evidence for their involvement in autoregulation are discussed in Chapter 2.

Rodbard (1971) proposed that autoregulation is caused by changes in tissue pressure. He suggested that the increase in transmural pressure produced by an increase in perfusion-pressure might increase tissue pressure and hence limit the increase in flow. This hypothesis is not supported by the findings of Driscol and co-workers (1964) who found that large coronary flow changes may occur uninfluenced by changes in tissue pressure.

The myogenic theory was first proposed by Bayliss (1902). He suggested that arterial pressure might act as a mechanical stimulus to the vascular smooth muscle and that like visceral smooth muscle it would respond to the stretch by contracting. There is much evidence for the involvement of a myogenic mechanism in the skeletal muscle and mesenteric beds (Johnson, 1980) but its contribution has not been rigorously evaluated in the coronary circulation.

When autoregulation occurs in the coronary bed the level at which flow is maintained depends on the metabolic requirements of the myocardium. At high metabolic demand autoregulation occurs at a high flow (Fig. 4.1,righthand panel) whilst at lower metabolic demands flow is reduced (Driscol et al., 1964; Mosher et al., 1964). Autoregulation can be abolished by vasodilating drugs or hypoxia (Weisfelt and Shock, 1970) and the relationship between perfusion pressure and coronary flow becomes linear or curvilinear (Klocke et al., 1981).

4.1.3 Minimum Perfusion Pressure Required for Flow

The coronary pressure flow curve plotted from mean values of perfusion pressure and coronary flow has a positive pressure intercept even when the coronary bed is maximally dilated. This is partly due to the beating of the heart. During systole intramyocardial pressure exceeds coronary perfusion pressure and intramural vessels are occluded (Sabiston and Gregg, 1957). This effectively reduces the flow at a given perfusion pressure. If end-diastolic values are used to construct the pressure-flow relationship the pressure intercept (P_{ZF}) is reduced but is still of the order of 10-15 mmHg (Sherman et al., 1980). This positive intercept has been taken as evidence of the existence of a vascular waterfall within the coronary circulation (Downey and Kirk, 1975). The effects of heart-beat and intramyocardial pressure on coronary perfusion and the concept of a vascular waterfall are dealt with more fully in Chapter 8.

4.1.4 Calculation of Coronary Resistance

Coronary vascular resistance has traditionally been calculated from the ratio of perfusion pressure to coronary flow (Gregg, 1950). This can give misleading results if a single measurement of perfusion pressure and coronary flow is used to calculate resistance. Take for instance the situation where an intervention has no effect on the slope or pressure intercept of the curve (Fig. 4.2a). If the vascular resistance is taken to be the ratio of perfusion pressure to coronary flow and measurement is made at a different perfusion pressure before (P_1) and after (P_2) the intervention resistance appears to

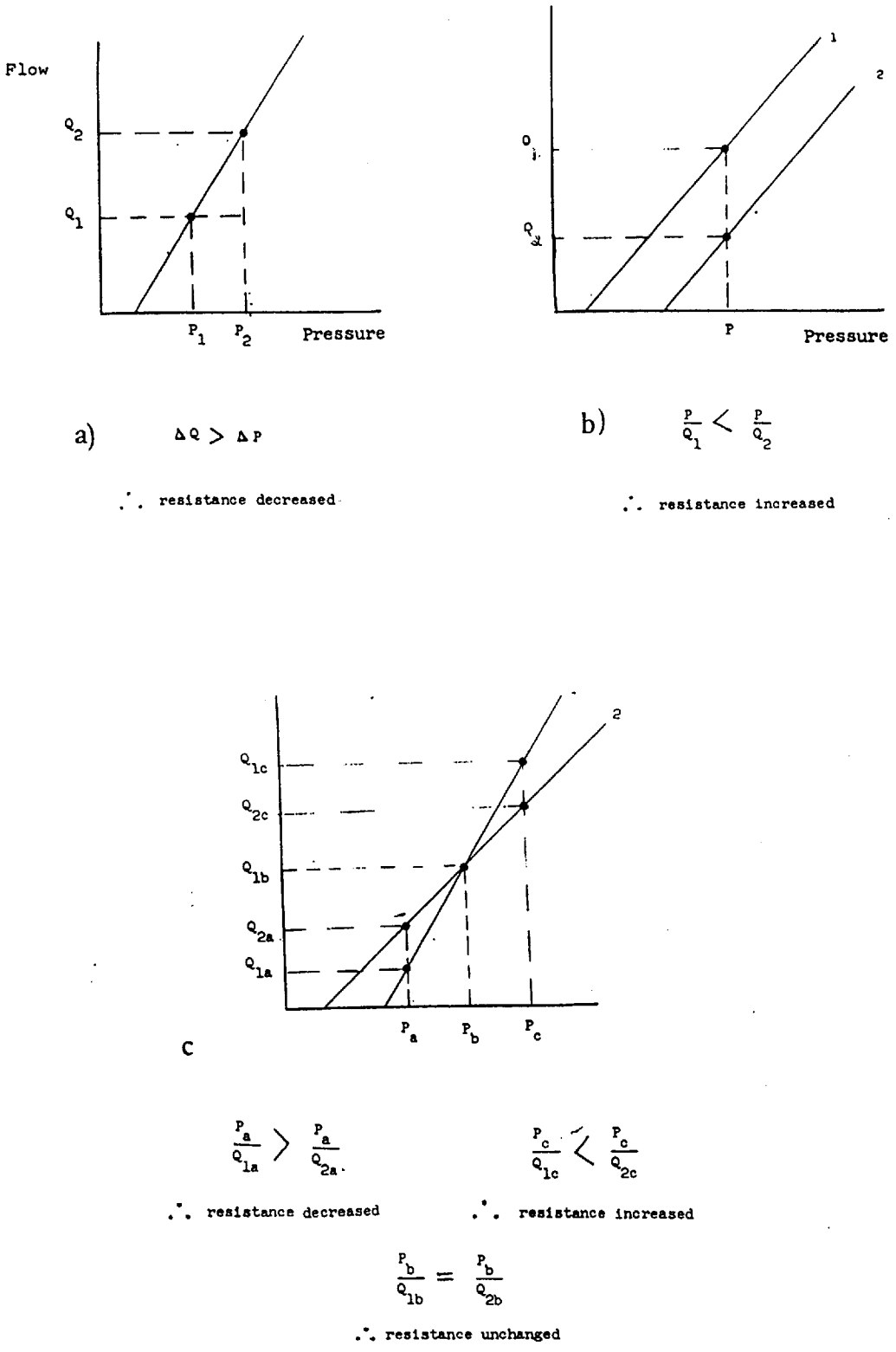


Figure 4.2 Errors inherent in estimating coronary resistance from single measurements of pressure and flow: (a) in the absence of a change in slope or intercept of the relationship between pressure and flow, (b) when only the intercept is altered and (c) when both slope and intercept are altered. Line 1 represents the relationship before an intervention, line 2 that after an intervention.

have decreased

$$\frac{P_1}{Q_1} > \frac{P_2}{Q_2}$$

Confusion can also occur if an intervention alters the pressure intercept (P_{ZF}) but does not change the slope (the resistance) (Fig. 4.2b). The ratio of the measured pressure to the flow before the intervention (P/Q_1) is less than that to the flow after the intervention (P/Q_2). The intervention appears to have increased the vascular resistance. The most complicated case is where an intervention alters both the slope and the pressure intercept (Fig. 4.2c). Here dependent on the pressure measured the intervention appears to have increased (P_c) or decreased (P_a) the resistance, or to have left it unchanged (P_b). These errors can be avoided if a series of values of perfusion pressure and coronary flow are measured and both the slope and the pressure intercept of the relationship calculated.

In the following chapter the relationship between end-diastolic perfusion pressure and end-diastolic coronary flow in the isolated rabbit heart is elucidated by setting up a series of perfusion pressures and measuring the corresponding flow and by setting up a series of flows and measuring the resultant perfusion pressure. The change in the relationship with time is analysed and the effect of adenosine, dipyridamole and nitroglycerin on the relationship investigated. The pressure-flow relationship in the rabbit heart is compared with that of the isolated guinea-pig heart.

In this and all subsequent chapters "resistance" is taken to be

the reciprocal of the slope of the plot of flow against pressure whilst "P_{ZF}" denotes the intercept of the curve with the pressure axis estimated by linear extrapolation of the curve.

4.2 Experimental

4.2.1 Determination of the Relationship Between Perfusion Pressure and Flow

Rabbit or guinea-pig hearts were perfused by the Langendorff method as outlined in Chapter 3. After a 20 min stabilisation period pressure or flow was altered in a stepwise fashion and the resulting changes in perfusion pressure and flow recorded when a steady state had been reached. The time for a steady state to be present was, in general, less than 2 min (Fig. 4.3).

4.2.2 Determination of the Effect of Length of Perfusion on the Relationship Between Pressure and Flow in the Rabbit Heart

After the initial equilibration and determination of the diastolic pressure-flow relationship as described in 4.2.1 systolic pressure was raised to 50 mmHg and the pressure-flow relationship redetermined at 45 and 60 min after cannulation.

4.2.3 Determination of the Effect of Vasodilators on the Relationship Between Pressure and Flow

After the initial equilibration and determination of the

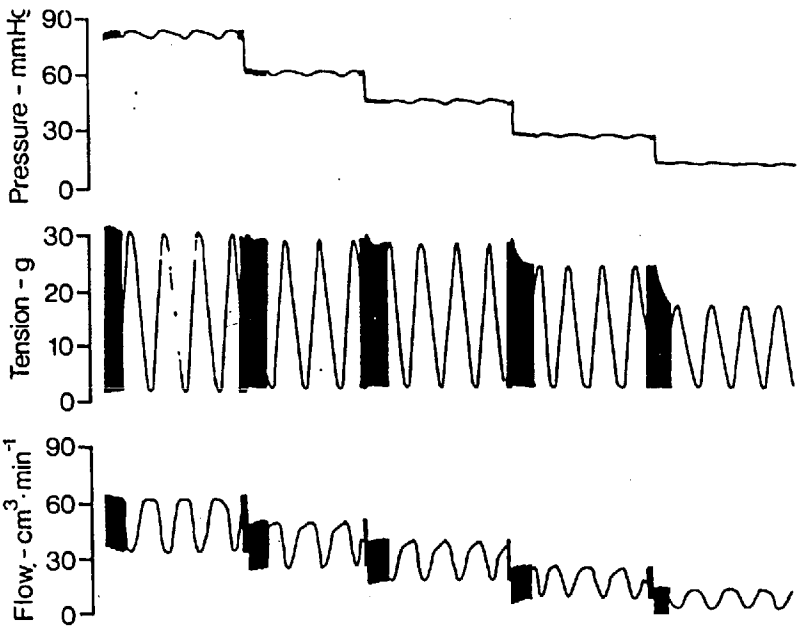


Figure 4.3 Example of the changes in perfusion pressure, tension and flow resulting from alteration of hydrostatic pressure.

pressure-flow relationship as described in 4.2.1 systolic pressure was raised to 50 mmHg and 10 min stabilisation allowed before the perfusate was changed for one containing adenosine (1.87×10^{-5} mol.litre^{-1}). After 5 min perfusion with this solution the relationship between perfusion pressure and flow was redetermined.In some experiments rabbit hearts were perfused with solutions containing dipyridamole (7.93×10^{-6} mol.litre^{-1}) or nitroglycerin (4.41×10^{-6} mol.litre^{-1}) in place of adenosine.

4.2.4 Treatment of Data

Before constructing pressure-flow curves from end-diastolic values pressure measurements were corrected for loss of pressure across the cannula, as explained in Chapter 3, and flows were standardised by expressing as flow per unit dry weight. Dry weights were determined by heating hearts at 100°C to constant weight.

Results are expressed as mean $\pm$ standard error of mean for n experiments. Tests of significance were made using Student's "t" test, taking $p = 0.05$ as the limit of significance. Regression lines were calculated by the "least squares" method.

4.2.5 Materials

Adenosine and nitroglycerin were dissolved in distilled water to give stock solutions of 1.87×10^{-2} mol.litre^{-1} and 4.41×10^{-3} mol.litre^{-1} respectively.Dipyridamole was dissolved in ethanol to give a stock solution of 7.93×10^{-3} mol.litre^{-1}.

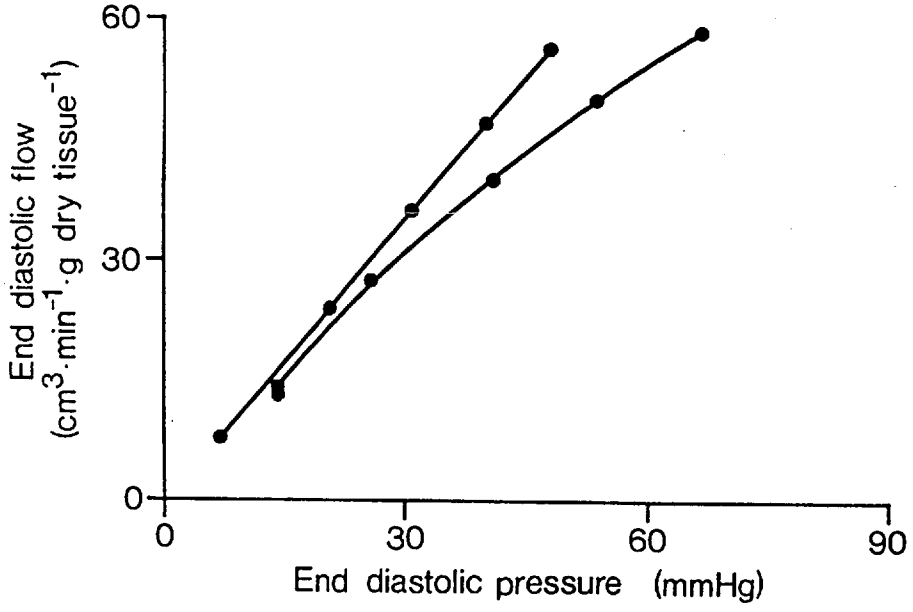


Figure 4.4 Examples of the relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated rabbit heart.

TABLE 4.1 EFFECT OF PERFUSION ON RESISTANCE TO FLOW IN THE RABBIT HEART

	Resistance (mmHg.min.g.litre^{-1})	P_{ZF} (mmHg)	n
Time after cannulation			
20 min	1330 $\pm$ 290	-9.3 $\pm$ 6.0	5
45 min	1530 $\pm$ 210	-12.0 $\pm$ 7.6	3
60 min	1850 $\pm$ 930	-9.5 $\pm$ 5.3	4

4.3 Results

4.3.1 Pressure and Flow in the Rabbit Heart

A step decrease in perfusion pressure, produced an immediate decrease in flow. Diastolic flow recovered slightly before becoming constant within 2 min of the pressure change (Fig. 4.3). The relationship between diastolic pressure and flow was either linear, or curved concavely towards the pressure axis at pressures between 75 and 40 mmHg (Fig. 4.4). The relationship was similar with either perfusion pressure, or flow as the independent variable, and the linear regression coefficient was greater than 0.94 in all experiments. The slope of the plot of end-diastolic flow against pressure was $0.70 \pm 0.05 \times 10^{-3}$ litre.min^{-1}.g^{-1}.mmHg^{-1} (n=15) when a fixed pressure-head was used, and $0.84 \pm 0.05 \times 10^{-3}$ litre.min^{-1}.g^{-1} mmHg^{-1} (n = 15) when fixed pump speed was used. The pressure at which flow ceased (P_{ZF}) for the group in which pressure was altered was -3.8 ± 2.8 mmHg by extrapolation from "best fit" regression lines, and that for the group in which flow was altered was -10.2 ± 2.5 mmHg. The dry weight of the hearts was 1.0090 ± 0.0488g (n = 5). The dry:wet weight ratio of the hearts was 0.111 ± 0.004 (n = 5).

4.3.2 Effect of Length of Perfusion

Coronary resistance (the ratio of change in pressure to change in flow) in the rabbit heart increased with the length of perfusion (Table 4.1). In the 40 min experimental period resistance increased

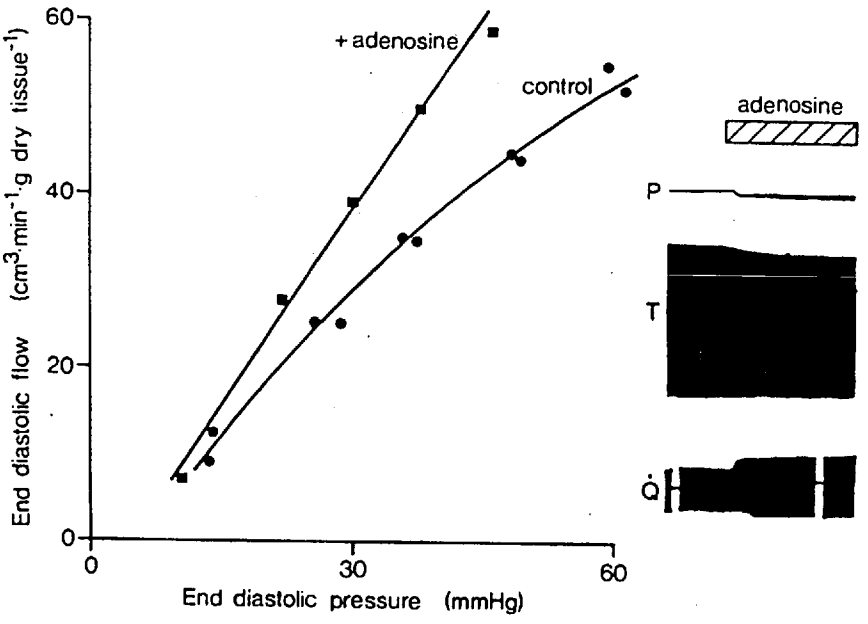


Figure 4.5 Example of the effect of adenosine (1.87×10^{-5} mol.litre^{-1}) on the relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated rabbit heart. Inset panel shows initial effect on perfusion pressure, tension and flow.

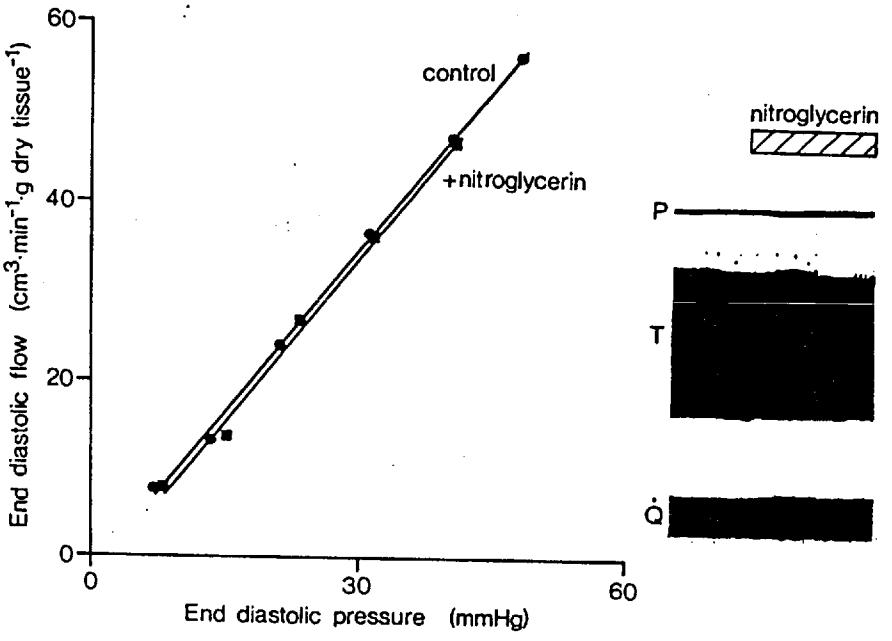


Figure 4.6 Example of the effect of nitroglycerin (4.41×10^{-6} mol. litre^{-1}) on the relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated rabbit heart. Inset panel shows initial effect of nitroglycerin on perfusion pressure, tension and flow.

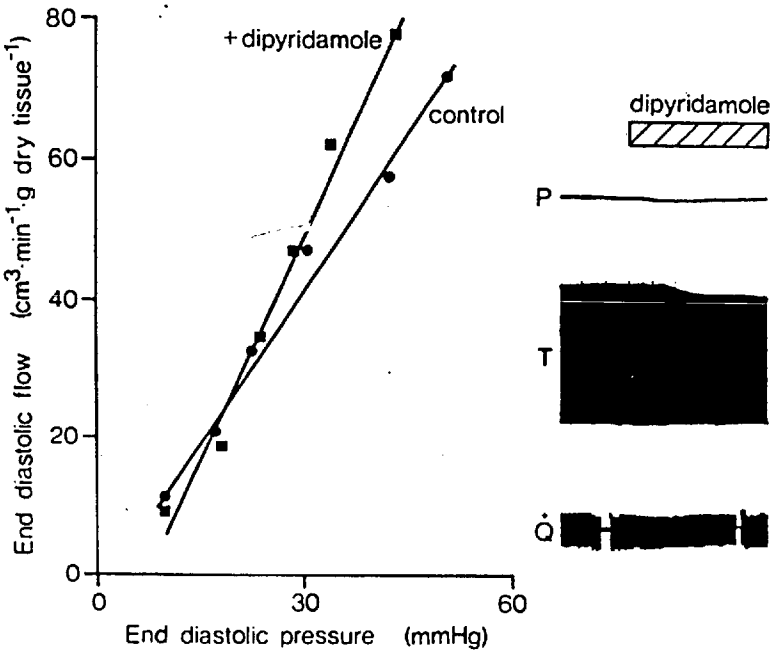


Figure 4.7 Example of the effect of dipyridamole (7.93×10^{-6} mol. litre^{-1}) on the relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated rabbit heart. Inset panel shows initial effect of dipyridamole on perfusion pressure, tension and flow.

TABLE 4.2 EFFECT OF VASODILATORS ON RESISTANCE TO FLOW IN THE RABBIT HEART

		Resistance (mmHg.min.g.litre^{-1})	P_{ZF} (mmHg)	n
Adenosine (1.8×10^{-6} mol.litre^{-1})	before	1070 ± 270	0.9 ± 2.1	5
	after	760 ± 140**	11.0 ± 3.9*	5
Dipyridamole (7.93×10^{-6} mol.litre^{-1})	before	760 ± 120	5.3 ± 2.5	8
	after	820 ± 160	4.9 ± 3.4	8
Nitroglycerin (4.41×10^{-6} mol.litre^{-1})	before	1220 ± 220	-4.7 ± 2.9	5
	after	1240 ± 160	4.0 ± 2.3	5

* = $P \leq 0.05$ paired t-test ** = $P \leq 0.01$ paired t-test

by 22.2 ± 18.3%(n = 4). Over the same period P_{ZF} did not alter. After 60 min perfusion the dry:wet weight ratio of the hearts (0.115 ± 0.006) was not significantly different from that after 20 min perfusion.

4.3.3 Effect of Vasodilators

Administration of 1.87 x 10^{-5}mol.litre^{-1} adenosine to the rabbit heart caused an increase in flow of 5.0±1.4 x 10^{-3} litre.min^{-1}.g^{-1} n = 5 (11.5 ± 2.9%) (Fig. 4.5). There was also a small decrease in diastolic perfusion pressure of 10.1 ± 3.9%. The relationship between pressure and flow was linear ($r > 0.99$), the slope of the plot of diastolic flow against pressure was increased by 34.8 ±9.4% ($p<0.05$,paired t-test)and P_{ZF} was increased by 10.1 ± 3.7 mmHg ($p<0.05$,paired t-test) (Table 4.2). No significant change in pressure or flow was observed when 4.41 x 10^{-6}mol.litre^{-1} nitroglycerin or 7.93 x 10^{-6}mol.litre^{-1} dipyridamole was added to the perfusate (Figs. 4.6, 4.7 and Table 4.2)

4.3.4 Pressure and Flow in the Guinea-Pig Heart

Alteration of perfusion pressure between 75 and 25 mmHg in the isolated guinea-pig heart resulted in little change in aortic flow. Outside this pressure range the change in flow produced by a given increment in pressure was much larger (Fig. 4.8).

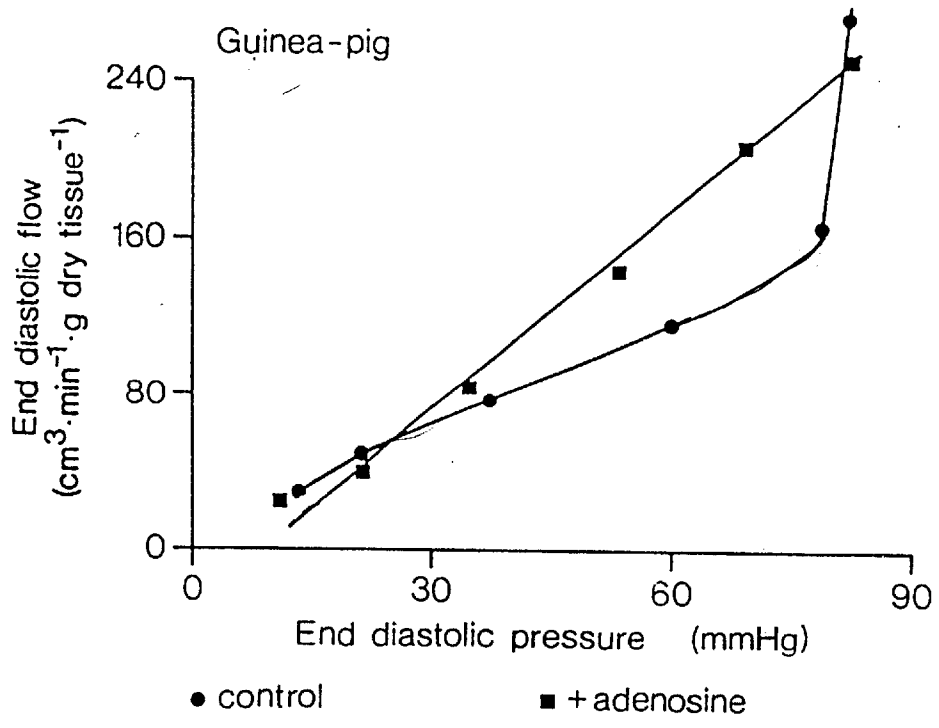


Figure 4.8 Example of the relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated guinea-pig heart. The effect of adenosine (1.87×10^{-5} mol.litre^{-1}) on this relationship is also shown.

4.3.5 Effect of Adenosine on the Pressure-Flow Relationship of the Guinea-Pig Heart

The relationship between perfusion pressure and aortic flow in 5 guinea-pig hearts which had been perfused with adenosine (1.87×10^{-5} mol.litre^{-1}) was linear ($r > 0.95$) (Fig. 4.8). The slope of the plot of diastolic flow against pressure was $3.36 \pm 1.06 \times 10^{-3}$ litre.min^{-1}.g^{-1}.mmHg^{-1} and P_{ZF} was 2.7 ± 1.8 mmHg.

4.4 Discussion

The range of hydrostatic pressures and coronary flows studied in these experiments was similar to that which has previously been used in the isolated rabbit heart (Day and Johnson, 1959), the isolated guinea-pig heart (Arnold et al., 1968) and the isolated rat heart (Weisfelt and Shock, 1970). The use of end-diastolic rather than mean values to eliminate the effects of myocardial contraction and the loss of driving pressure in the tubing connecting the perfusion reservoir to the heart (see Chapter 3) resulted in pressure-flow plots at lower pressures and flows than those previously reported. When this was taken into consideration coronary resistance estimates were similar to those previously obtained in the isolated rabbit heart (Day and Johnson, 1959; Bergmann et al., 1979) and the isolated guinea-pig heart (Schror et al., 1976; Onuaguluchi and Tanz, 1981).

4.4.1 Autoregulation

The guinea-pig hearts showed autoregulation at diastolic perfusion pressures between 25 and 70 mmHg. In the rabbit hearts, however, the only evidence of residual autoregulation was the slight alinearity of the pressure-flow relationship and the slight recovery in flow following an initial drop in response to a reduction in perfusion pressure (Fig. 4.3). The absence of autoregulation in the isolated buffer perfused rabbit heart was also found by Day and Johnson (1959) and may be a consequence of the low oxygen content of the perfusate. The oxygen capacity of blood is greater than 20 $cm^3/100\ cm^3$ blood whilst that of a physiological salt solution is only 0.30 $cm^3/100\ cm^3$. As coronary tone is regulated in response to the oxygen consumption of the myocardium (see Chapter 2) decreased delivery of oxygen will result in a compensatory decrease in coronary tone and will if severe enough result in loss of autoregulation. In the smaller guinea-pig heart where flow per gram of dry tissue is about twice that in the rabbit heart, oxygen supply is more than sufficient to meet metabolic demands and autoregulation still occurs. An alternative explanation for the absence of autoregulation has been suggested by Moretti (Moretti and Abraham, 1978). He found that in the isolated rabbit heart vasoconstriction and autoregulation can be augmented by the addition of blood plasma to the perfusate. He has suggested that a plasma constituent stimulates the production of an arachidonate metabolite and that this normally controls coronary vascular tone in the rabbit heart. This constituent does not seem to be required for coronary autoregulation in the isolated guinea-pig heart (Schrader et al., 1977b) or in the anaesthetised dog (Rubio and Berne,

1975).

4.4.2 Applicability of Linear Regression Analysis

Linear regression analysis was applied to the pressure-flow points obtained from the rabbit experiments in order to estimate coronary diastolic resistance and the minimum perfusion pressure required for flow to occur (P_{ZF}). In some experiments residual autoregulation was present, evidenced by slight curvature of the pressure-flow relationship and application of linear regression resulted in underestimation of P_{ZF}. The correlation coefficient for linearity was always greater than 0.90 and maximal dilation with adenosine produced only a small increase in P_{ZF}.

4.4.3 Prolonged Perfusion

The increase in coronary resistance which occurred after prolonged perfusion of the isolated rabbit heart is unlikely to have been caused by active constriction of the vascular smooth muscle. The most probable causes are increased intramyocardial pressure ,due to oedema or blockage of small vessels by particles in the perfusate. Oedema in isolated hearts perfused with no macromolecules was first reported by Bleeham and Fisher (1954). In rabbit hearts the weight gain due to this increase in tissue water content has been estimated to be 6% per hour (Vargas and Blackshear, 1981) and is independent of flow rate (Henry et al., 1977). In the hearts used in this study there was no decrease in dry:wet weight ratio over the prolonged period of perfusion. However, the tissue water content was greater

than that reported for freshly isolated hearts and hence oedema could have contributed to the increase in resistance to flow.

The coronary vessels of the heart act as a filter preventing the passage of particles of greater size than the vessel diameter. Thus any particles larger than 5 μm in diameter in the perfusate would have become lodged in the arterioles and capillaries and reduced or completely prevented flow-through that vessel. The proportion of the capillary bed affected would have increased with the length of the perfusion period thus accounting for the increase in coronary resistance with time.

4.4.4 Vasodilators

Adenosine is a potent vasodilator. Amongst the earliest reports of its effects on the coronary circulation are those of Dury and Szent-Gyorgyi (1929) and Wedd (1931). Adenosine acts preferentially on small vessels (Schnaar and Sparks, 1972) but higher concentrations will dilate large coronary arteries (Winbury et al., 1969; Schnaar and Sparks, 1972). Dilation results from interaction of adenosine with a purinergic receptor to produce an increase in intracellular cAMP or Ca^{2+} concentration (see Chapter 2). Dipyridamole potentiates vasodilation produced by adenosine and is thought to act by inhibiting the uptake of adenosine by erythrocytes, smooth muscle cells and endothelium (Stafford, 1966; Alfonso and O'Brien, 1967). It also inhibits phosphodiesterase and may produce vasodilation by facilitating accumulation of cAMP (Hamilton, 1972). The vasodilatory effect occurs mainly at the level of the small resistance vessels (Fam and McGregor, 1968; Tomoike et al., 1981). Nitroglycerin, on the

other hand, acts primarily on the intramural conductance vessels (Fam and McGregor, 1968; Schnaar and Sparks, 1972; Bache et al., 1975; Feldman et al., 1981) although it produces a transient reduction in resistance vessel tone (Cohen and Kirk, 1973). The action is thought to be mediated directly, by oxidation of sulphydril groups at vascular receptor sites (Johnson, 1975), or indirectly, through release of prostaglandin E (Morcillio et al., 1980). When used to relieve angina this direct action of nitroglycerin on the coronary vasculature becomes of secondary importance in the presence of widespread venodilation (Abrams, 1980; Cohn and Braunwald, 1980).

In the experiments carried out on the isolated guinea-pig heart adenosine (1.87×10^{-5} mol.litre^{-1}) abolished autoregulation. The same concentration caused only a small reduction in the resistance of the coronary bed of the rabbit. A similar concentration of adenosine has been shown to produce maximal relaxation of canine coronary artery strips contracted with 35 mmol.litre^{-1} K^+ (Schnaar and Sparks, 1972) and to block the generation of the action potential of smooth muscle cells of canine coronary artery (Harder et al., 1979). Thus this dose of adenosine produces maximal relaxation of the vascular smooth muscle and the coronary vasculature of the isolated rabbit heart can be assumed to be maximally dilated.

Dipyridamole (7.93×10^{-6} mol.litre^{-1}) and nitroglycerin (4.41×10^{-6} mol.litre^{-1}) had no significant effect on the resistance or P_{ZF} of the coronary bed of the isolated rabbit heart. A similar dose of dipyridamole has been found to produce a marked increase in flow and potentiate the response to adenosine (1.87×10^{-5} mol.litre^{-1}) by almost 40% in the isolated guinea-pig heart (Nayler et al., 1967), whilst 1×10^{-6} mol.litre^{-1}

nitroglycerin produces a 60% relaxation of canine conduit artery strips (Schnaar and Sparks, 1972).

These results suggest that the arterial resistance vessels of the isolated rabbit heart have a small vasodilatory reserve whilst the large coronary vessels are already maximally dilated. The absence of dilation in the presence of dipyridamole suggests that there is not a continuous basal production of adenosine in the isolated rabbit heart.

4.4.5 Conclusions

The isolated perfused guinea-pig heart shows autoregulation which can be abolished by adenosine. In the isolated rabbit heart the relationship between perfusion pressure and diastolic coronary flow is almost linear although a small vasodilatory reserve exists which can be exposed by adenosine. Application of linear regression to the pressure-flow relationship of the rabbit heart may result in underestimation of the minimum pressure required for flow. In the presence of adenosine there is a small positive intercept with the pressure axis. Coronary resistance increases with length of perfusion and this must be taken into account when the effects of interventions on coronary resistance are being assessed.

CHAPTER 5

PERFUSION PARAMETERS AND MYOCARDIAL CONTRACTION

5.1 Introduction

As stated in previous chapters myocardial contraction influences coronary perfusion. Contraction of the myocardium increases extravascular pressure and reduces perfusion during systole whilst the tone of the vascular smooth muscle is highly influenced by the metabolic activity of the heart. There is, however, also evidence that myocardial contraction is influenced by coronary flow and by perfusion pressure.

5.1.1 Influence of Coronary Flow

Although coronary blood flow is regulated to supply sufficient oxygen to meet metabolic demands (Chapter 2) there is considerable evidence which suggests that the blood flow provided is somewhat less than that required to optimise cardiac metabolism and hence performance. Gregg (1958) reported that myocardial oxygen consumption could be increased by independently increasing coronary blood flow above the control level. Other workers have also found myocardial oxygen consumption and/or ventricular performance to be limited by flow (e.g. Bacaner et al., 1965; Opie, 1965; Weisfelt and Shock, 1970) but Ross and co-workers (1963) and Sarnoff and co-workers (1963)

failed to find such a relationship. Downey (1976) suggested that these differences were due to the use of nonautoregulating deteriorating hearts and showed that when autoregulation was present reducing flow below the autoperfused level resulted in a decrease in contractile force but that increasing flow above the autoperfused level had no effect on myocardial performance. The decrease in myocardial force produced by decreased flow is thus a reflection of insufficient oxygen/substrate supply and myocardial force is hence influenced by oxygen/substrate delivery not flow per se.

5.1.2 Influence of Perfusion Pressure

There is evidence that perfusion pressure has a direct effect on both resting tension and the force developed by the myocardium. The reduction in developed tension produced by lowering perfusion pressure can be readily reversed by injection of a viscous dextran solution at the same flow rate (Apstein et al., 1979) thus suggesting that the initial reduction in force is not caused by insufficient oxygen/substrate supply. Salisbury and co-workers (1960) reported that increasing perfusion pressure resulted in an increase in left ventricular diastolic pressure measured by an air filled balloon within the left ventricle of an anaesthetised dog. They proposed that this was due to an increase in the volume of blood retained in the vessels at higher perfusion pressures. Arnold and co-workers confirmed this finding in the isolated guinea-pig heart (Arnold et al., 1968) and named the phenomenon "the garden hose effect". They also suggested that the increase in fibre tension caused by extension of the coronary vessels would increase developed tension by the

Frank-Starling effect (Morgenstern et al., 1973). In this chapter the effects of perfusion pressure and coronary flow on the resting tension and the force developed by the isolated rabbit heart are investigated. The effect of prolonged perfusion on tension is also determined.

5.2 Experimental

5.2.1 Determination of the Relationship Between Pressure, Flow and Tension

Rabbit hearts were perfused by the Langendorff method as outlined in Chapter 3. After a 20 min stabilisation period pressure or flow was altered in a stepwise fashion and the resulting changes in tension recorded when a steady state had been reached. The time for a steady state to be present was, in general less than 2 min, although at low perfusion pressures and flows no stabilisation of the developed tension occurred.

5.2.2 Determination of the Effect of Prolonged Perfusion on Tension Development

After the initial stabilisation and determination of the effect of perfusion parameters on tension outlined in 5.2.1 systolic pressure was raised to 50 mmHg and the relationship redetermined 45, and 60 min after cannulation.

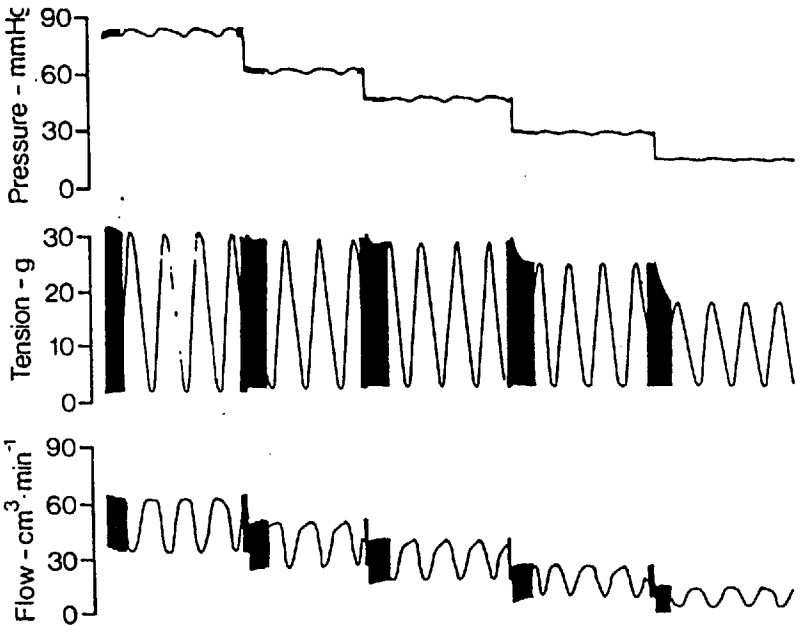


Figure 5.1 Example of the changes in perfusion pressure, tension and flow resulting from alteration of hydrostatic pressure.

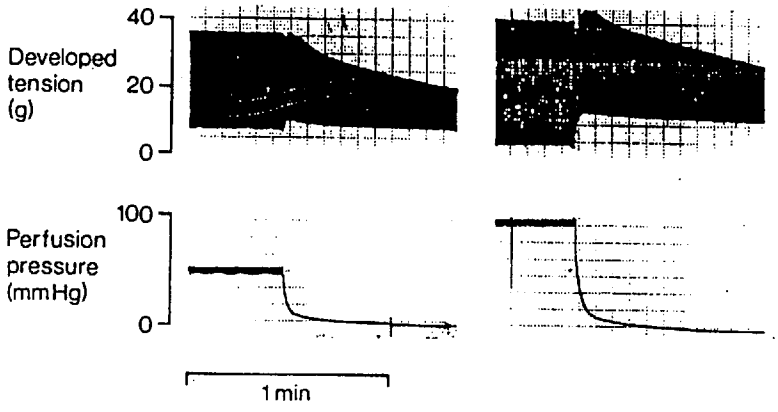


Figure 3.2 Effect of perfusion pressure on tension. In this example the length of aorta between cannula and aortic valves was increased to exaggerate the changes in resting tension produced by altering perfusion pressure. Note that the rise in resting tension produced by reducing the perfusion pressure from 50 mmHg to 0 mmHg (lefthand panel) is less than that produced when perfusion pressure is reduced from 100 mmHg (righthand panel).

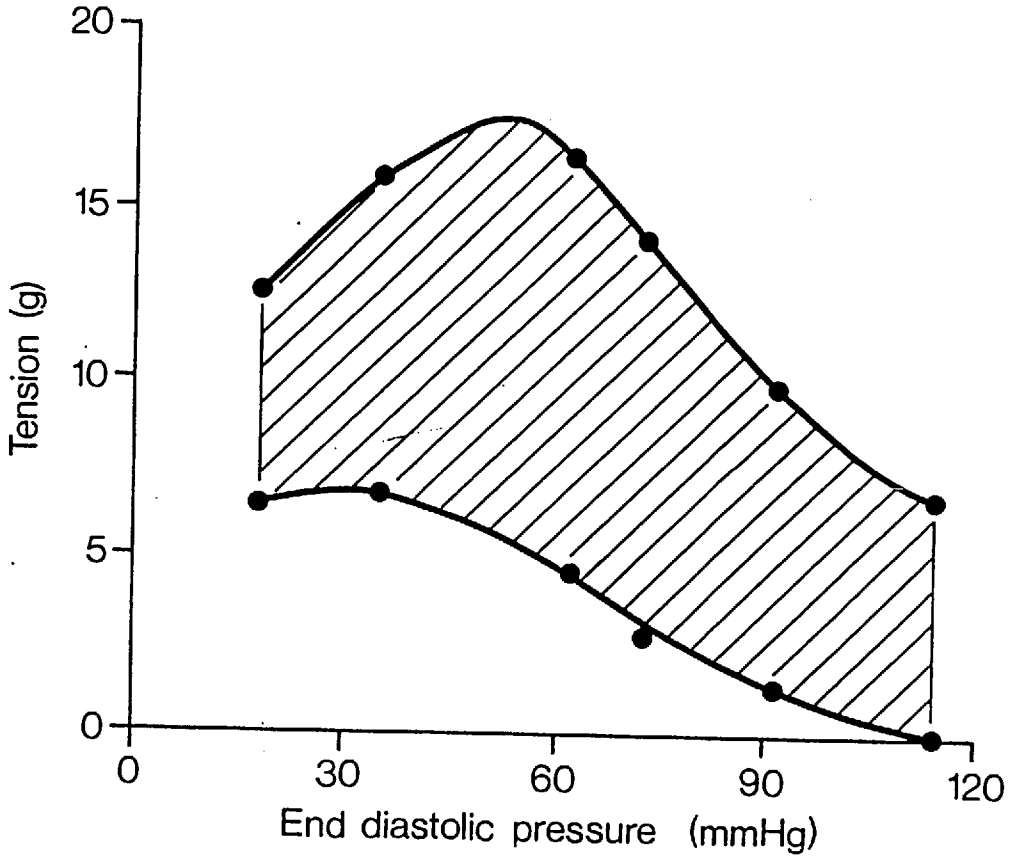


Figure 5.3 Example of the relationship between perfusion pressure and tension. The lower line shows resting tension. Developed tension is shown by the difference between lower and upper line.

5.3 Results

5.3.1 Effect of Perfusion Parameters on Tension

A negative relationship existed between perfusion pressure or flow and resting tension. This relationship was independent of perfusion method and non linear (Fig. 5.1 and 5.3). The magnitude of the change in resting tension for a given change in perfusion parameters differed widely. In one group of experiments the length of aorta between the aortic valves and the cannula was increased. This resulted in an exaggerated change in resting tension for a given change in perfusion parameters (Fig. 5.2).

Maximum developed tension occurred at a perfusion pressure of 50.5 ± 3.1 mmHg (range 23.5 - 88.5 mmHg) which corresponds to a flow of $44.1 \pm 2.8 \times 10^{-3}$ litre.min^{-1} (range 26.1-69.2 $\times 10^{-3}$ litre.min^{-1}) n = 30. At higher or lower perfusion parameters developed tension was reduced (Fig. 5.3). At perfusion pressures below 20 mmHg a fall in developed tension was observed and no steady state was reached; this effect was probably due to ischaemia.

5.3.2 Effect of Prolonged Perfusion on Tension

Resting tension was unchanged throughout the 60 min of perfusion as was the relationship between perfusion parameters and resting tension. Developed tension declined steadily throughout the perfusion period (Table 5.1). The basic relationship between the perfusion parameters and developed tension was unchanged by the length of the perfusion period.

TABLE 5.1 EFFECT OF PERFUSION ON TENSION

	resting tension g	developed tension %	n
Time after cannulation			
20 min	3.0 ± 0.5	100	5
45 min	2.7 ± 0.6	84.8 ± 5.8	5
60 min	2.8 ± 0.5	74.2 ± 4.0	5

5.4 Discussion

No differentiation can be made between the effects of changes in perfusion pressure and/or coronary flow on tension because of the positive linear relationship between them (Chapter 4).

5.4.1 Effects on Resting Tension

The length of aorta between the cannula and the aortic valves played an important part in the magnitude of the changes in resting tension in response to changes in perfusion parameters. As the perfusion parameters are increased in this preparation the aorta expands and lengthens; the resting tension measured from the apex of the heart is decreased. The longer the aorta the larger the effect. Increasing perfusion parameters also increases ventricular wall thickness and changes heart shape by the "garden hose effect" (Morgenstern et al., 1973). This results in an increase in left ventricular diastolic pressure (Salisbury et al., 1960) and an increase in heart volume (Morgenstern et al., 1973) hence decreasing resting tension measured from the apex of the heart. Resting tension measured by an intraventricular balloon is increased resulting in the positive relationship between perfusion pressure and resting tension seen in the open-chested dog by Salisbury and co-workers (1960).

5.4.2 Effects on Developed Tension

The gross changes in resting tension produced by altering

perfusion parameters will result in alteration of developed tension by Frank-Starling effect (Frank, 1895; Starling, 1918). It has also been suggested that extension of the coronary vessels increases fibre tension and again results in increased force of contraction by the Frank-Starling effect (Arnold et al., 1968). The former effect will increase developed tension as perfusion parameters are reduced in the preparation used in these experiments whilst the latter will have the opposite effect. The observed relationship is the result of interaction of these two opposing effects. At low perfusion pressures and flows oxygen/substrate supply becomes insufficient and a decrease in developed tension caused by ischaemia is superimposed on the relationship.

A time dependent decrease in ventricular function has been observed in other isolated buffer perfused preparations e.g. rat hearts (Aronson and Serlick, 1976) and guinea-pig hearts (Bunger et al., 1975). It is accompanied by an increase in tissue water content (see Chapter 4). The oedema is extracellular (Vargas and Blackshear, 1981) and hence decreases the efficiency of myocardial contraction. The decrease in tension with time may also be due to slight hypoxia caused by the low oxygen capacity of the perfusate made more severe by the increase in resistance to flow which occurs after prolonged perfusion (Chapter 4).

5.4.3 Conclusions

Both resting and developed tension of the isolated rabbit heart are affected by perfusion parameters. The way in which the tension record is altered in response to a change in perfusion parameters

depends on the method used to measure tension and on the length of the aorta between cannula and aortic valves. If interventions are being assessed in this preparation for their effects on resting tension or force of contraction alterations in perfusion parameters and their effects on tension must be taken into account.Many previous studies of myocardial ischaemia have ignored these factors. In studies where the interventions applied have altered perfusion parameters the changes observed in mechanical performance do not necessarily reflect changes in contractility of the myocardial cells and more caution is necessary in their interpretation.

CHAPTER 6

EFFECT OF HYPOXIA AND ISCHAEMIA ON MYOCARDIAL CONTRACTION AND CORONARY RESISTANCE

6.1 Introduction

The flow of blood through the coronary circulation provides the myocardium with oxygen and other substrates. The circulation also removes the harmful metabolic products. Cellular oxygen delivery may become insufficient if blood flow is impaired (ischaemia) or if the oxygen content of the blood is inadequate (hypoxia). Both myocardial ischaemia and hypoxia can occur clinically; both result in hypoxia of the myocardial cell. Ischaemia also results in a deficiency of substrates for energy production and an accumulation of metabolic products and other substances from injured myocardial cells. In hypoxia substrate supply is maintained and metabolic products removed.

Experimentally myocardial ischaemia can be studied by inducing regional ischaemia (by occluding a single epicardial coronary artery) or global ischaemia (by reducing the blood flow to the whole heart). Global hypoxia can be induced by perfusion with a solution of low oxygen content. Removal of substrate from this perfusate allows the effects of depletion of energy stores to be studied in the absence of a build up of metabolic products.

The mammalian heart is almost exclusively aerobic and ischaemia and hypoxia rapidly produce detrimental effects. Contraction in the

ischaemic area declines rapidly after coronary artery ligation and within 30 - 60 s there is a passive systolic expansion of the ischaemic area resulting from the pressure produced from the normally perfused myocardium (Tennant and Wiggers, 1935). If a sufficient quantity of myocardium is involved the ability of the heart to act as a pump is impaired and cardiac output declines. Induction of global myocardial ischaemia or hypoxia also results in rapid contractile failure and after a prolonged period contracture develops (Hearse et al., 1977). Coronary vasodilation takes place on initiation of ischaemia (Broadley, 1976) but after prolonged ischaemia an increase in coronary resistance may occur (Gorman and Sparks, 1982).

Recovery of mechanical function and coronary flow on reperfusion/reoxygenation after short periods of ischaemia/hypoxia is almost complete. After longer periods, however, mechanical recovery is impaired and resistance to flow is elevated - the "no reflow" phenomenon (Willerson et al., 1975; Ganote et al., 1977).

This chapter describes experiments which were undertaken to determine the effect of ischaemia and hypoxia on the isolated rabbit heart. Intracellular hypoxia was produced by three methods:

a) oxygen and glucose were removed from the perfusate - global no substrate hypoxia;

b) perfusion pressure was reduced to 20 mmHg - low flow global ischaemia;

c) perfusion was stopped - no flow ischaemia.

The effects of these interventions on coronary resistance and mechanical function was determined.

6.2 Experimental

6.2.1 Determination of the Effect of Hypoxia and Reoxygenation

Isolated rabbit hearts were perfused by the pressure controlled Langendorff method described in Chapter 3. After 20 min perfusion the hydrostatic pressure was altered in a stepwise fashion and the resultant changes in perfusion pressure and flow recorded. After 10 min perfusion at a systolic pressure of 50 mmHg the perfusate was replaced with one in which mannitol (11.1 mmol.litre^{-1}) had been substituted for glucose and which had been equilibrated with 95% N_2,5% CO_2.
Perfusion was continued with this "hypoxic" solution for 25 min and perfusion pressure, flow and tension were continuously recorded. The heart was then perfused with the control solution for 30 min before the relationship between perfusion pressure and flow was redetermined.

6.2.2 Determination of the Effect of Low Flow Ischaemia

Rabbit hearts were perfused by the pressure controlled Langendorff method described in Chapter 3. The relationship between perfusion pressure and flow was determined after an initial 20 min stabilisation. Systolic perfusion pressure was then reduced to 20 mmHg and the changes in flow and tension recorded over the subsequent 90 min. The relationship between perfusion pressure and flow was then redetermined.

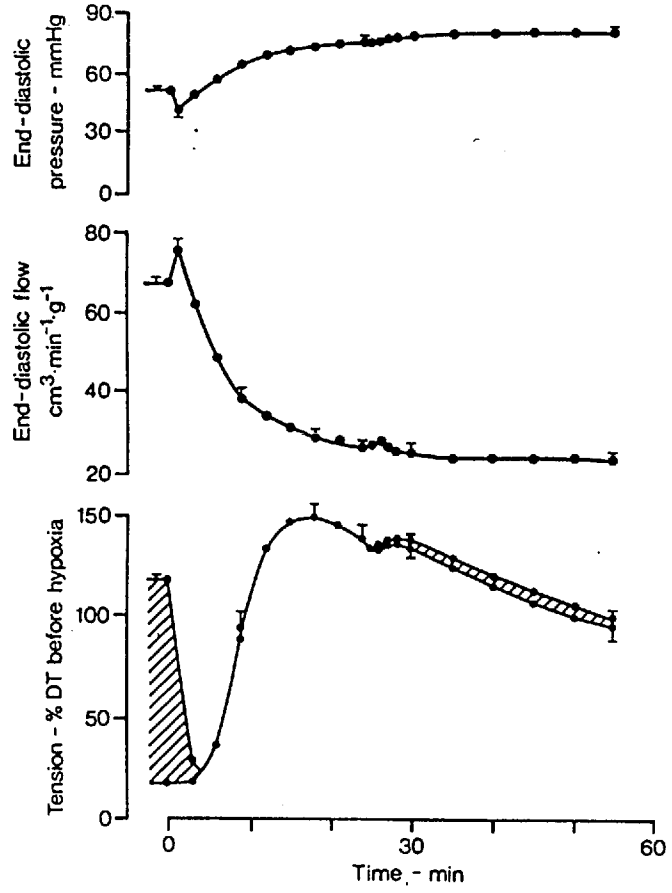


Figure 6.1 Summed results (n = 5) of the effect of 25 min hypoxia (attained by perfusion with a solution gassed with 95% N_2, 5% CO_2 in which glucose had been replaced by mannitol) followed by 30 min reperfusion on end-diastolic perfusion pressure, end-diastolic flow and tension of the isolated rabbit heart. Hypoxic perfusion was initiated at 0 min. For clarity standard errors are only shown at selected points.

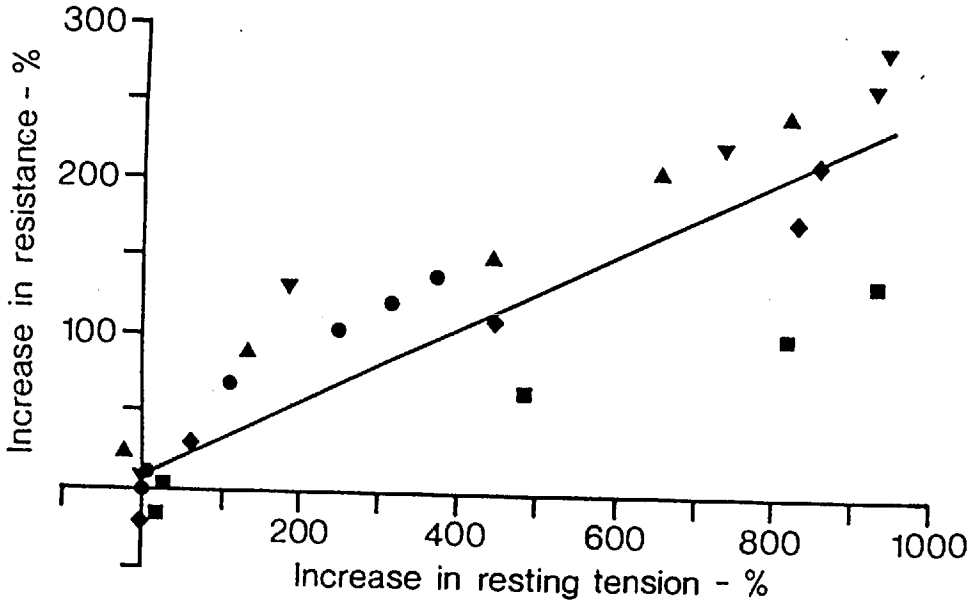


Figure 6.2 Relationship between the increase in resting tension and the increase in end-diastolic coronary resistance during the first 15 min of hypoxia. Values were taken every 3 min. Each symbol represents a different heart. The continuous line was calculated from these values by linear regression. Note that for each heart the relationship was linear.

6.2.3 Determination of the Effect of No-Flow Ischaemia

Rabbit hearts were perfused by the Langendorff method. Either hydrostatic pressure or flow was controlled (see Chapter 3). After 20 min stabilisation and determination of the pressure-flow relationship systolic pressure was set to 70 mmHg and after a stabilisation period of 5 min the flow of fluid to the muscle was stopped. Global ischaemia was maintained for 15, 27 or 60 min. This was followed by a 30 min reperfusion period at either the same pump speed or the same reservoir height as before ischaemia. The relationship between pressure and flow was then redetermined.

6.3 Results

6.3.1 Effect of Hypoxia

On initiation of hypoxia with no substrate there was an immediate decrease in perfusion pressure and an increase in end-diastolic flow (Fig. 6.1). Perfusion pressure then increased gradually over the remainder of the hypoxic period reaching 75.8 $\pm$ 2.0 mmHg (n = 5) (51$\pm$7% higher than control) after 25 min (Fig. 6.1). Flow declined rapidly after the initial increase and after 25 min was reduced to 27.2 $\pm$ 1.7 $cm^3.min^{-1}.g^{-1}$ (40.7 $\pm$ 3.2% of control values). Developed tension declined to zero within 5 min of initiation of hypoxia (Fig. 6.1). Resting tension began to rise

TABLE 6.1 EFFECT OF MYOCARDIAL CELLULAR HYPOXIA ON DIASTOLIC RESISTANCE TO FLOW IN THE ISOLATED RABBIT HEART

	control		After Cellular Hypoxia		n
	resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	
25 min hypoxia + 30 min reperfusion					
	720 ± 90	1.0 ± 2.5	3970 ± 400†	-7.8 ± 6.1	5
90 min 'low flow' ischaemia					
	1390 ± 210	-1.9 ± 1.1	2180 ± 470	2.1 ± 2.5	4
15 min total global ischaemia + 30 min reperfusion					
Q	1340 ± 130	-14.9 ± 2.9	2240 ± 60†	-11.5 ± 6.9	5
P	1490 ± 180	-9.6 ± 5.1	2290 ± 180†	-3.1 ± 3.2	5
27 min total global ischaemia + 30 min reperfusion					
Q	1220 ± 70	-4.4 ± 2.1	3300 ± 450†	15.7 ± 6.7	5
P	1850 ± 260	-16.6 ± 10.3	3990 ± 450†	0.7 ± 8.7	5
60 min total global ischaemia + 30 min reperfusion					
Q	1490 ± 140	-11.7 ± 5.5	3910 ± 380†	35.6 ± 8.8†	5
P	940 ± 70	-7.6 ± 1.9	4710 ± 730†	14.6 ± 6.4	5

Q = hearts reperfused at constant pump speed
P = hearts reperfused at constant hydrostatic pressure
† = significantly different ($p \leq 0.01$) from value before intracellular hypoxia

within 4 min of initiation of hypoxia (Fig. 6.1) and reached a peak, 46.5 ± 8.3% higher than the initial developed tension, after 15 min. Reoxygenation resulted in a small decline in contracture (Fig. 6.1) and a 5.0 ± 2.0% recovery of developed tension. There was a further decrease in diastolic flow (10.5 ± 3.7%) and a small increase in perfusion pressure (8.0 ± 1.1%) (Fig. 6.1). A period of 25 min hypoxia followed by 30 min reperfusion increased coronary diastolic resistance by 467 ± 68% ($p<0.01$, paired t-test)(Table 6.1). P_{ZF} was not significantly changed (Table 6.1).

Assuming that P_{ZF} did not change throughout the hypoxic period and was not significantly different from zero the ratio of diastolic perfusion pressure to diastolic flow reflects coronary resistance. This measure of instantaneous resistance correlated strongly ($r = 0.89$) with the increase in resting tension during the first 15 min of hypoxia (Fig. 6.2):

$$\text{diastolic resistance} = 0.23 \times \text{resting tension} + 17.5$$

This indicates that diastolic coronary resistance increased as resting tension increased.

6.3.2 Effect of Low Flow Ischaemia

When "low flow" perfusion was initiated ($n = 4$) end-diastolic pressure was 19.6 ± 1.8 mmHg, end-diastolic flow declined rapidly to 15.5 ± 2.4 $cm^3.min^{-1}.g^{-1}$ and developed tension declined to a new steady state (50% of that before the intervention) over a 5 min period (Fig. 6.3). Over the period of "low flow" perfusion

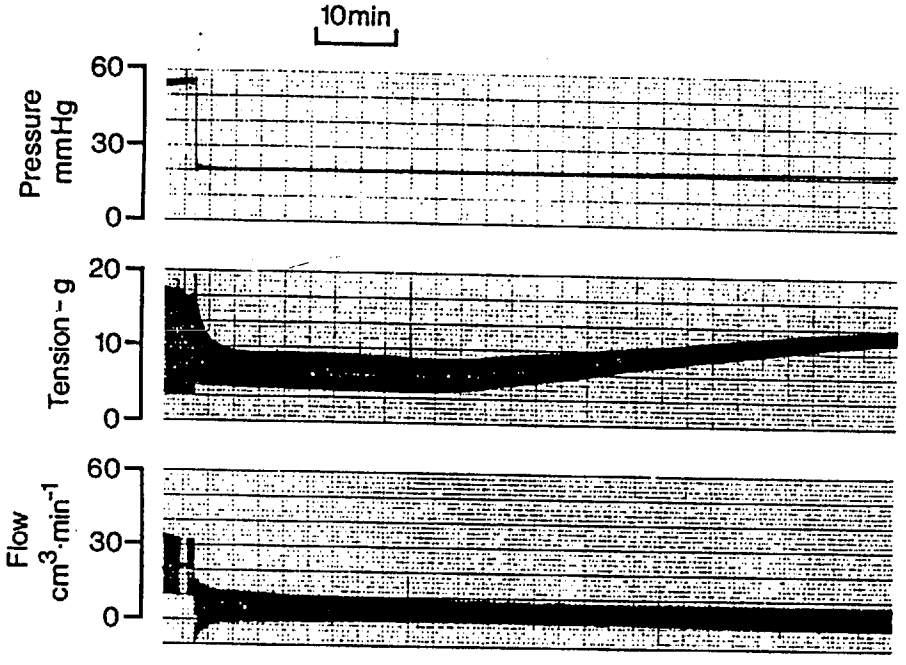


Figure 6.3 Example of the effect of reducing systolic perfusion pressure to 20 mmHg in the isolated rabbit heart. The upper trace shows perfusion pressure, the middle trace tension and the lower trace flow over 90 min of 'low flow' ischaemia.

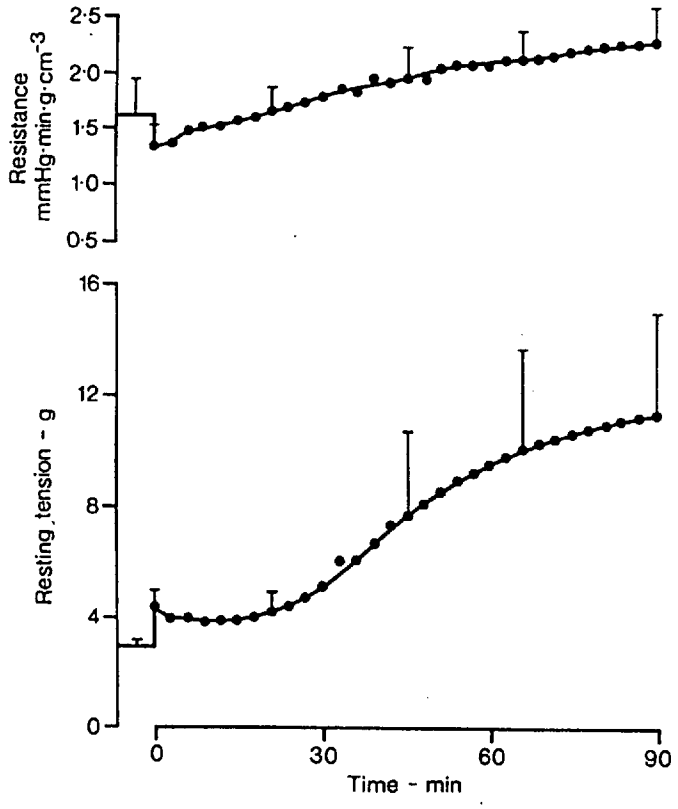


Figure 6.4 Summed results (n = 4) of the effect of 90 min 'low flow' ischaemia on end-diastolic coronary resistance (upper panel) and resting tension (lower panel) in the isolated rabbit heart. Low flow ischaemia was initiated at 0 min. For clarity standard errors are only given at selected points.

end-diastolic flow gradually decreased and end-diastolic pressure increased (Fig. 6.3) so that after 90 min end-diastolic perfusion pressure was 21.6 ± 2.3 mmHg and flow was 10.2 ± 2.3 $cm^3.min^{-1}.g^{-1}$. Over the 90 min resistance increased by 53.4 ± 8.8% ($p<0.01$, paired t-test)(Table 6.1) and P_{ZF} was not significantly altered (Table 6.1). Over the same period resting tension rose by 138.8 ± 61.6% (Fig. 6.3). In the absence of a change in P_{ZF} and as P_{ZF} was not significantly different from zero the ratio of diastolic perfusion pressure to diastolic coronary flow during the "low flow" ischaemia period was taken as an estimate of instant coronary resistance. If the time course of the rise in this value is compared with the rise in resting tension (Fig. 6.4) diastolic resistance appears to increase before resting tension, indicating that the increase in resting tension is the initial cause of the increase in coronary resistance.

6.3.3 Effect of No-Flow Ischaemia

On initiation of ischaemia (Fig. 6.5 and 6.6) the perfusion pressure dropped rapidly to zero (within 1 min) and there was an initial rise in resting tension and an increase in developed tension. Over the next 15 to 20 min developed tension fell to zero, whilst resting tension declined (Fig. 6.5 and 6.6). After total ischaemia for 20 min resting tension began to increase reaching a maximum (about 80% of tension developed before ischaemia) after 50 min total ischaemia (Fig. 6.6).

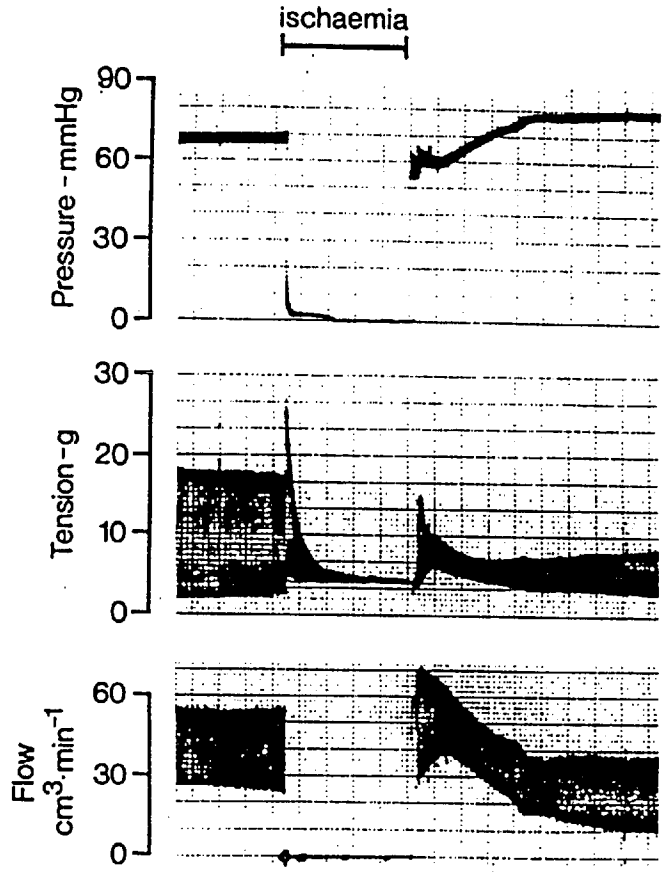


Figure 6.5a · Example of the effect of 15 min total global ischaemia followed by 30 min reperfusion on perfusion pressure (upper panel), tension (middle panel) and flow (lower panel) in the isolated rabbit heart.

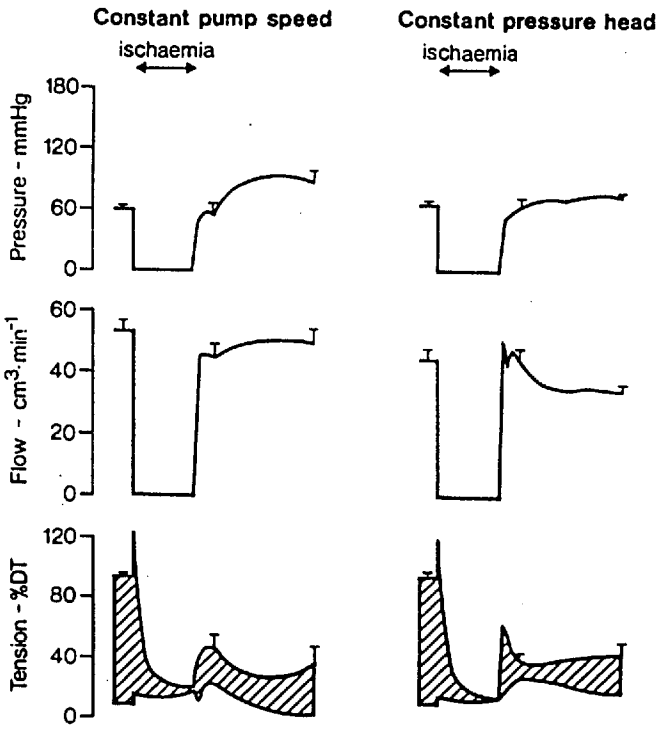


Figure 6.5b Summed results (n = 5) of the effect on end-diastolic perfusion pressure, tension and end-diastolic flow of 15 min total ischaemia followed by reperfusion at the same pump speed (left hand panel) or the same hydrostatic pressure (right hand panel).
For clarity standard errors are only given at selected points.

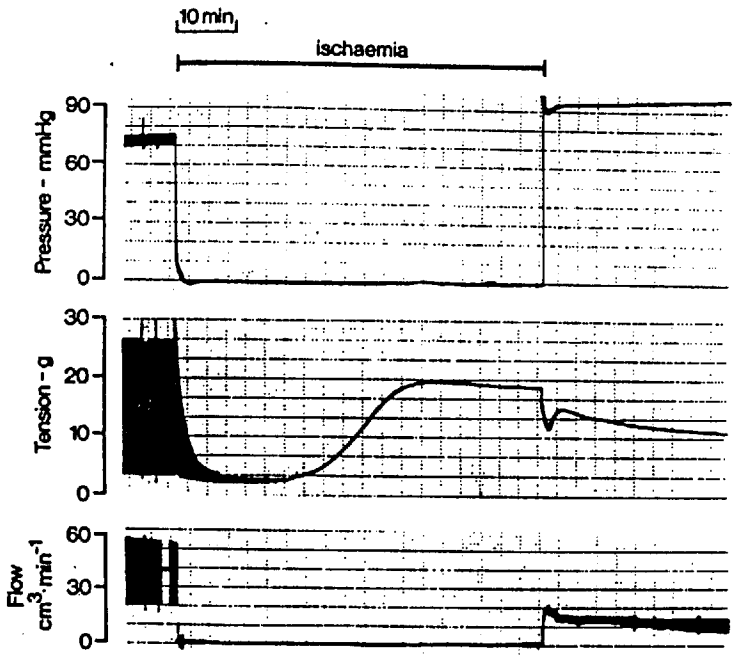


Figure 6.6a Example of the effect of 60 min total global ischaemia followed by 30 min reperfusion on perfusion pressure (upper panel), tension (middle panel) and flow (lower panel) in the isolated rabbit heart.

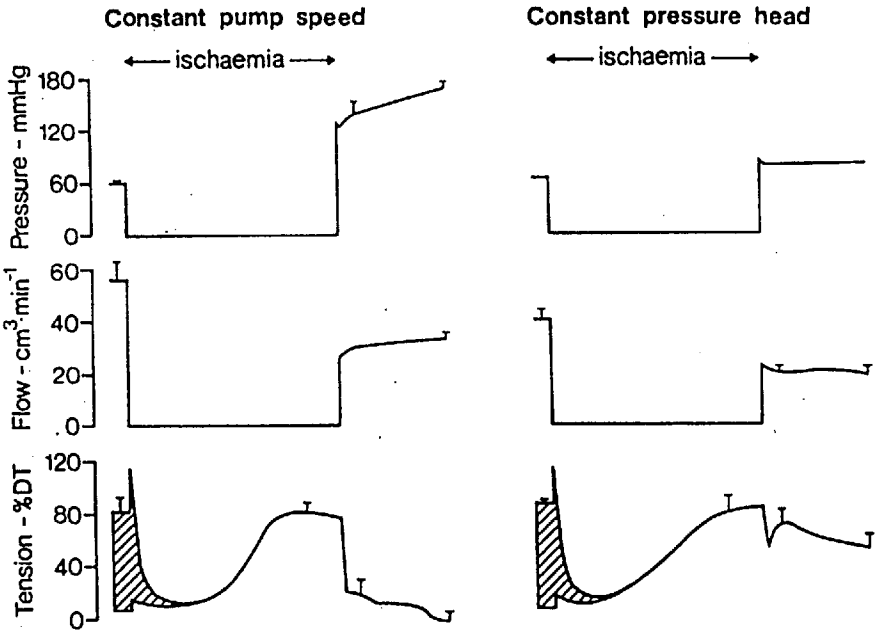


Figure 6.6b Summed results (n = 5) of the effect on end-diastolic perfusion pressure, end-diastolic flow and tension of 60 min total global ischaemia followed by reperfusion at the same pump speed (left hand panel) or the same hydrostatic pressure (right hand panel). Note that contracture on reperfusion at the same pump speed appears to be depressed relative to that recorded at the same hydrostatic pressure.

TABLE 6.2 EFFECT OF TOTAL GLOBAL ISCHAEMIA AND METHOD OF REPERFUSION ON RESTING TENSION IN THE ISOLATED RABBIT HEART

Length of ischaemic period (mins)		Resting Tension (g) control	end of ischaemia	reperfusion 5 min	30 min	n
15 min	Q	1.9 ± 0.1	3.1 ± 0.5	4.3 ± 0.9	0.8 ± 0.6*	5
	P	2.3 ± 0.4	3.1 ± 0.4	6.3 ± 0.4†	4.3 ± 0.7*	5
27 min	Q	1.4 ± 0.4	3.1 ± 0.5	7.1 ± 2.2	0.5 ± 1.0*	5
	P	2.5 ± 0.3	4.1 ± 0.6	12.5 ± 2.3†	7.5 ± 1.9*	5
60 min	Q	1.3 ± 0.3	14.9 ± 0.6†	3.6 ± 2.7*	0.2 ± 1.4*	5
	P	1.4 ± 0.2	16.6 ± 1.9†	14.0 ± 2.4†*	10.3 ± 2.1†*	5

Q = hearts reperfused at constant pump speed
P = hearts reperfused at constant hydrostatic pressure
† = significantly different ($p \leq 0.05$) from value before ischaemia
* = significantly different ($p \leq 0.05$) from other group given a similar period of ischaemia

6.3.3.1 Effect of Reperfusion on Resting Tension

When reflow was started there was an initial sudden decrease in resting tension which was followed by a steady increase until a maximum was reached 5 to 10 min after the start of reflow (Fig. 6.5 and 6.6, Table 6.2). Over the remaining period of reflow a slow decline in resting tension occurred. The extent of the increase in resting tension was dependent both on the method of perfusion, and on the length of the ischaemic period: a greater increase being seen after longer periods of ischaemia (Table 6.2). The rise in resting tension was less on reperfusion at the same pump speed than if the same hydrostatic pressure was used (Table 6.2). The depression of the rise in resting tension on reperfusion at constant pump speed could be accounted for by the increase in perfusion pressure which occurred when this method of reperfusion was used.

6.3.3.2 Effect of Reperfusion on Developed Tension

Synchronous contraction of the heart occurred only on reperfusion after the shortest period of ischaemia. No contraction was seen on reperfusion after 27 or 60 min ischaemia. Ventricular fibrillation occurred in 13 of the 23 hearts reperfused after 15 min ischaemia. In the group reperfused at constant hydrostatic pressure after 15 min ischaemia 5 did not fibrillate, 4 remained in ventricular fibrillation throughout the reflow period and 3 spontaneously reverted to paced rhythm after 15 min reperfusion. In the group reperfused at constant pump speed after 15 min ischaemia, 5 did not fibrillate and 1 of the

TABLE 6.3 EFFECT OF TOTAL GLOBAL ISCHAEMIA AND METHOD OF REPERFUSION ON DIASTOLIC PERFUSION PRESSURE IN THE ISOLATED RABBIT HEART

Length of ischaemic period (mins)		End-Diastolic Perfusion Pressure (mmHg) control	reperfusion 5 min	reperfusion 30 min	n
15 min	Q	59.5 ± 2.2	55.4 ± 10.8	88.5 ± 11.7	5
	P	66.7 ± 2.3	66.0 ± 6.1	75.9 ± 3.0	5
27 min	Q	56.7 ± 2.4	84.5 ± 5.6†	120.2 ± 4.4†*	5
	P	56.2 ± 2.6	73.5 ± 1.4†	76.5 ± 2.6†*	5
60 min	Q	59.9 ± 2.6	138.7 ± 13.3†*	166.8 ± 7.7†*	5
	P	63.6 ± 1.7	78.3 ± 2.7†*	79.2 ± 2.2†*	5

Q = hearts reperfused at constant pump speed
P = hearts reperfused at constant hydrostatic pressure
† = significantly different ($p \leq 0.05$) from value before ischaemia
* = significantly different ($p \leq 0.05$) from other group given a similar period of ischaemia

6 hearts which developed fibrillation regained synchronous ventricular contraction before the end of the reperfusion period.

Results from the 10 hearts which did not fibrillate on reperfusion after 15 min ischaemia are shown in Fig. 6.5. In the 5 hearts which did not fibrillate when reperfused at constant hydrostatic pressure developed tension rose to 45.2 ± 11.9% of that developed on initiation of ischaemia within 1 min of reperfusion. It then fell to a minimum of 10.7 ± 2.9% (after 10 min reperfusion) before steadily rising to reach 24.9 ± 8.8% by the end of the reperfusion period. By contrast in the 5 hearts which did not fibrillate when reperfused at constant pump speed after 15 min ischaemia developed tension rose to 29.0 ± 9.1% of that developed on initiation of ischaemia within one minute of reperfusion. After falling to 18.2 ± 6.5% after 15 min reflow developed tension rose to 33.5 ± 10.1% of that developed on initiation of ischaemia by the end of the reperfusion period.

6.3.3.3 Effect of Reperfusion on Perfusion Pressure

Perfusion pressure reached a stable level within 5 min of the start of reflow when the hydrostatic pressure was kept constant (Fig. 6.5 and 6.6). This pressure was significantly higher than the diastolic pressure before ischaemia ($P < 0.05$ paired t-test, for 15 min ischaemia, $P < 0.01$ for 27 or 60 min ischaemia) (Table 6.3) due to decreased flow causing less pressure loss between reservoir and cannula (see Chapter 3).

When pump speed was kept constant, pressure increased throughout the reflow period (Fig. 6.5 and 6.6). The rise in pressure

TABLE 6.4 EFFECT OF TOTAL GLOBAL ISCHAEMIA AND METHOD OF REPERFUSION ON DIASTOLIC CORONARY FLOW IN THE ISOLATED RABBIT HEART

Length of ischaemic period (mins)		End-Diastolic Flow ($cm^3.min.g^{-1}$) control	reperfusion 5 min	reperfusion 30 min	n
15 min	Q	53.1 ± 3.8	45.3 ± 4.4	49.7 ± 5.1	5
	P	44.6 ± 3.7	44.7 ± 4.2	35.8 ± 1.9	5
27 min	Q	60.0 ± 4.9*	42.3 ± 5.2*	46.9 ± 4.5*	5
	P	42.2 ± 2.1*	23.9 ± 1.7†*	20.9 ± 0.9†*	5
60 min	Q	55.7 ± 6.8	30.0 ± 1.4†*	33.2 ± 2.0†*	5
	P	39.9 ± 4.1	20.2 ± 2.2†*	19.0 ± 2.6†*	5

Q = hearts reperfused at constant pump speed
P = hearts reperfused at constant hydrostatic pressure
† = significantly different ($p \leq 0.05$) from value before ischaemia
* = significantly different ($p \leq 0.05$) from other group given a similar period of ischaemia

on reflow after 15 min ischaemia was less ($P < 0.01$) than that after 27 or 60 min ischaemia (Table 6.3). There was no significant difference between the rise in pressure on reflow after 27 and 60 min ischaemia (Table 6.3).

6.3.3.4 Effect of Reperfusion on Flow

Flow was significantly lower on reflow after 27 or 60 min ischaemia ($P < 0.01$) when hydrostatic pressure was kept constant (Fig. 6.6, Table 6.4). On reflow after 15 min ischaemia flow returned to pre-ischaemic values (Fig. 6.5, Table 6.4).

When pump speed was kept constant flow returned to values which were not significantly different from pre-ischaemic levels on reflow after 15 and 27 min ischaemia (within 0.5 and 10 min respectively) (Table 6.4). However, flow after 60 min ischaemia failed to reach pre-ischaemic levels (Fig. 6.6, Table 6.4).

6.3.3.5 Effect of Reperfusion on Resistance

All three periods of ischaemia produced an increase of coronary resistance in the Langendorff preparation. The magnitude of this increase was related to the length of the ischaemic period. Both methods of reperfusion produced an equal increase in resistance for a given period of ischaemia (Table 6.1). Reperfusion at constant hydrostatic pressure gave a large decrease in flow with a small increase in perfusion pressure whilst reperfusion at constant pump speed resulted in a large increase in pressure and a small decrease

in flow. The minimum pressure required for flow to occur(P_{ZF}) varied widely after ischaemia (range -50 to 66.7 mmHg) but was only significantly different from zero ($p < 0.01$) on reflow after 60 min ischaemia when pump speed was kept constant (Table 6.1).

6.4 Discussion

6.4.1 Criticism of Methods

6.4.1.1 Hypoxia

The hypoxic solution was bubbled with 95% N_2,5% CO_2 and should have contained no oxygen. However, as the solution passed through the perfusion circuit oxygen may have diffused through the tubing into the perfusate. This solution was therefore labelled "hypoxic" rather than "anoxic".Low values of oxygen content are difficult to measure accurately.Little oxygen was likely to be in the perfusate since developed tension fell rapidly.

Glycogen stores within the heart are sufficient to maintain glycolysis for 1 - 2 hours (Neely et al., 1969). Removal of the exogenous substrate accelerates use of these stores resulting in accumulation of lactate. In hypoxia the resultant acidosis is partly tempered by continued flow but mild acidosis still occurs (Cobbe and Poole-Wilson, 1980b). The effects of no substrate hypoxia are thus equivalent to no flow ischaemia with removal of some metabolic products.

6.4.1.2 Pressure and Flow Measurement

As resistance changes were occurring within the coronary circulation during hypoxia and ischaemia neither perfusion pressure nor flow could be kept precisely constant on reoxygenation/reperfusion although hydrostatic pressure or pump speed was maintained (see Chapter 3). These small changes were a consiquence of the design of the equipment.The changes were sufficiently small to have little influence on the results.

6.4.2 Effect of Ischaemia on Mechanical Function

The mechanical records obtained during and on reperfusion after myocardial cellular hypoxia are qualitatively similar to those obtained by others from the Langendorff preparation (e.g. Apstein et al., 1977; Hearse et al., 1977; Henry et al., 1977) and from the isolated arterially perfused interventricular septum (Harding and Poole-Wilson, 1980). Quantitative differences can be accounted for by differences in temperature, species and method of measuring tension.

6.4.2.1 Decline of Contraction during Ischaemia

On initiation of hypoxia, low flow or no flow ischaemia developed tension declined rapidly. The rate of decline of developed tension in the face of inadequate oxygen supply is dependent on the species, the temperature, the heart rate, the mechanical work being performed and the inotropic state of the heart (Bing et al., 1975). In any given

experimental system the rate of decline in developed tension is greater in ischaemia than hypoxia (Cobbe and Poole Wilson, 1980). Four possible causes of the decline in contractility have been proposed; a decrease in the intracellular ATP concentration, intracellular acidosis, a decline in myocardial wall tension, and a decrease in the amount of Ca^{2+} available for excitation-contraction coupling.

a) ATP

Exogenous ATP or creatine phosphate have been shown to maintain contractile function in anoxia and ischaemia (Parratt and Marshall, 1974). The intracellular ATP concentration declines to 75% of control within 5 s of anoxia in the isolated working rat heart (Hearse, 1979). However both analytical techniques (Kubler and Spieckeman, 1970) and ^{31}P nuclear magnetic resonance studies of hypoxia (Matthews et al., 1981) show that the ATP concentration is reduced by only 20 - 30% when tension is greatly reduced. Thus unless a compartment exists within the myocardial cell where the ATP concentration has fallen to much lower levels, as proposed by Gudbjarnason et al. (1970), or a reaction involved in contraction is extremely sensitive to ATP concentration ATP depletion is unlikely to be the sole cause of early contractile failure.

b) Acidosis

If intracellular pH is decreased a decline in developed tension occurs which is proportional to the degree of acidosis (Jacobus et al., 1982). In ischaemia a profound acidosis has been measured (Jennings, 1963; Cobbe and Poole-Wilson, 1980a) due to retention of H^+ and lactate whilst in hypoxia a less severe acidosis occurs

(Cobbe and Poole-Wilson, 1980b; Matthews et al., 1981) despite continued washout of metabolic products. Acidosis is thus a possible cause of the contractile failure. During ischaemia interstitial pH falls before the onset of contractile failure (Cobbe and Poole-Wilson, 1980a) but the decrease in intracellular pH is only sufficient to account for 40 - 50% of the immediate negative inotropic effect (Jacobus et al., 1982). In hypoxia tension falls before a decrease in intracellular pH is seen (Orchard et al., 1983). Thus although acidosis may be involved in early contractile failure it cannot be the sole determinant.

c) Myocardial Wall Tension

The initial decline in developed tension during ischaemia can be reversed by injection of a viscous dextran solution into the coronary arteries (Apstein et al., 1979). This suggests that the initial decrease in developed tension is not due to metabolic changes but is caused by a decrease in wall tension resulting from the decreased perfusion pressure - the "garden hose" effect (Arnold et al., 1968) (see Chapter 5). A similar mechanism could be involved in the initial decline in tension during hypoxia if vasodilation caused redistribution of intracoronary pressure.

d) Ca^{2+} Availability

Ca^{2+} is required to initiate contraction (Weber and Herz, 1963; Ebashi and Endo, 1968) a decline in contractile force could thus be due to a decrease in the intracellular availability of Ca^{2+} or to a decrease in the sensitivity of the contractile elements to Ca^{2+}. This may occur as a consequence of intracellular acidosis,

depletion of ATP or by another mechanism.

There is no change in the net influx of ^{45}Ca (Nayler et al., 1979; Harding and Poole-Wilson, 1980) or total tissue Ca^{2+} concentration (Vleugels et al., 1976) during the first few minutes of hypoxia. Neither is the capacity of isolated sarcoplasmic reticulum to take up Ca^{2+} impaired (Nayler et al., 1979). This suggests that gross changes in intracellular Ca^{2+} concentration are not occurring at the time when developed tension is declining. The methods used, however, are not sensitive enough to detect changes within an intracellular pool and depletion of "activator" Ca^{2+} may occur. Acidosis has been shown to decrease the Ca^{2+} sensitivity of the myofilaments (Fabiato and Fabiato, 1978) but as already stated the degree of acidosis during ischaemia is insufficient to account for the contractile failure and thus a decrease in Ca^{2+} sensitivity due to acidosis cannot totally account for the decrease in tension.

It has been suggested that energy is needed for Ca^{2+} accumulation at the sarcolemma and for transport across it (Sperelakis and Schneider, 1976). Philipson and Langer (1979) have proposed that the amount of Ca^{2+} bound to the sarcolemma influences contractile state. Local depletion of ATP at the sarcolemma could therefore result in a decrease in sarcolemmal bound Ca^{2+} and hence a decrease in contractile state.

6.4.2.2 Development of Contracture During Ischaemia

Comparison of the experiments where hydrostatic pressure was kept constant shows that development of contracture was dependent on the

length of the ischaemic period; more contracture occurring after longer periods of ischaemia. Contracture developed more quickly during no substrate hypoxia than in no flow ischaemia and was less severe in low flow ischaemia.

The rise in resting tension in hypoxia or ischaemia is probably due to excess Ca^{2+} at the myofilaments (Henry et al., 1977) or a decrease in the availability of ATP at the myofilaments,resulting in the formation of rigor bonds (Hearse et al., 1977 ;Holubarsch et al., 1982),or both (Jarmakani et al., 1978). The more rapid development of contracture in hypoxia than in no flow ischaemia suggests that the presence of metabolic products or the prevention of loss of molecules from the heart is important in delaying the development of contracture.

6.4.2.3 Mechanical Recovery on Reperfusion

a) Developed Tension

Contraction was only seen on reperfusion after the shortest period of no flow ischaemia. Mechanical performance on reperfusion after ischaemia correlates with tissue ATP content (Reibel and Rovetto, 1978; Apstein and Ogilby, 1980). Reperfusion restores the supplies of oxygen and substrates to the myocardium which are necessary for the synthesis of ATP. The stores of ATP might therefore be expected to increase on reperfusion. Although some repletion does occur full restoration of ATP stores does not (Kubler and Spieckeman, 1970; Reibel and Rovetto, 1978). The lack of recovery may be due to impaired mitochondrial function (Nayler et al., 1980) or to insufficient availability of purine bases (Reibel and Rovetto, 1978).

Fibrillation occurred in about one half of the hearts reperfused after 15 min no flow ischaemia. The exclusion of fibrillating hearts may have caused overestimation of the degree of recovery after ischaemia,because this constitutes selection of results. Fibrillation causes an increase in resting tension and alters the pressure-flow relationship (see Chapter 8),inclusion of these results would therefore have further biased the results.

b) Resting Tension

There was an initial increase in resting tension on reperfusion after 15 min ischaemia before resting tension returned to control values. This increase has been described by others (Weisfelt et al., 1974; Henry et al., 1977) and has been attributed to a slowing in the rate of relaxation of the myofibrils resulting in incomplete relaxation between beats (Weisfeldt et al., 1974). This slowing is reversed after about 5 min of reperfusion and true resting tension is again recorded.

In hearts that were reperfused at constant hydrostatic pressure after prolonged ischaemia or hypoxia (where contracture had already developed) no reduction in resting tension occurred on reperfusion. Irreversible rigor bonds probably formed during ischaemia in these hearts. In the hearts reperfused at constant pump speed contracture appeared to be reduced on reperfusion. In these experiments the increase in perfusion pressure on reperfusion was greater and the decrease in flow less than those perfused at constant hydrostatic pressure. The relationship between the perfusion parameters and tension (Chapter 5) results in lower measured tension at higher pressures and flows. The degree of contracture in the group where pump

speed was maintained therefore appeared to be reduced.

6.4.3 Coronary Resistance During and After Ischaemia

The method of reperfusion had no effect on the increase in diastolic coronary resistance after total global ischaemia. This increase was however related to the length of the ischaemic period, a greater increase occurring after a longer period of ischaemia (Fig. 6.7). Diastolic coronary resistance also increased after low flow ischaemia and no substrate hypoxia. The increase observed after 90 min low flow ischaemia was similar to that seen after 15 min total ischaemia whilst that after hypoxia was more compatible with that after 60 min ischaemia.

Increases in coronary resistance after prolonged ischaemia have previously been attributed to intracellular swelling or extracellular oedema (Willerson et al., 1975; Ganote et al., 1977), ischaemic damage to the vasculature (Kloner et al., 1980; Ku, 1982) or myocardial contracture (Apstein et al., 1977; Humphrey et al., 1981). In the present experiments myocardial contracture could account for the increase in resistance after prolonged ischaemia or hypoxia. However after 15 min ischaemia an increase in coronary resistance occurs in the absence of an increase in resting tension thus suggesting that other mechanisms must be involved.

The minimum pressure required for flow (P_{ZF}) was not significantly different from zero and was not altered by 15 min total global ischaemia, 25 min hypoxia or 90 min low flow ischaemia. P_{ZF} can thereforebe assumed to be unchanged throughout the experimental period. The ratio of diastolic perfusion pressure to diastolic

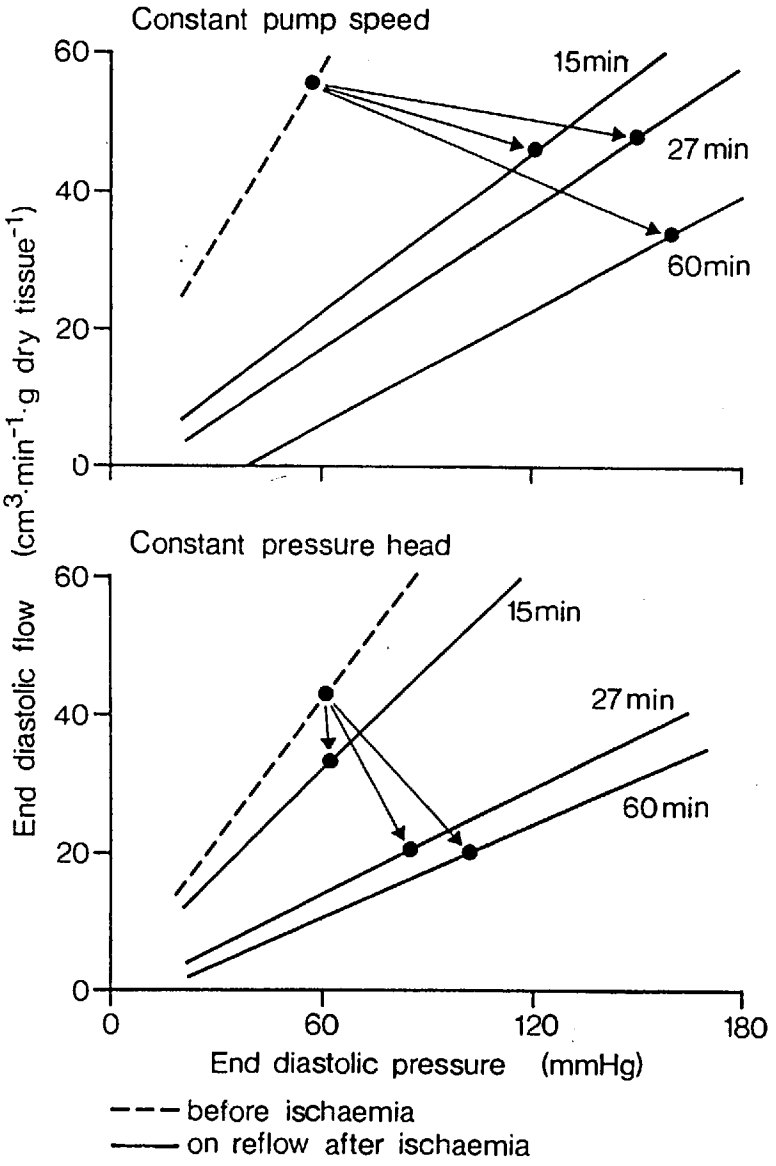


Figure 6.7 Effect of total global ischaemia on the relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated rabbit heart. Lines were constructed using the mean values for slope and P_{ZF} from 5 experiments. Arrows indicate the changes in pressure and flow occurring on reperfusion at the same pump speed (upper panel) or the same hydrostatic pressure (lower panel). Note that the resistance changes increase with the length of the ischaemic period but are independent of the method of reperfusion.

coronary flow at a single point can therefore be used to estimate changes in coronary resistance in the experimental period. These estimates show that on reperfusion after 15 min total global ischaemia resistance was initially reduced and only increased as reperfusion continued. This suggests that the increase in coronary resistance does not occur during the ischaemic period but is a consequence of reperfusion. Oedema has been shown to occur on reperfusion (Kloner et al., 1974; Whalen et al.,1974; Ganote et al.,1977) and the increase in coronary resistance may be a consequence of both endothelial and myocardial cell swelling.Instantaneous estimates of resistance during hypoxia show a rapid increase in diastolic resistance during the first 15 min which correlate well with the increase in resting tension suggesting that in hypoxia it is the development of contracture which restricts myocardial blood flow. Similar estimates made during low flow ischaemia suggest that diastolic coronary resistance starts to increase before resting tension starts to rise. In this situation direct effects within the coronary vasculature may be occurring.

The results of these experiments thus suggests that the increase in coronary resistance after prolonged ischaemia is due to myocardial contracture, although cell swelling and extracellular oedema may also be involved. Coronary constriction or obstruction contributes after shorter periods of ischaemia. Preventing myocardial contracture and/or administration of vasodilators (Chapter 7) should improve restoration of blood flow after ischaemia.

CHAPTER 7

EFFECT OF DRUGS ON POST-ISCHAEMIC CORONARY RESISTANCE

7.1 Introduction

Restoration of blood flow after ischaemia is an important requisite for salvage of the ischaemic area. It has been reported that reperfusion after coronary artery occlusion is detrimental and increases the size of the infarcted area (Bresnahan et al., 1972; Lang et al., 1974). However, a decrease in damage has also been reported (Maroko et al., 1972). Hofmann and his co-workers (1980) carried out a study in which two similar arteries in the same heart were occluded only one of which was subsequently reperfused. Reperfusion did not change infarct size after three or six hours of occlusion. These workers concluded that the benefits of early reperfusion far exceeded the possible side effects, and that as long as myocardial oxygen requirements were controlled, early reperfusion could only be beneficial.

On reperfusion after ischaemia coronary resistance may be elevated (Willerson et al., 1975; Ganote et al., 1977; Gaasch et al., 1978; Chapter 6). This could be due to increased extravascular forces (see Chapter 8) or vasoconstriction. If the increase in resistance is caused by coronary vasoconstriction the "no reflow" phenomenon should be susceptible to treatment with vasodilators. The experiments

described in this chapter were performed to find out if the increase in diastolic coronary resistance observed after ischaemia in the isolated rabbit heart was due to vascular constriction and to determine whether vasodilators which reduced vascular tone could improve mechanical recovery after ischaemia.

Adenosine has already been shown to produce vasodilation in the isolated rabbit heart under control conditions (Chapter 4). In this chapter its ability to reduce coronary resistance after ischaemia is assessed.

The calcium antagonists have been shown to be beneficial in the treatment of myocardial ischaemic syndromes (Ellrodt et al., 1980). One of the major actions of this group is vasodilation. The vasodilatory action of nifedipine predominates over myocardial effects. This drug was therefore used to assess whether the beneficial effects of the calcium antagonists could be due to actions on the vasculature.

If the increase in coronary resistance on reperfusion after ischaemia is due to vasoconstriction it is probable that the increase in vascular smooth muscle tone is caused by release of a chemical mediator. Both prostaglandin $F_{2\alpha}$ and thromboxane A_2 have been shown to contract vascular smooth muscle and reduce coronary flow (Moretti et al., 1976; Samuelsson et al., 1978). Indomethacin inhibits synthesis of both prostaglandins and thomboxanes and was therefore used to determine whether these agents are involved in the post-ischaemic increase in coronary resistance in the isolated rabbit heart.

7.2 Experimental

7.2.1 Determination of the Effects of Adenosine, Nifedipine and Indomethacin on the Changes in Coronary Resistance and Mechanical Function Produced by Global Ischaemia

Rabbit hearts were perfused by the pressure controlled Langendorff method outlined in Chapter 3. After the initial 20 min stabilisation the relationship between end- diastolic perfusion pressure and end-diastolic flow was determined by decreasing the hydrostatic pressure in steps and recording the resultant changes in perfusion pressure and coronary flow. Systolic perfusion pressure was then set to 70 mmHg. After a stabilisation period of 10 min the flow of fluid to the muscle was stopped. Global ischaemia was maintained for 15 or 60 min. This was followed by a 30 min reperfusion period at the same hydrostatic pressure as before ischaemia. The relationship between pressure and flow was then redetermined. Adenosine (1.87×10^{-5} $mol.litre^{-1}$), nifedipine ($1.44 \times 10^{-8} mol.litre^{-1}$ or $1.44 \times 10^{-7} mol.litre^{-1}$) or indomethacin ($1.40 \times 10^{-5}$ $mol.litre^{-1}$) was added to the perfusate 10 min before ischaemia or at reperfusion.

7.2.2 Materials and Methods

Adenosine was dissolved in distilled water to give a stock solution of $1.87 \times 10^{-2} mol.litre^{-1}$. Indomethacin and nifedipine were dissolved in ethanol to give stock solutions of $1.40 \times 10^{-2} mol.litre^{-1}$ and $1.44 \times 10^{-4} mol.litre^{-1}$ respectively.

Nifedipine is light sensitive. In experiments in which this drug

was used the perfusion apparatus was covered in aluminium foil to restrict the exposure to light.

7.3 Results

7.3.1 Effects of Drugs on Control Values

7.3.1.1 Adenosine

Addition of adenosine (1.87×10^{-5} mol.litre^{-1}) to the perfusate caused an increase in diastolic flow of 15.1 ± 6.4% (n = 4) and a reduction in diastolic perfusion pressure of 10.8 ± 8.6% Developed tension and resting tension were unaltered. These results were similar to those already reported in Chapter 4.

7.3.1.2 Nifedipine

Under control conditions the low dose of nifedipine (1.44×10^{-8} mol.litre^{-1}) caused a small decrease in developed tension (18.9±3.7%) and a decrease in resting tension of 0.3 ± 0.2 g (Fig. 7.2b and 7.5a). End-diastolic flow was increased by 15.2±3.8% and end-diastolic pressure was reduced by 17.9 ± 3.3% (Fig. 7.2b and 7.5a).

The high dose of nifedipine (1.44×10^{-7} mol.litre^{-1}) caused a larger decrease in developed tension (86.1 ± 1.6%) and reduced resting tension by 1.1 ± 0.3 g (Fig. 7.5b). After an initial increase end-diastolic flow fell to reach a value 7.7 ± 2.8% lower than control after 10 min perfusion. End-diastolic pressure was

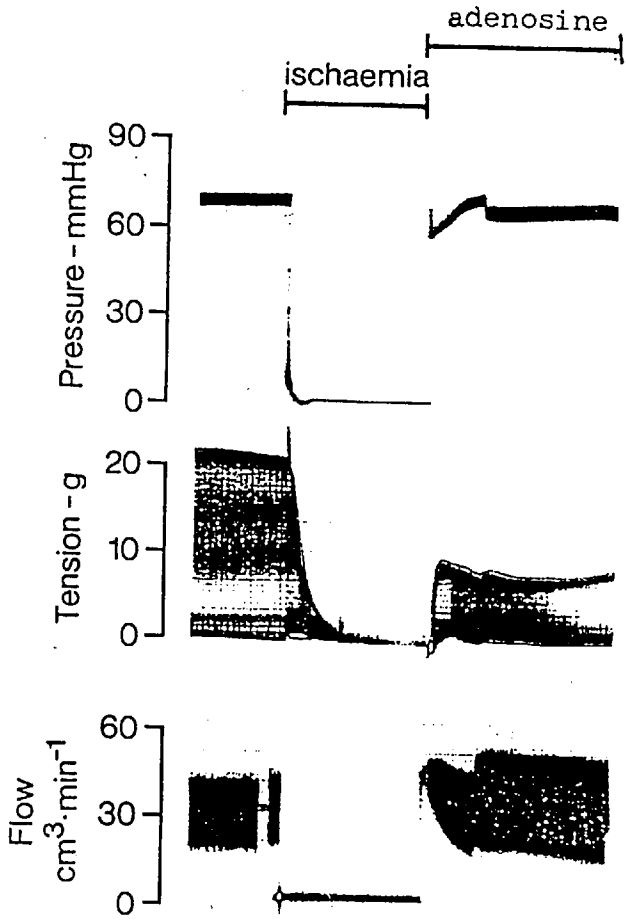


Figure 7.1a Example of the effect of adenosine on the changes in perfusion pressure, tension and flow produced by 15 min total global ischaemia. Adenosine (1.87×10^{-5} mol.litre^{-1}) was added to the perfusate on reperfusion.

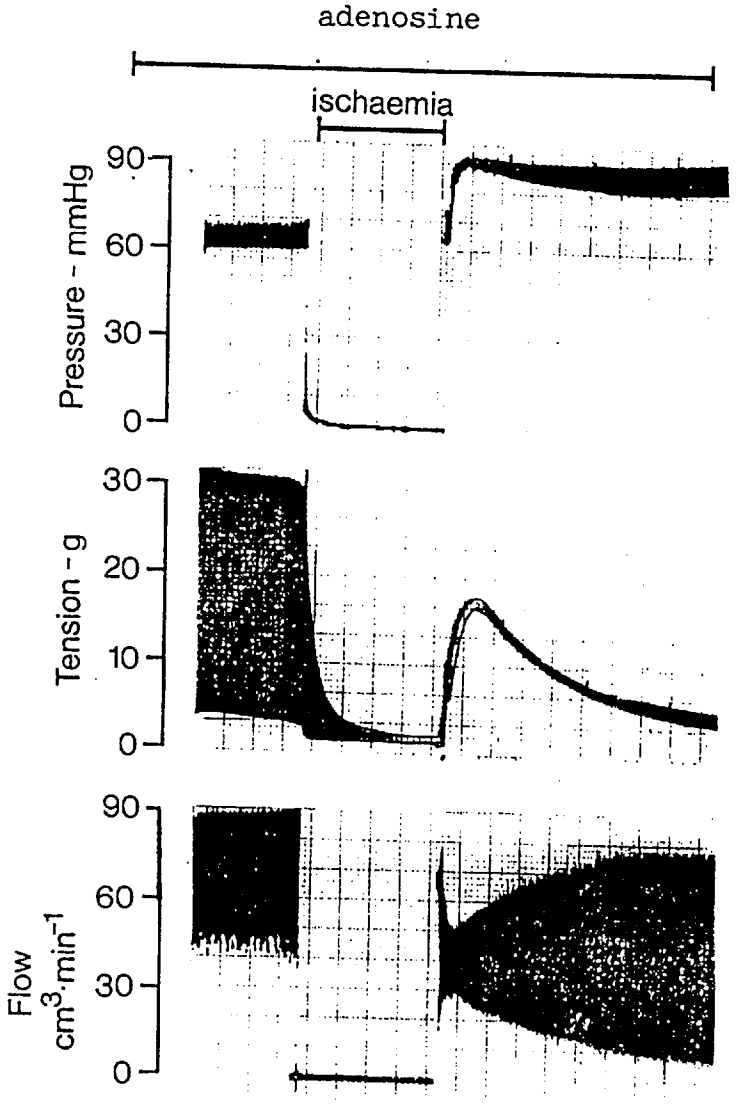


Figure 7.1b Example of the effect of adenosine on the changes in perfusion pressure, tension and flow produced by 15 min total ischaemia. Adenosine (1.87×10^{-5} mol.litre^{-1}) was added to the perfusate 10 min before the ischaemic period.

initially reduced, but returned to control values within 10 mins (Fig. 7.5b).

7.3.1.3 Indomethacin

In the 5 experiments where indomethacin (1.40×10^{-5} mol.litre^{-1}) was added to the perfusate control values for diastolic perfusion pressure and diastolic flow were not significantly changed although there was a trend towards a decrease in perfusion pressure and an increase in flow (Fig. 7.3). When the drug was added there was a decrease in developed tension of 12.8 $\pm$ 4.3%, resting tension was unaltered (Fig. 7.3).

7.3.2 Effects of Drugs After 15 min Ischaemia

7.3.2.1 Adenosine

When adenosine (1.87×10^{-5} mol.litre^{-1}) was added to the perfusate on reflow after 15 min ischaemia the initial recovery of perfusion pressure, flow and tension was similar to that seen in the absence of adenosine (Chapter 6). After about 5 min of reperfusion the adenosine began to take effect and there was an increase in flow accompanied by a decrease in perfusion pressure (Fig. 7.1a). These effects persisted so that at the end of the reperfusion period diastolic perfusion pressure was 57.7 $\pm$ 2.1 mmHg (9.7 $\pm$ 3.2% lower than that before ischaemia) and diastolic flow was 50.0 $\pm$ 0.7 cm^3.min.$^{-1}$g.$^{-1}$(31.3 $\pm$ 6.1% higher than that

before ischaemia). After 30 min reperfusion resting tension was unchanged from that before ischaemia and developed tension had recovered to 30.2 ± 3.4% of that before ischaemia. Resistance after the ischaemic period in the presence of adenosine was 64.5± 7.3% of that before ischaemia ($p<0.01$, paired t-test), P_{ZF} was increased by 11.2 ± 3.2 mmHg ($p<0.05$, paired t-test) (Table 7.1).

When adenosine (1.87×10^{-5} mol.litre^{-1}) was given before ischaemia the effects observed during the 15 min ischaemic period were similar to those observed in the absence of adenosine (Fig. 7.1b) i.e. pressure and flow dropped rapidly to zero and after an initial increase developed tension also fell to zero whilst resting tension was unchanged (Fig. 7.1b).

On reperfusion resting tension climbed rapidly and reached 15.2 ± 3.0 g (68.5 ± 12.1% of the tension developed before ischaemia) after 5 min (Fig. 7.1b). Resting tension then declined steadily so that by the end of the 30 min reperfusion period it had reached 5.4 ± 2.0 g (113.1 ± 85.9% higher than before ischaemia) (Fig. 7.1b). Developed tension recovered to 13.7 ± 8.7% of that before ischaemia. Perfusion pressure climbed rapidly to a peak on initiation of reperfusion and then remained stable for the remainder of the reperfusion period (Fig. 7.1b). After 30 min reperfusion diastolic perfusion pressure was 65.5± 5.5 mmHg (25.4 ± 9.3% higher than before ischaemia). After an initial peak on reperfusion diastolic flow declined as contracture developed (Fig. 7.1b). As resting tension declined flow recovered so that by the end of reperfusion diastolic flow was 80.9 ± 10.2 cm^3.min.$^{-1}$g.$^{-1}$ (90.5 ± 2.3% of that before ischaemia).

The perfusion pressure and flow values reflected the diastolic

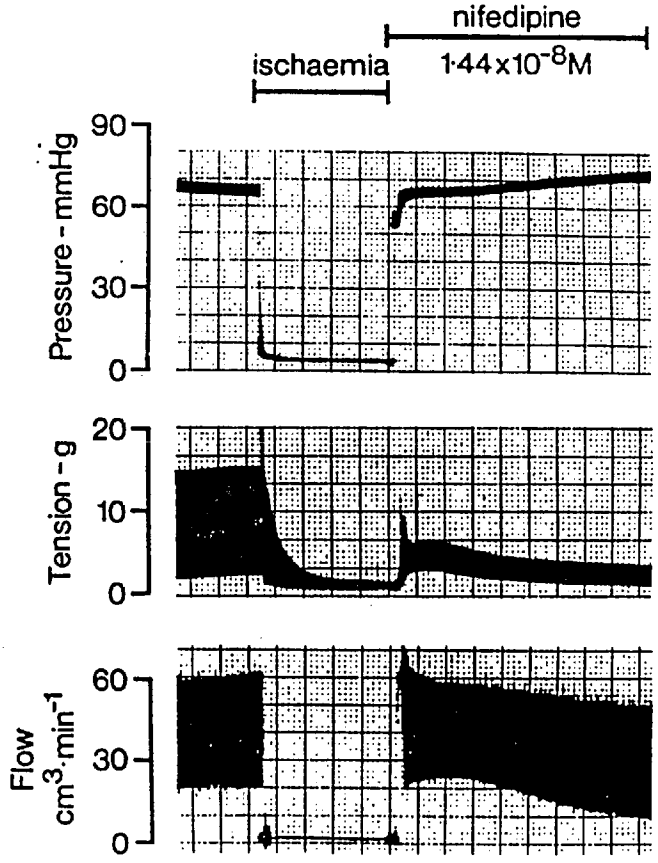


Figure 7.2a Example of the effect of nifedipine on the changes in perfusion pressure, tension and flow produced by 15 min total global ischaemia. Nifedipine (1.44×10^{-8} mol.litre^{-1}) was added to the perfusate on reperfusion.

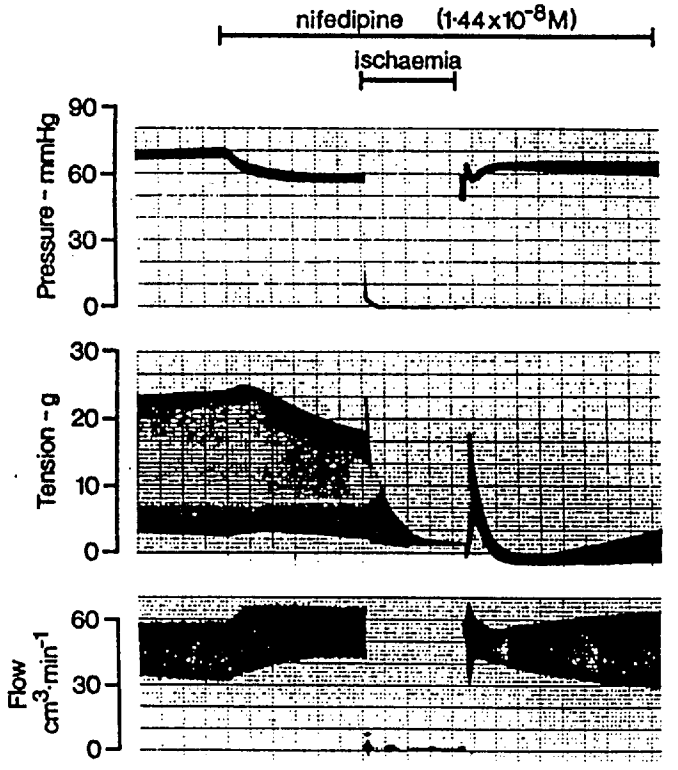


Figure 7.2b Example of the effect of nifedipine on the changes in perfusion pressure, tension and flow produced by 15 min total global ischaemia. Nifedipine (1.44×10^{-8} mol.litre^{-1}) was added to the perfusate 10 min before ischaemia.

resistance which was not significantly different from that before ischaemia (Table 7.1). P_{ZF} was increased by 4.3 $\pm$ 0.8 mmHg ($p<0.01$ paired t-test)(Table 7.1).

7.3.2.2 Nifedipine

After 15 min ischaemia ventricular fibrillation occurred within 5 min of reperfusion in 12 of the 17 hearts in the group in which nifedipine (1.44×10^{-8} mol.litre^{-1}) was given on reperfusion. Ventricular fibrillation did not occur in the 5 hearts to which nifedipine (1.44×10^{-8} mol.litre^{-1}) was given before ischaemia. When nifedipine (1.44×10^{-8} mol.litre^{-1}) was only present during reperfusion (Fig. 7.2a) the changes in end-diastolic pressure and end-diastolic flow were similar to those seen in the absence of the drug (Chapter 6). In the 5 hearts which did not fibrillate end-diastolic pressure was 63.6 $\pm$ 3.9 mmHg after 30 min reperfusion (14.6 $\pm$ 9.0% higher than before ischaemia). End-diastolic flow was 45.9 $\pm$ 4.6 $cm^3.min.^{-1}g.^{-1}$ (13.3$\pm$ 9.5% lower than before ischaemia). Developed tension was reduced by 80 $\pm$ 4%. Resting tension did not differ significantly from values recorded before ischaemia. Coronary resistance increased by 83 $\pm$ 27% ($p<0.05$,paired t-test) (Table 7.1), P_{ZF} was unchanged (Table 7.1).

In the experiments where nifedipine (1.44×10^{-8} mol.litre^{-1}) was present throughout both ischaemia and reperfusion (n = 5) (Fig. 7.2b) the decline in tension during ischaemia was similar to that in the absence of the drug (Chapter 6, Fig. 7.2a). On reperfusion perfusion pressure rose and reached a steady state within 10 min. After 30 min reperfusion end-diastolic perfusion

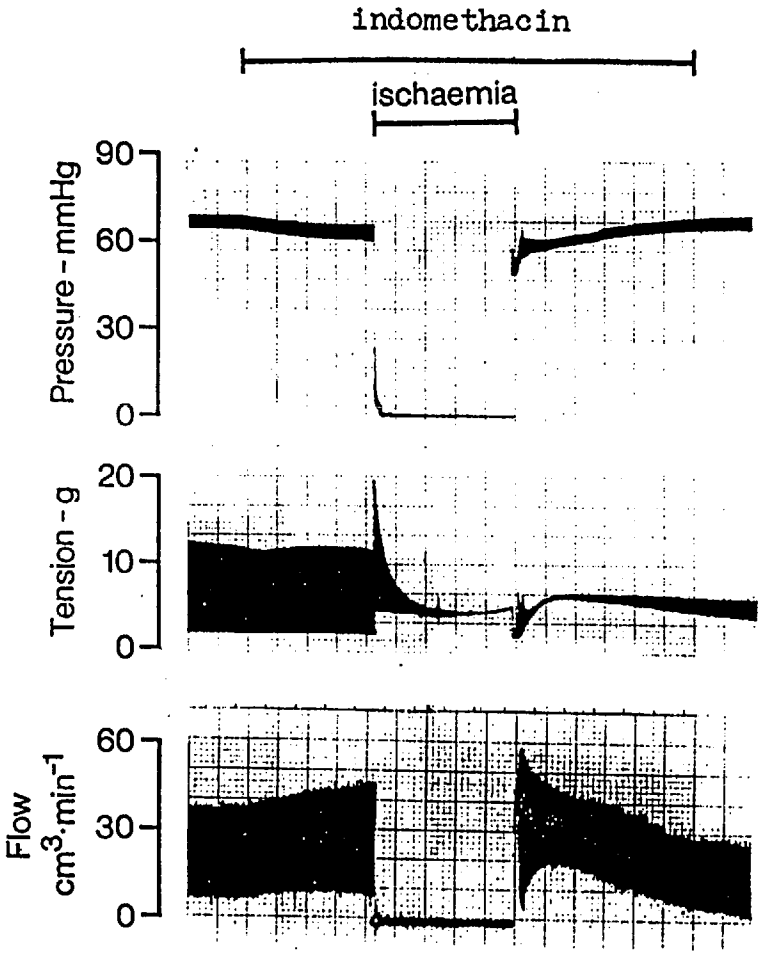


Figure 7.3 Example of the effect of indomethacin on the changes in perfusion pressure, tension and coronary flow produced by 15 min total global ischaemia. Indomethacin (1.40×10^{-5} mol.litre^{-1}) was added to the perfusate 10 min before the ischaemic period.

pressure was 50.9 ± 4.8 mmHg (not significantly different from that before ischaemia). End-diastolic flow was reduced to 55.0 ± 3.4 $cm^3.min.^{-1}g.^{-1}$ (by 5.1±1.6%) but was still higher than before nifedipine was administered.
Developed tension recovered transiently on reperfusion but after 30 min had declined to 38.0 ± 7.9% of that before ischaemia. Resting tension was depressed by 1.7 ± 0.4 g. Coronary resistance was 20.6± 9.6% lower than before nifedipine was administered (Table 7.1). The minimum pressure required for flow (P_{ZF}) was increased by 4.9±1.6 mmHg ($p<0.05$, paired t-test)(Table 7.1).

7.3.2.3 Indomethacin

During ischaemia perfusion pressure and flow dropped rapidly to zero (Fig. 7.3). After an initial increase in resting tension and developed tension resting tension declined towards control values and developed tension was reduced to zero (Fig. 7.3) i.e. the results were similar to those observed in the absence of indomethacin (Chapter 6). On reperfusion (Fig. 7.3) changes in perfusion pressure, flow and contraction were also similar to the results obtained after a similar period of ischaemia in the absence of indomethacin (Chapter 6). Ventricular fibrillation occurred in 7 of the 12 hearts reperfused after 15 min ischaemia. In the 5 hearts which did not fibrillate developed tension recovered to 14.4 ± 2.7% of that developed before ischaemia by the end of the reperfusion period. Resting tension declined to a value insignificantly different from that before ischaemia. Diastolic perfusion pressure was 66.5 ± 2.6 mmHg (11.8± 0.9% above control values) diastolic flow was 47.5 ± 6.5

$cm^3.min.^{-1}g^{-1}$ (31.2 ± 8.6% below control values). In the 5 hearts which did not fibrillate diastolic resistance tended to increase after ischaemia (by 64.2 ± 24.3%) (Table 7.1). There was also an increasing trend in P_{ZF} (10.5 ± 4.3 mmHg) but this was not significant (Table 7.1).

7.3.2.4 Comparison with Results Obtained in the Absence of Drugs

After 15 min ischaemia and 30 min reperfusion in the absence of drugs diastolic coronary resistance was increased by 53.3 ± 16.3% ($p<0.05$, paired t-test) (see Chapter 6). The increase in resistance seen when indomethacin (1.4×10^{-5} mol.litre^{-1}) was present throughout the ischaemic period (64.2 ± 24.3%) was not significantly different from this, nor was the value obtained when nifedipine (1.44×10^{-8} mol.litre^{-1}) was given on reperfusion (83.0 ± 27.0%). Adding nifedipine (1.44×10^{-8} mol.litre^{-1}) or adenosine (1.87×10^{-5} mol.litre^{-1}) to the perfusate before the ischaemic period, however, prevented the increase in resistance, as did administration of adenosine on reperfusion (resistance decreased by 20.6 ± 9.6%, 4.8 ± 4.0% and 35.5 ± 7.3% respectively).

Values taken from Chapter 6 show that the minimum pressure required for flow (P_{ZF}) increased by 6.5 ± 4.5 mmHg after 15 min ischaemia. Administration of adenosine, nifedipine or indomethacin did not significantly alter this increase.

In the absence of drugs mechanical function improved on reperfusion after 15 min ischaemia so that after 30 min resting tension was not significantly different from that before ischaemia and developed tension had recovered to 30.0 ± 9.5% of

TABLE 7.1 EFFECT OF DRUGS ON THE INCREASE IN RESISTANCE TO FLOW AFTER 15 MIN TOTAL GLOBAL ISCHAEMIA IN THE ISOLATED RABBIT HEART

Before Ischaemia		After Reperfusion		n
resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	
Control (from Chapter 6)				
1490 ± 180	-9.6 ± 5.1	2290 ± 180	-3.1 ± 3.2	5
Adenosine (1.87×10^{-7} mol.litre^{-1})				
before ischaemia				
880 ± 100*	-0.4 ± 1.3	850 ± 130**	3.9 ± 0.7	4
after ischaemia				
1840 ± 240	1.3 ± 0.9	1130 ± 40**	11.2 ± 1.8**	5
Nifedipine (1.44×10^{-8} mol.litre^{-1})				
before ischaemia				
1280 ± 140	-4.1 ± 1.8	990 ± 120**	0.8 ± 1.0	5
after ischaemia				
920 ± 80*	2.1 ± 0.5	1650 ± 250	5.9 ± 3.1	5
Indomethacin (1.40×10^{-5} mol.litre^{-1})				
before ischaemia				
1130 ± 140	-11.4 ± 4.5	1750 ± 170	-1.6 ± 1.9	5

* = significantly different ($p \leq 0.05$) from corresponding value in the absence of the drug
** = significantly different ($p \leq 0.01$) from corresponding value in the absence of the drug

that before ischaemia (Chapter 6). Perfusion with adenosine, nifedipine or indomethacin did not significantly alter this recovery.

Thus whilst adenosine and nifedipine given before the ischaemic period prevented the increase in resistance to flow which occurred on reperfusion indomethacin, or nifedipine given on reperfusion were ineffective.None of the drugs administered improved mechanical recovery on reperfusion.

7.3.3 Effects of Drugs After 60 min Ischaemia

7.3.3.1 Adenosine

In the experiments where adenosine (1.87×10^{-5} mol.litre^{-1}) was given on reperfusion after 60 min ischaemia (n = 5) (Fig. 7.4) effects were similar to those seen in the absence of adenosine (Chapter 6). No recovery of developed tension occurred and after 30 min reperfusion, resting tension was 10.7 ± 1.6 g (63.0 ± 11.8% of developed tension before ischaemia).Diastolic perfusion pressure was 92.6 ± 3.5 mmHg (66.8±10.0% higher than before ischaemia) and diastolic flow was 17.4 ± 2.9 $cm^3.min.^{-1}g^{-1}$ (69.0 ± 6.8% lower than before ischaemia).
Coronary resistance increased by 679 ± 150% ($p<0.05$,paired t-test) (Table 7.2), P_{ZF} also tended to increase (by 7.2 ± 3.6 mmHg) (Table 7.2).

7.3.3.2 Nifedipine

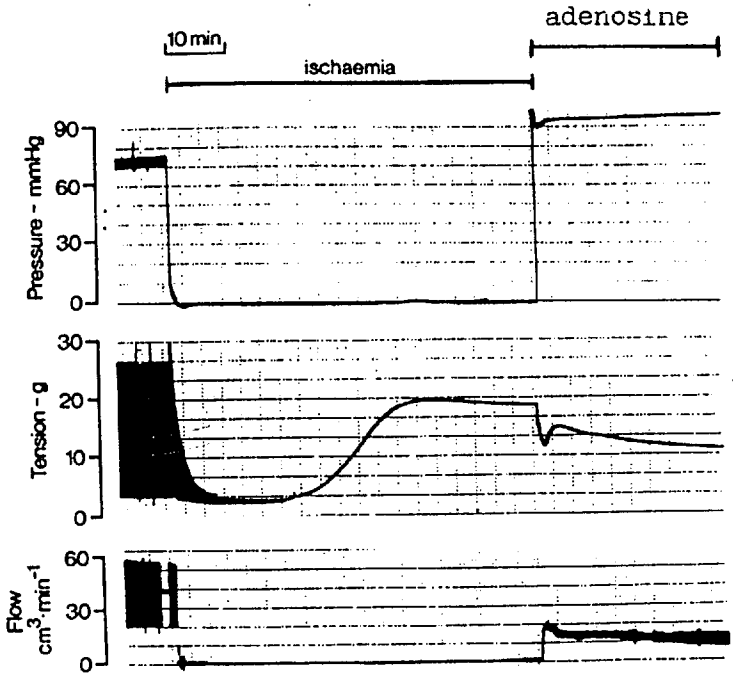


Figure 7.4 Example of the effect of adenosine on the changes in perfusion pressure, tension and coronary flow produced by 60 min total global ischaemia. Adenosine (1.87×10^{-5} mol.litre^{-1}) was added to the perfusate on reperfusion.

On reperfusion after 60 min ischaemia synchronous contraction occurred in the group which had been perfused with 1.44×10^{-8} mol.litre^{-1} nifedipine before the ischaemic period. The tension developed was 5.3 ± 2.1% of that developed before ischaemia. No contraction was seen during reperfusion in the group which had been perfused with 1.44×10^{-7} mol.litre^{-1} nifedipine before ischaemia.

The effects of nifedipine on recovery after 60 min ischaemia are shown in Figure 7.5. Given before the ischaemic period nifedipine delayed the development of contracture during ischaemia. The delay was greater with the higher concentration of nifedipine (1.44×10^{-7} mol.litre^{-1}). After 30 min reperfusion the degree of contracture was similar to that seen in the absence of nifedipine (Chapter 6). Resting tension had increased by 6.8 ± 0.6 g in the "low dose" nifedipine (1.44×10^{-8} mol.litre^{-1}) group and by 11.5 ± 1.6 g in the "high dose" nifedipine (1.44×10^{-7} mol.litre^{-1}) group.

Perfusion with nifedipine (1.44×10^{-8} or 1.44×10^{-7} mol.litre^{-1}) before ischaemia reduced the increase in end-diastolic pressure and the decrease in end-diastolic flow seen after 60 min ischaemia in the absence of the drug (Chapter 6). After 30 min reperfusion end-diastolic pressure was 77.9 ± 3.1 mmHg in the "low dose" group and 75.5 ± 1.1 mmHg in the "high dose" group (an increase of 64.7 ± 9.1 and 47.4 ± 7.8% respectively). End-diastolic flow was 26.6 ± 3.2 $cm^3.min.^{-1}g^{-1}$ in the "low dose" group and 27.7 ± 3.4 $cm^3.min.^{-1}g^{-1}$ in the "high dose" group (a decrease of 59.9 ± 4.9 and 50.3 ± 3.4% respectively). Coronary resistance increased by 195 ± 31% ($p<0.01$, paired t-test) in the "low dose" group whilst P_{ZF} increased by 9.4 ± 1.7 mmHg

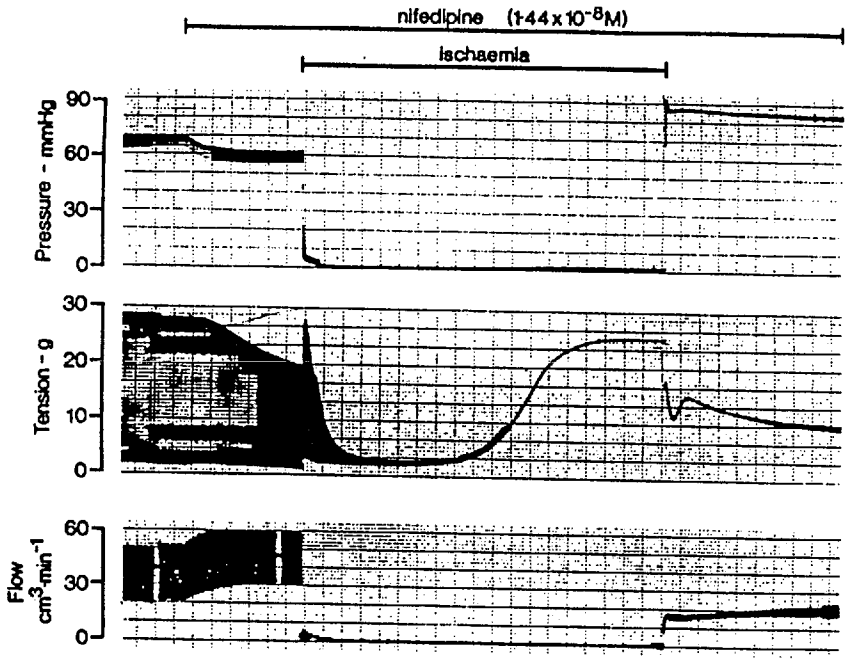


Figure 7.5a Example of the effect of nifedipine on the changes in perfusion pressure, tension and coronary flow produced by 60 min total global ischaemia. Nifedipine (1.44×10^{-8} mol.litre^{-1}) was added to the perfusate 10 min before the ischaemic period.

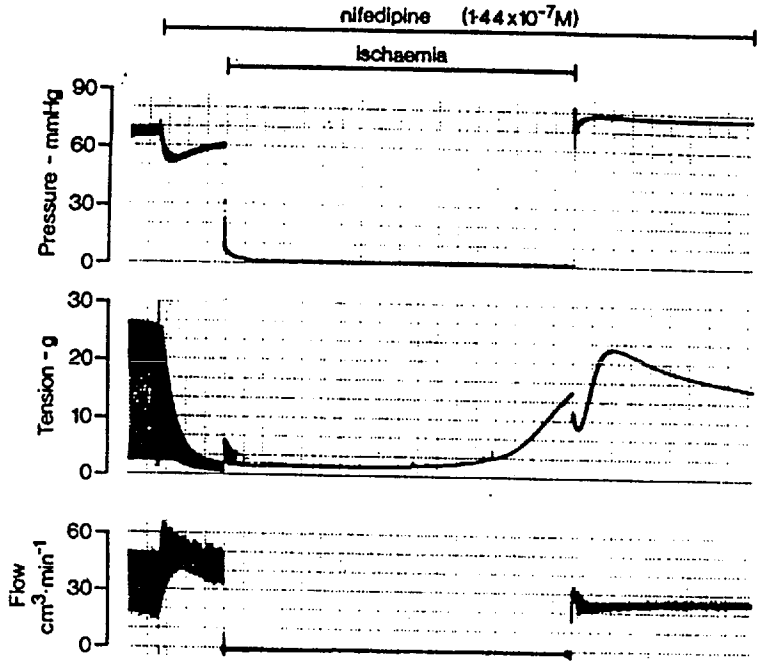


Figure 7.5b Example of the effect of nifedipine on the changes in perfusion pressure, tension and coronary flow produced by 60 min total global ischaemia. Nifedipine (1.44×10^{-7} mol.litre^{-1}) was added to the perfusate 10 min before the ischaemic period.

($p<0.01$,paired t-test) (Table 7.2). In the "high dose" group resistance increased by 234 ± 53% ($p<0.05$,paired t-test) and P_{ZF} increased by 5.9 ± 1.9 mmHg ($p<0.05$,paired t-test) (Table 7.2).

7.3.3.3 Comparison with Results Obtained in the Absence of Drugs

In a group of 5 experiments 60 min total global ischaemia followed by 30 min reperfusion at the same hydrostatic pressure resulted in an increase in resting tension of 9.9 ± 2.6 g. Diastolic coronary resistance was increased by 410 ± 99% ($p<0.05$,paired t-test) whilst P_{ZF} was not significantly altered (P_{ZF} decreased by 6.8 ± 5.0 mmHg,$p>0.05$,paired t-test) (Table 7.2). The increase in resting tension observed when nifedipine (1.44×10^{-8} mol.litre^{-1} or 1.44×10^{-7} mol.litre^{-1}) or adenosine (1.87×10^{-5} mol.litre^{-1}) was present was not significantly different from this control value (6.8± 0.8, 11.5 ± 1.6 and 8·7±1·4g respectively). The increase in diastolic resistance seen in the presence of adenosine (679 ± 150%) was also similar to that in the absence of the drug. Both concentrations of nifedipine appeared to reduce the increase in resistance but this reduction was only significant with the higher concentration (Table 7.2).Adenosine and nifedipine significantly increase P_{ZF}.

Thus myocardial contraction and the increase in resistance after 60 min total global ischaemia could not be prevented by prior administration of adenosine. Nifedipine may have reduced the increase in resistance to flow but did not improve mechanical recovery.

7.4 Discussion

TABLE 7.2 EFFECT OF DRUGS ON THE INCREASE IN RESISTANCE TO FLOW AFTER 60 MIN TOTAL GLOBAL ISCHAEMIA IN THE ISOLATED RABBIT HEART

Before Ischaemia		After Reperfusion		n
resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	
Control				
940 ± 70	-7.6 ± 1.9	4710 ± 730	-14.4 ± 6.4	5
Adenosine (1.87 x 10^{-7}mol.litre^{-1}) after ischaemia				
800 ± 110	4.9 ± 1.6**	5650 ± 820	12.1 ± 3.3**	5
Nifedipine (before ischaemia) 1.44 x 10^{-8}mol.litre^{-1}				
1000 ± 70	-1.3 ± 1.0*	2950 ± 380	8.0 ± 1.1**	5
1.44 x 10^{-7}mol.litre^{-1}				
830 ± 70	0.0 ± 1.4*	2720 ± 370*	5.9 ± 1.8*	5

* = significantly different from corresponding value in the absence of the drug. * = $p \leq 0.05$; ** = $p \leq 0.01$

7.4.1 Effect of Adenosine on Coronary Resistance After Global Ischaemia

Adenosine is a potent vasodilator which has been proposed as the chemical mediator released in response to insufficient oxygen supply to the myocardium (see Chapter 2). In the isolated rabbit heart adenosine (1.87×10^{-5} mol.litre^{-1}) maximally dilates the coronary circulation under control conditions (Chapter 4). The results of the experiments described in this chapter show that the same concentration of adenosine partly prevents or reverses the increase in diastolic coronary resistance which occurs on reperfusion after 15 min ischaemia in this preparation. The increase in resistance to flow after 60 min ischaemia is not susceptible to treatment with this concentration of adenosine. This suggests that the increase in diastolic coronary resistance observed after 15 min ischaemia is due to an increase in vascular smooth muscle tone whilst that after 60 min ischaemia is not.

7.4.2 Effect of Nifedipine on Coronary Resistance After Global Ischaemia

Nifedipine dilates large coronary arteries (Kroneberg, 1975), decreases coronary arteriolar resistance (Vater et al., 1972) and is negatively inotropic in vitro (Schwartz et al., 1981). These effects are consequences of inhibition of calcium influx through voltage-dependent channels in the cell membrane (Fleckenstein, 1977). Other compounds with a similar mechanism of action (e.g. verapamil, diltiazem) have been found to prevent tissue damage and reduce

calcium accumulation on reperfusion if given before a period of ischaemia (Smith et al., 1977; Bourdillon and Poole-Wilson, 1982). When added during ischaemia or at the moment of reperfusion no protection is observed (Smith et al., 1977; Watts et al., 1980; Bourdillon and Poole-Wilson, 1982). These results have lead to the hypothesis that the calcium antagonists are protective because they decrease the energy requirement (Smith et al., 1977; Watts et al., 1980; Bourdillon and Poole-Wilson, 1982) or modify transmembrane ionic fluxes (Bush et al., 1982; Weintraub et al., 1982) during ischaemia, rather than blocking Ca^{2+} influx during reperfusion or increasing reperfusion to an ischaemic area. The "low dose" of nifedipine used in the experiments described in this chapter was similar to that used by Kissin and Kilpatrick (1982) in the rabbit Langendorff. It had a small effect on developed tension whilst producing maximal vasodilation. The effects of this dose could be attributed to vasodilatory actions rather than "energy sparing" effects. This "low dose" of nifedipine was effective in preventing the increase in diastolic coronary resistance if given before 15 min ischaemia and possibly if given before 60 min ischaemia but not if given only on reperfusion. The "protective" effect of nifedipine after 15 min ischaemia was probably due to its vasodilatory action. Nifedipine given on reperfusion may not have been able to reach its site of action or it may be unable to reverse the increase in coronary resistance. The results obtained in the presence of adenosine suggest that the increase in coronary resistance on reperfusion after 60 min ischaemia is not due to vasoconstriction. The protective effect of nifedipine after this prolonged period of ischaemia is due to a property other than vasodilation.

7.4.3 Effect of Indomethacin on Coronary Resistance After Global Ischaemia

Indomethacin is an inhibitor of cyclooxygenase and hence inhibits synthesis of prostaglandins and thromboxanes (Vane, 1971). The dose used in this study was similar to that used by others to prevent prostaglandin synthesis in the coronary circulation (e.g. Block et al., 1975; Schror et al., 1976). Administration of indomethacin to the isolated guinea-pig heart results in an increase in coronary resistance (Schror et al., 1976). Block and colleagues (1975), however, found no change in coronary flow when indomethacin was given to the buffer perfused rabbit heart. The experiments described in this chapter suggest that there is a low production of a vasoconstrictor prostaglandin like substance in the isolated rabbit heart under control conditions. Inhibition of cyclooxygenase did not prevent diastolic coronary resistance increasing after ischaemia suggesting that the vasoconstriction is not due to production of prostaglandins or thromboxanes.

7.4.4 Recovery of Mechanical Function after Ischaemia

Although both adenosine and nifedipine improved reperfusion after ischaemia no resultant increase in mechanical recovery was observed. Inadequate reperfusion was therefore not the cause of the incomplete recovery of contractile force. Similar conclusions have been drawn from other studies. Kloner (1982) found that the area of "no reflow" was restricted to the centre of an area of myocardial damage and

ultrastructural studies have shown that myocardial damage occurs before vascular damage (e.g. Kloner et al., 1980).

Adenosine has previously been shown to improve recovery of mechanical function after ischaemia (Foker et al., 1980; Humphrey and Seelye, 1982). In both these studies adenosine deaminase was inhibited, preventing the conversion of adenosine to inosine and hence promoting adenosine uptake and intracellular conversion to ATP. The improved mechanical performance can thus be attributed to maintenance of intracellular ATP concentrations. In the experiments described in this chapter adenosine deaminase was not inhibited and conversion of adenosine to ATP was probably insufficient to maintain intracellular stores.

Verapamil has been reported to increase recovery of developed tension in the working rat heart (Watts et al., 1980). Bourdillon and Poole-Wilson (1982), however, found no improvement in mechanical performance in the interventricular septum of the rabbit. Nifedipine has been reported to prevent contracture on reperfusion after 60 min ischaemia in the isolated rabbit heart (Henry et al., 1977). In the experiments described in this chapter a similar dose of nifedipine was used. The rise in resting tension during a 60 min ischaemic period was delayed but there was no significant difference in the degree of contracture seen on reperfusion. Nifedipine did, however, reduce the incidence of reperfusion arrhythmias if given before ischaemia as previously reported (Henry et al., 1977; Bush et al., 1982) probably by increasing the ventricular fibrillation threshold during ischaemia (Thandroyen, 1982).

7.4.5 Conclusions

The increase in diastolic coronary resistance on reperfusion after 15 min global ischaemia in the isolated rabbit heart is mainly due to coronary vasoconstriction whilst that after 60 min ischaemia is not. The increase in vascular smooth muscle tone is not caused by release of prostaglandins or thromboxanes. The constriction can be prevented by treatment with adenosine or nifedipine (if given before the period of ischaemia). Impaired myocardial function after ischaemia is not improved in the absence of an increase in coronary resistance and is therefore not a direct consequence of insufficient reperfusion.

CHAPTER 8

EXTRAVASCULAR FACTORS AND CORONARY RESISTANCE

8.1 Introduction

The resistance to blood flow of the coronary circulation is determined by extravascular factors as well as by the contractile activity of the smooth muscle cells within the vascular walls. Under normal conditions changes in extravascular resistance are compensated for by changes in vascular smooth muscle tone. However, when the coronary vessels are maximally dilated increases in extravascular resistance cannot be compensated and extravascular forces become important determinants of coronary blood flow. Extravascular forces are highest during systole but they can also contribute to diastolic coronary resistance.

8.1.1 Extravascular Forces in Systole

Inhibition of coronary inflow during systole was conclusively demonstrated by Sabiston and Gregg in 1957, thus ending almost a century of debate between those who supported this view (Scaramucci, 1687; Rebatel, 1872; Anrep et al., 1927) and those who proposed that the heart-beat helped to propel blood through the coronary circulation by a milking action (Porter, 1898; Hockrein et al., 1930; Wiggers and

Cotton, 1933). Systole increases resistance to flow by causing mechanical deformation of the coronary vasculature. This could result from an increase in shear strains, an increase in traction forces or an increase in tissue pressure. Shear strains increase because adjacent cardiac muscle fibres slide past one another during contraction and vessels running between them are distorted and may become kinked or totally occluded at the interface of the two fibres (Feigl and Fry, 1964). Traction forces may be important in epicardial vessels which become stretched longditudinally as ventricular wall thickness increases during systole. Increased intraventricular pressure during systole might be expected to be transferred through the soft tissue of the myocardium and hence increase intramyocardial tissue pressure. Current evidence suggests that the majority of the increase in resistance during systole is caused by the increase in tissue pressure rather than that in shear strain or traction force (Downey et al., 1974; Williams et al., 1981).

During systole there is a linear gradient of tissue pressure across the left ventricular wall ranging from a low subepicardial value to a high subendocardial one (Baird, 1980). In the subendocardium tissue pressure accounts for almost half of the coronary resistance whilst accounting for virtually none in the subepicardium (Russell et al., 1977). Peak subendocardial tissue pressure has been estimated to reach values in excess of left ventricular pressure (e.g. Johnson and Dipalma, 1939; Gregg and Eckstein, 1941; Stein et al., 1980), however, tissue pressure recorded by a foreign object placed in the myocardial wall is a function of its size (Brandi and McGregor, 1969) and if the data obtained are corrected for the volume of the measuring instrument subendocardial

tissue pressure is found to be close to ventricular pressure. This estimate of subendocardial tissue pressure is supported by the results of Heineman and co-workers (1979) who used a micropipette of tip size an order of magnitude lower than that previously used and by those obtained Baird and co-workers (1970) from vessel segments implanted in the ventricular wall. Intramyocardial tissue pressure is proportional to left ventricular pressure (Baird et al., 1975) and both the magnitude and gradient across the left ventricular wall can be changed by altering contractility (Marzilli et al., 1979; Trimble and Downey, 1979). However, the pressure gradient across the ventricular wall is maintained when the ventricle is vented (Baird, 1980) indicating that intramyocardial pressure is not solely a function of intra-ventricular pressure.

8.1.2 Extravascular Forces in Diastole

The diastolic pressure-flow relationship of the maximally vasodilated dog heart shows a positive pressure intercept of 15 - 18 mmHg (Archie, 1978). As coronary tone is minimal in this preparation this pressure must be that required to overcome extravascular forces. Thus extravascular forces influence coronary flow during diastole as well as during systole. In diastole intramyocardial tissue pressure is greater in the subepicardium than the subendocardium (Rouleau et al., 1979; Baird, 1980; Stein et al., 1980). It is affected by changes in preload (Ellis and Klocke, 1979), coronary sinus pressure (Bellamy et al.,1980) and resting tension (Baird et al., 1975; Downey et al., 1979).

8.1.3 Vascular Waterfalls

Current evidence suggests that intramyocardial tissue pressure alters coronary flow by forming vascular waterfalls. As fluid passes through a tube energy is lost overcoming frictional resistance. This causes pressure to fall from the inlet to the outlet of the tube. If the tube is a soft walled collapsible vessel to which external pressure can be applied the tube will collapse if external pressure (P_T) exceeds inflow pressure (P_A). Waterfall theory as presented by Holt (1941) says that if P_T is less than P_A but greater than the outflow pressure (P_V) the tube will collapse where the pressure inside the tube becomes less than P_T. Collapse of the tube prevents flow through it so the pressure at the collapse point becomes equal to P_A. The tube is therefore reopened. An equilibrium is set up where a short region of partial collapse is formed as near the outflow end of the tube as is physically possible. This region of partial collapse effectively drops the pressure within the vessel from P_T to P_V whilst the resistance of the vessel upstream of the collapsed section is unaltered. Thus by a modification of Poiseuille's Law flow through the tube is proportional to $P_A - P_T$ rather than $P_A - P_V$. Changing P_V has no effect on flow as long as P_V is less than P_T. This is analagous to a waterfall; raising the level of the river below the falls does not influence the flow of water over it.

The applicability of this model to the vascular bed has been extensively studied in the lung (Permutt et al., 1962) and the kidney (Hinshaw et al., 1963) and was applied to the coronary circulation by Downey and Kirk (1975). They found that if a maximally dilated dog

heart was arrested the pressure-flow relationship was shifted to the left but there was no change in slope (Downey and Kirk, 1975). They suggested that this indicated that systole altered the pressure intercept but did not alter resistance, something that would be expected if a vascular waterfall was operating.

In 1978 Bellamy determined the pressure-flow relationship of a canine coronary artery during a prolonged diastole and found a linear relationship with a zero flow intercept (P_{ZF}) of 40 mmHg. This intercept could be reduced to 10 - 15 mmHg by pharmacological vasodilation or reactive hyperaemia. Bellamy suggested that these results indicated that vascular tone played a part in determining the height of the vascular waterfall. It has been suggested, however, that the high values of P_{ZF} obtained during prolonged diastoles in the autoregulating dog heart are due to capacitive effects (Eng et al., 1981; Dole and Bishop, 1982) and that when these are taken into account P_{ZF} is similar to that in the maximally dilated coronary bed. Other workers have claimed that the artifact due to the capacitance of the left ventricular circulation is less than 10 mmHg (Kirkeeide et al., 1981) and cannot fully account for the high values of P_{ZF} observed during prolonged diastoles, thus indicating that coronary tone does play a part in determining the height of the vascular waterfall.

If intramyocardial tissue pressure controls coronary flow by forming vascular waterfalls the position of partial collapse is likely to be in the venules or the small veins. Pressures in subepicardial venules are about 25/10 mmHg (Tillmanns et al., 1981), well above that in the coronary sinus. Although pressure loss will occur due to frictional resistance as blood passes from these venules into the

large veins and eventually to the coronary sinus the observed pressure difference is too large to be accounted for by this mechanism.Venule pressures probably reflect local tissue pressures and are therefore probably partially collapsed i.e. the point at which the waterfall acts.

8.1.4 Arguments Against the Operation of a Vascular Waterfall

Spaan and co-workers (1981) have argued that the vascular waterfall model proposed by Downey and Kirk (1975) cannot account for the predominantly systolic venous outflow from the coronary circulation, for the negative systolic flows which occur at low perfusion pressures or for the continuation of pressure oscillations in the coronary vessels after coronary occlusion. To account for these observations they proposed the existence of an intramyocardial pump (Spaan et al., 1981). Recently Westerhof and co-workers (1983) have re-examined the two models and have shown that a vascular waterfall model can account for systolic reversal of flow and continued pressure oscillations during coronary occlusion. The observation that coronary sinus flow is predominantly systolic can only be explained if a venous compliance is added to the hydraulic waterfall (Westerhof et al., 1983).

8.1.5 Physiological Contribution of Extravascular Forces

As already stated changes in extravascular forces are normally compensated for by alterations in coronary tone and only become important determinants of resistance to flow when the coronary bed is

maximally vasodilated. Ischaemia is a potent vasodilator and it has been suggested that extravascular forces may be involved in exacerbating myocardial ischaemia and in the flow defect observed on subsequent reperfusion. An increase in extravascular forces may be a direct consequence of ischaemia, due to oedema or myocardial contracture. Alternatively, raised intramyocardial pressure may result in exhaustion of vasodilatory reserve and precipitate an ischaemic event.

8.1.5.1 Oedema

Ischaemia in the kidney is accompanied by cell swelling (Leaf, 1973). A similar situation in the heart would increase extravascular pressure and hence further inhibit blood flow. Direct evidence that myocardial hypoxia produces cell swelling is not strong. Limited evidence of interstitial and cellular swelling has been found after ligation of the coronary artery of the dog (Kloner et al., 1974; Whalen et al., 1974) and during anoxic perfusion of the isolated rat heart (Brachfeld et al., 1975). However, Polimeni (1975) and Pine and his co-workers (1979) failed to find any increase in the tissue water content of hypoxic myocardial tissue from the rat.

Although the evidence of cell swelling and oedema during myocardial ischaemia is limited oedema on reperfusion/reoxygenation of the heart is well documented (e.g. Kloner et al., 1974; Whalen et al., 1974; Ganote et al., 1977). Thus although increased intramyocardial forces due to cell swelling and oedema may play no role in exacerbating ischaemia they probably contribute to reperfusion defects.

8.1.5.2 Myocardial Contracture

Prolonged ischaemia leads to myocardial contracture (see Chapter 6) which squeezes the coronary vessels and hence increases resistance to flow through them. A further increase in contracture may occur in early reperfusion and is associated with a further increase in resistance to flow (Chapter 6; Apstein et al., 1977). The increase in resistance to flow can be prevented by keeping the heart isovolumic during the ischaemic period and hence preventing the decrease in the compliance of the myocardium (Humphrey et al., 1981).

Local myocardial contracture may contribute to increases in resistance to flow seen after short periods of ischaemia. Anginal attacks are accompanied by a decrease in blood flow to the ischaemic area (Mazeri et al., 1972) and it has been proposed that this decrease in blood flow is caused by concurrent myocardial contraction restricting flow through small vessels (Harris, 1975). Harris suggested that the resultant situation might exacerbate ischaemia, with contraction of myocytes around vessels giving rise to further ischaemia which in turn produces further myocardial contraction eventually leading to myocardial infarction if the circuit is not broken. Thus increased intramyocardial forces in the form of increased myocardial contraction can both exacerbate ischaemia and reduce tissue reperfusion.

8.1.5.3 Exhaustion of Vasodilatory Reserve

As already mentioned intramyocardial pressure is affected by preload (Ellis and Klocke, 1979) and coronary sinus pressure (Bellamy et al., 1980). In situations where diastolic ventricular pressure is

elevated, e.g.heart failure and aortic valve disease, compensation for the increased intramyocardial pressure will reduce vasodilatory reserve and the ability of the coronary circulation to respond to increased myocardial demand. Reserve will be more quickly exhausted and an ischaemic event may be precipitated.

In the following chapter the contribution of extravascular forces to the coronary resistance of the isolated rabbit heart is determined. Intramyocardial pressure is increased by increasing the stimulation rate and by perfusion with FCCP. Left ventricular pressure is increased by insertion of a balloon into the left ventricle.

8.2 Experimental

8.2.1 Determination of the Effect of Intraventricular Pressure

Rabbit hearts were perfused by the pressure controlled Langendorff method described in Chapter 3. After 10 min perfusion a latex balloon was inserted into the left ventricle. The balloon was kept in place with a purse-string suture around the mitral valve. Water was injected through a catheter attached to the balloon until the balloon just filled the ventricular cavity (approximately 0.5 cm^3). After 10 min stabilisation adenosine (1.87×10^{-5} mol.litre^{-1}) was added to the perfusate. After a further 5 min pressure was altered in a stepwise fashion and the resulting changes in perfusion pressure and aortic flow were recorded when a steady state had been reached. After a 5 min stabilisation period in the presence of adenosine, the volume of the balloon was increased to 2 cm^3 and after a further 5 min the relationship between perfusion pressure and flow

was redetermined.

8.2.2 Determination of the Effect of Increased Stimulation Rate

Rabbit hearts were perfused by the pressure controlled Langendorff method described in Chapter 3. After the 20 min stabilisation stepwise alterations in hydrostatic pressure were made and the resulting changes in perfusion pressure and flow recorded. Systolic pressure was then adjusted to 50 mmHg and after 5 min stabilisation stimulation rate was increased from 3 to 30 Hz. When a steady state had been reached the relationship between perfusion pressure and flow was redetermined.

8.2.3 Determination of the Effect of FCCP

Rabbit hearts were perfused by the pressure controlled Langendorff method described in Chapter 3. The relationship between perfusion pressure and flow was determined by altering the hydrostatic pressure and recording the resultant changes in perfusion pressure and flow. Systolic pressure was adjusted to 50 mmHg and 10 min stabilisation allowed before FCCP (1×10^{-6} mol.litre^{-1}) was added to the perfusate. After 20 min perfusion the pressure-flow relationship was redetermined. In one group of experiments adenosine (1.87×10^{-5} mol.litre^{-1}) was added to the perfusate 5 min before FCCP.

8.2.4 Materials

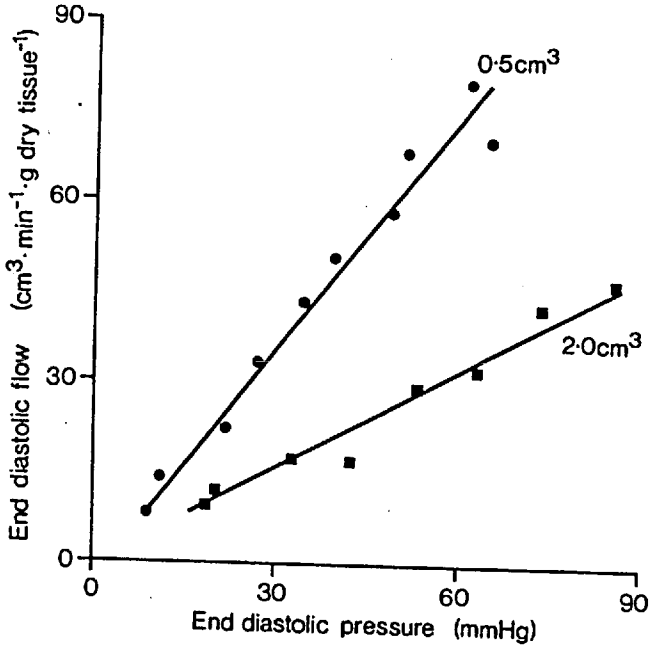


Figure 8.1 Example of the effect of increasing left ventricular pressure on the relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated rabbit heart. Left ventricular pressure was increased by increasing the volume of a balloon within the left ventricle from 0.5 cm^3 to 2.0 cm^3.

FCCP was dissolved in ethanol to give a stock solution containing 2.5×10^{-4} mol.litre^{-1}. Adenosine was dissolved in distilled water to give a stock solution containing 1.87×10^{-2} mol.litre^{-1}.

8.3 Results

8.3.1 Effect of Increased Left Ventricular Pressure

Insertion of a balloon into the left ventricle caused no significant change in the slope of the diastolic pressure-flow relationship or the pressure axis intercept. Resistance was 880 ± 80mmHg.min.g.litre^{-1} ($r > 0.98$) and P_{ZF} was 9.1 ± 2.7 mmHg ($n = 5$), i.e. similar to values previously obtained in the presence of adenosine (see Chapter 4). Increasing the balloon volume to $2cm^3$ increased resistance by 121 ± 28% ($p<0.05$, paired t-test)(Table 8.1), P_{ZF} was unchanged (Table 8.1).

8.3.2 Effect of Increased Stimulation Rate

When the stimulation rate was increased from 3 to 30 Hz resting tension increased by 298 ± 55% (Fig. 8.2). Contraction was almost abolished (Fig. 8.2). Diastolic resistance increased by 37 ± 10% ($p<0.05$, paired t-test), P_{ZF} was not significantly changed (Table 8.1).

8.3.3 Effect of FCCP

On administration of FCCP (1×10^{-6} mol.litre^{-1}) the developed tension of the isolated rabbit heart fell rapidly to zero.

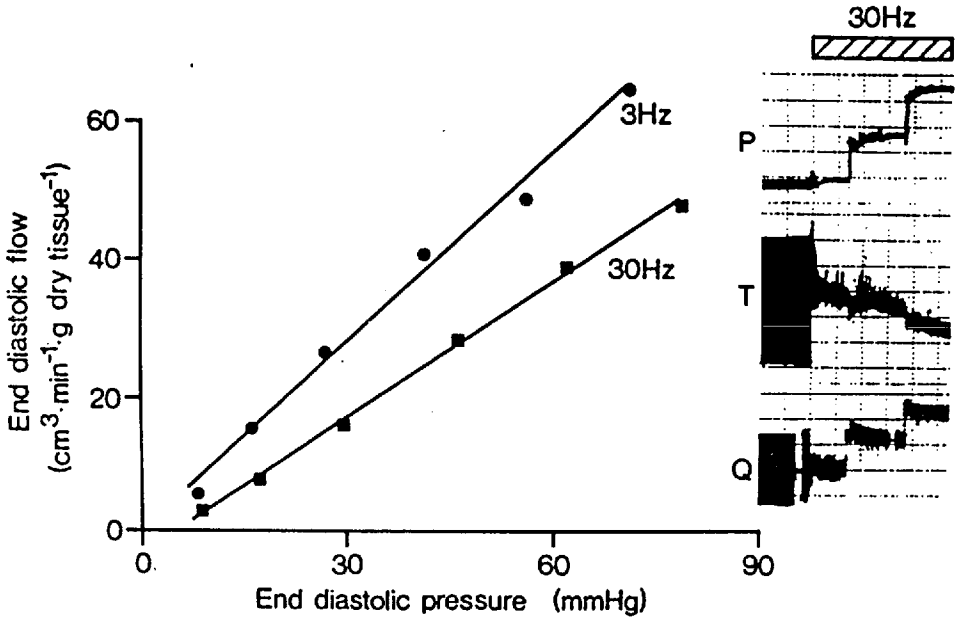


Figure 8.2 Example of the effect of increasing stimulation rate from 3 to 30 Hz on the relationship between end-diastolic pressure and end-diastolic flow in the isolated rabbit heart. The inset trace shows the effect of increased stimulation rate on perfusion pressure (P), tension (T) and flow (Q).

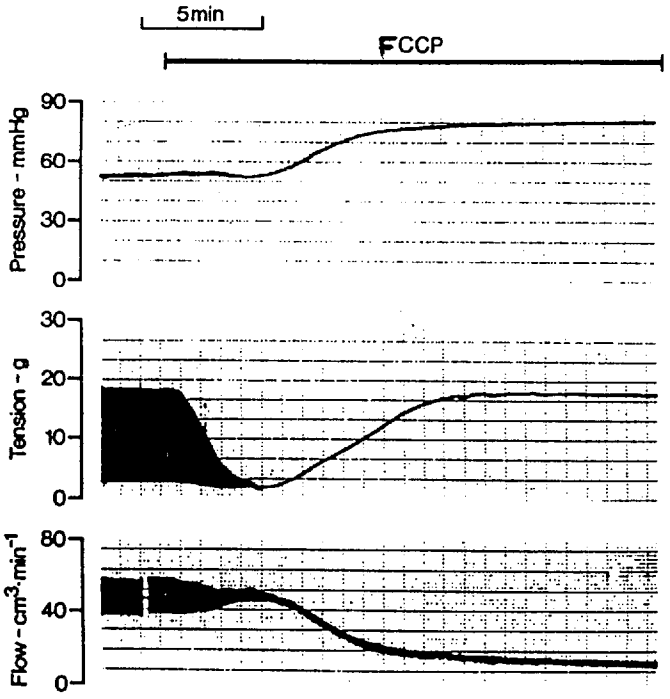


Figure 8.3 Example of the effect of FCCP (1×10^{-6} mol.litre^{-1}) on perfusion pressure (upper trace), tension (middle trace) and coronary flow (lower trace) in the isolated rabbit heart.

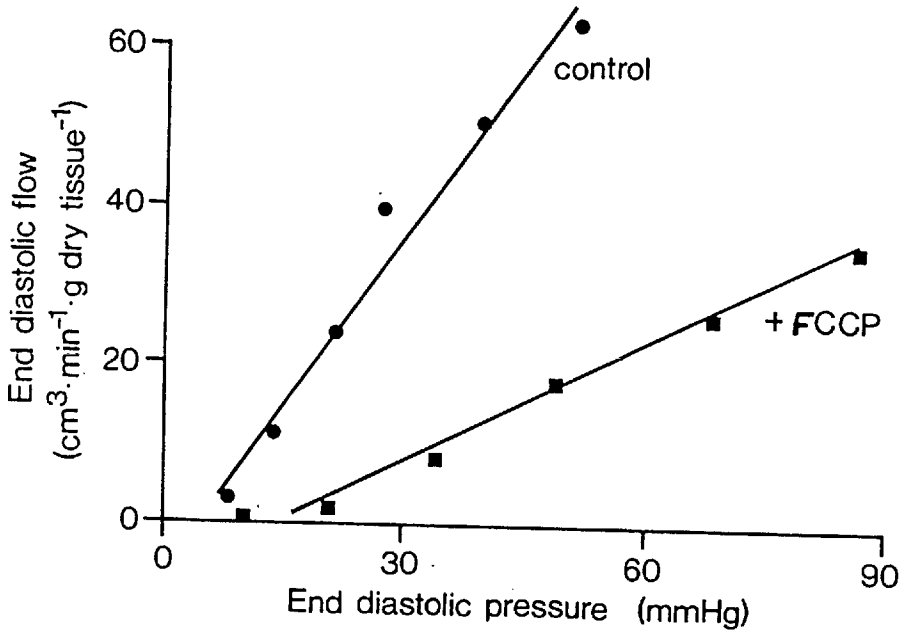


Figure 8.4 Example of the effect of FCCP (1×10^{-6} mol.litre^{-1}) on the relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated rabbit heart.

After about 5 min perfusion resting tension began to increase reaching a maximum (767 ± 98% higher than that before FCCP was added) after 15 min (Fig. 8.3). The increase in resting tension was paralleled by an increase in perfusion pressure and a decrease in coronary flow (Fig. 8.3). The plot of diastolic perfusion pressure against flow showed a decrease in slope and an increase in the pressure axis intercept (Fig. 8.4). Resistance increased by 169 ± 27% ($p<0.01$,paired t-test) (Table 8.1), P_{ZF} increased by 11.8 ± 1.6 mmHg ($p<0.01$,paired t-test)(Table 8.1). Administration of adenosine (1.87×10^{-5} mol.litre^{-1}) exacerbated the increase in resistance caused by FCCP (Table 8.1).

8.4 Discussion

8.4.1 Increased Left Ventricular Pressure

If a fluid filled balloon is used to measure or alter left ventricular pressure it is important that the balloon should occupy the whole of the ventricular space. To ensure that this was so a selection of balloons were made and a balloon a little larger than the left ventricular volume used in each experiment. Before determination of the control pressure-flow relationship the balloon was inflated until it just filled the left ventricular cavity, i.e. when myocardial pressure was fully transmitted to the fluid within the balloon. Care was taken to maintain left ventricular diastolic pressure below 10 mmHg. The adenosine in the perfusate caused maximal vasodilation (see Chapter 4) and hence maximised the effect of increased intramyocardial pressure on coronary flow. Increasing the volume of the fluid within the balloon increased left ventricular pressure and hence increased

TABLE 8.1 EFFECT OF PARAMETERS WHICH INCREASE EXTRAVASCULAR FORCES ON RESISTANCE TO CORONARY FLOW

control		After Intervention		n
resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	
Increasing left ventricular balloon volume to 2cm^3				
880 ± 80	9.1 ± 2.7	1930 ± 290*	7.9 ± 3.6	5
Increasing stimulation frequency from 3 to 30 Hz				
1150 ± 190	-6.5 ± 3.6	1540 ± 210	0.5 ± 1.9	5
Addition of FCCP (1 x 10^{-6}mol.litre^{-1}) to perfusate				
880 ± 70	1.1 ± 1.4	2370 ± 320*	12.9 ± 1.2**	5
Addition of adenosine (1.87 x 10^{-5}mol.litre^{-1}) before FCCP				
660 ± 100	9.8 ± 1.4	4830 ± 720**	17.1 ± 0.8*	5

* significantly different from value before intervention * = $p<0.05$ ** = $p<0.01$

intramyocardial pressure. In these experiments this resulted in an increase in resistance with no change in P_{ZF} which is consistent with the increase in intramyocardial pressure being greater towards the endothelium and increasing the transmural resistance gradient. Other workers have observed a parallel shift, i.e. an increase in P_{ZF} with no change in resistance, (Kjekshus, 1973; Ellis and Klocke, 1979) or no change (Archie and Brown, 1974) in the pressure-flow relationship when preload was increased. The results were obtained in vivo and it has been proposed that the parallel shift in the pressure-flow curve was due to the concurrent increase in right atrial pressure rather than a direct effect of the increase in left ventricular pressure (Ellis and Klocke, 1979). Raised right atrial pressure increases coronary venous pressure which has been shown to increase P_{ZF} without affecting the slope of the pressure-flow relationship (Bellamy et al., 1980). Right atrial pressure was unaffected by inflating a balloon within the left ventricle of the isolated rabbit heart and the effects observed were therefore a direct result of increasing left ventricular pressure i.e. an increase in coronary resistance caused by an increase in the transmural resistance gradient.

8.4.2 Stimulation Rate

Tone in the isolated extramural rabbit coronary strip is increased by increasing the rate of stimulation from 3 to 30 Hz (Nakayama et al., 1978) but the voltage required to initiate this contraction is of the order of 5 $V.cm^{-1}$.This was only achieved in the small area between the stimulating electrodes in the isolated

rabbit heart. Increasing the stimulation rate can therefore be assumed to have no direct effect on coronary smooth muscle tone in the experiments described in this chapter. Fibrillation has been shown to increase intramyocardial pressure (Baird et al., 1975; Downey et al., 1979). The mechanism by which this occurs is unknown but it is not due to an increase in radial stress as the effect persists when the ventricle is vented (Downey et al.,1979).In the isolated rabbit heart increasing the stimulation frequency caused a small increase in both resistance and P_{ZF}. If intramyocardial pressure was increased equally across the ventricular wall a parallel rightward shift in the pressure-flow curve would occur. The effect observed is more in accordance with a greater increase in intramyocardial pressure occurring within the endocardium resulting in an increase in the transmural resistance gradient. Downey and his co-workers (Downey et al., 1979) have measured the flow in different layers within the myocardium during fibrillation and have observed a greater reduction in flow in the endocardium. Thus it appears that increasing the heart-rate of the isolated rabbit heart to 30 Hz results in an increase in intramyocardial pressure which is greater in the endocardium than in the epicardium and hence increases coronary resistance.

8.4.3 FCCP

Carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) is a metabolic uncoupler which increases the proton permeability of the mitochondrial membrane and dissipates the protonic electrochemical gradient, thus uncoupling respiration from ATP synthesis (Williamson, 1979). Uncouplers also promote a glycolytic "energy leak" (McDonald

and MacLeod, 1973) and are thought to bind non-specifically to proteins, peptides and cytochromes and have different solubilities in aqueous and lipid cellular compartments (Rose et al., 1977). The rapid development of contracture in the isolated rabbit heart exposed to FCCP could be attributed to the development of rigor bands caused by the lack of ATP (see Chapter 6), however rundown of other reactions dependent on ATP may contribute to the effect and other less specific actions of the uncoupler may be involved.

FCCP has no effect on the basal tone of an isolated epicardial coronary segment from the rabbit and relaxes segments previously contracted with potassium chloride (see Appendix 1). This inability to produce rigor probably reflects the low energy requirement of vascular smooth muscle, or a reduced permeability to FCCP. The reduction in flow and increase in perfusion pressure seen in the isolated rabbit heart perfused with FCCP must therefore be due to an increase in extravascular forces rather than vasoconstriction. The failure of adenosine to reduce the resistance also indicates involvement of intramyocardial rather than vascular factors.

FCCP increased the resistance and raised the minimum pressure required for flow (P_{ZF}) of the isolated rabbit heart. This effect cannot be due to greater compression of the vessels resulting in an increase in the height of the vascular waterfall as this would result in an increase in P_{ZF} with no change in resistance. The substantial contracture produced in these experiments probably alters the interaction between adjacent cardiac muscle fibres. Vessels running between these fibres will become kinked or totally occluded and hence resistance to flow through them will be increased. As well as increasing shear strains the contracture may have a greater effect in

the endocardium and increase the transmural resistance gradient which would also increase resistance.

8.4.4 Conclusions

The results obtained in the experiments described in this chapter seem at first to be incompatible with control of coronary flow by a vascular waterfall as this theory predicts that increasing extravascular pressure will increase P_{ZF} whilst leaving the slope of the relationship between perfusion pressure and flow unchanged. However, when the effects of the interventions in the different layers of the myocardium are considered neither the effects of increasing left ventricular pressure or stimulation rate are at variance with this theory. Experiments with FCCP represent an extreme situation where factors which normally control coronary flow may no longer do so.

The results suggest that extravascular forces can and do contribute to the resistance to flow through the coronary bed of the isolated rabbit heart. Any intervention which alters intramyocardial forces will affect coronary perfusion. One situation where intramyocardial forces are raised is after prolonged ischaemia. After 60 min global ischaemia (Chapter 6) coronary resistance quadrupled and resting tension increased by 1000%. Perfusion with FCCP produced a similar degree of contracture but only doubled coronary resistance. Increased extravascular forces therefore contributed to the increase in coronary resistance but were not suffient to account for the whole of the increase.Other factors must therefore be involved.

After 15 min global ischaemia there was little change in resting tension but coronary resistance increased (CHapter 6).Intramyocardial pressure

was only slightly changed and the increase in resistance to flow was probably due to vascular constriction.

CHAPTER 9

VASOCONSTRICTION AND CORONARY RESISTANCE

9.1 Introduction

There are two pathological situations in which active coronary constriction can be cited as a cause of myocardial injury. In the first - coronary artery "spasm",active constriction is the primary cause of myocardial ischaemia. In the second - the no reflow phenomenon,the increase in coronary resistance occurs as a result of ischaemia but may also be the cause of further myocardial damage.

9.1.1 Coronary "Spasm"

Myocardial ischaemia is usually considered to be due to narrowed coronary vessels which cannot dilate in response to increased myocardial oxygen requirements as a consequence of atherosclerosis. There is, however, increasing evidence that some cases of myocardial ischaemia are the result of coronary constriction. Vasospasm has long been postulated as a possible cause of acute coronary artery obstruction (e.g. Latham, 1876; Prinzmetal et al., 1959) but the almost universal presence of atherosclerotic lesions at autopsy led to widespread acceptance that no mechanism other than impaired function due to atherosclerosis was required to explain myocardial

ischaemia. There are isolated autopsy reports where no atherosclerotic lesions were found (e.g. Leary, 1935; Bulkley et al., 1978) and El-Maraghi and Sealey (1980) recorded persistent spasm of the left anterior descending artery of a 25 year old man 16 hours after death.

Development of non-invasive techniques such as arteriography have enabled the coronary vasculature to be observed during anginal attacks and have revealed coronary constriction to be the primary cause of ischaemia in Prinzmetal's variant angina (e.g. Oliva et al., 1973; Chahine et al., 1975) and to be involved in some cases of exertional angina (Yasue et al., 1979), unstable angina and myocardial infarction (Maseri et al., 1978).

Coronary spasm usually affects the main coronary arteries and therefore has a large effect on the resistance to flow through an extensive area of myocardium. As the constriction is proximal to the resistance arterioles,where functional vasodilation occurs,the normal response of the myocardium to inadequate perfusion, i.e. vasodilation, is ineffective. In any given patient spasm is usually localised to one major vessel and often repeated attacks occur at the same location. There are however reports of multivessel involvement (e.g. Higgins et al., 1976; El-Maraghi and Sealey, 1980). Chahine and associates (1975) recorded that of the 45 instances of coronary spasm described in the literature 78% occurred in the right coronary artery.

The cause of coronary spasm is unknown. In susceptible patients a similar constriction to that normally observed during variant angina can be induced with ergonovine (Curry et al., 1977), methacholine (Endo et al., 1976) or concurrent administration of propranolol and adrenaline (Yasue et al., 1976). Immersion of a hand in iced water -

the cold pressor test (which induces generalised systemic reflex sympathetic vasoconstriction [Mudge et al., 1976]) has also been used to evoke coronary artery constriction (e.g. Raizner et al., 1980). It has been reported (Yasue et al., 1978) that hyperventilation during intravenous infusion of Tris-buffer induces coronary constriction. In patients with a history of variant angina this procedure which increases arterial pH and reduces blood PCO_2 induced coronary spasm. It has also been suggested that ecosanoids are involved in the pathogenesis of coronary artery spasm (Theroux et al., 1982). The diverse properties of the agents which have been shown to induce constriction in susceptible patients suggests that the response is due to some defect in vascular physiology rather than an inappropriate response to stimulation of a specific receptor.

9.1.2 The No-Reflow Phenomenon

Several groups have reported an increase in resistance to flow through the coronary circulation after myocardial ischaemia. In the most severe cases no reperfusion of the ischaemic area occurs. After prolonged ischaemia this increase in resistance is probably due to contracture of the myocardium restricting blood flow in the coronary vessels passing through it (Apstein et al., 1977; Humphrey et al., 1981; Chapter 8). The increase in resistance described after shorter periods of ischaemia has been attributed to oedema or cell swelling (Willerson et al., 1975; Ganote et al., 1977), vascular damage (Kloner et al., 1980) or occlusion of capillaries by erythrocytes (Krug et al., 1966). This increase in coronary resistance could also be due to altered vascular reactivity resulting in vascular constriction (Ku,

1982; Chapter 7).

In this chapter the effect of coronary constriction on the diastolic coronary resistance of the isolated rabbit heart is investigated. Vasopressin and an increased perfusate K^+ concentration are used to increase coronary resistance. Extracellular K^+ concentration has been shown to increase during ischaemia (Weiss and Shine, 1982) and the possibility that this rise in K^+ concentration is responsible for increased coronary resistance after ischaemia is examined.

9.2 Experimental

9.2.1 Determination of the Effect of Vasopressin

Rabbit hearts were perfused by the non-recirculating pressure controlled Langendorff method described in Chapter 3. After 20 min stabilisation the pressure-flow relationship was determined by decreasing the hydrostatic pressure in steps and recording the resultant changes in perfusion pressure and coronary flow. Systolic perfusion pressure was then set to 50 mmHg and after 5 min equilibration the perfusate was changed for one containing vasopressin (0.5 I.U. $litre^{-1}$). Perfusion was continued for 5 min with this solution before the pressure-flow relationship was redetermined.

9.2.2 Determination of the Effect of Increased Perfusate K^+ Concentration

Rabbit hearts were perfused from an elevated reservoir as

described in Chapter 3. After twenty minutes perfusion the pressure-flow relationship of the coronary bed was determined by altering the height of the reservoir above the heart and recording the resultant changes in perfusion pressure and coronary flow. The systolic perfusion pressure was then raised to 50 mmHg and after 5 min perfusion with a "high potassium" solution was initiated. The "high potassium" solution was perfusate in which the K^+ concentration had been increased to 19.2 $mmol.litre^{-1}$ by addition of KCl. The NaCl concentration of this solution was reduced by an equivalent amount to prevent changes in the Cl^- ion concentration and ionic strength. Perfusion with the "high potassium" solution was continued for 15 min before the relationship between perfusion pressure and coronary flow was redetermined.

In one group of experiments adenosine ($1.87 \times 10^{-5} mol.litre^{-1}$) was added to the perfusate (and the diastolic pressure-flow relationship redetermined) before the K^+ concentration was increased.In another group adenosine ($1.87 \times 10^{-5} mol.litre^{-1}$) was added to the perfusate after 15 min perfusion with the "high potassium" solution. Perfusion was continued for 5 min before the pressure-flow relationship was redetermined.

9.2.3 Determination of Extracellular K^+ Concentration During Total Global Ischaemia and Hypoxia

Rabbit hearts were perfused by the pressure controlled Langendorff method described in Chapter 3. Extracellular K^+ concentration was recorded with valinomycin K^+ sensitive electrodes (Webb et al., 1983) which were inserted into the ventricular wall. After a 20 min

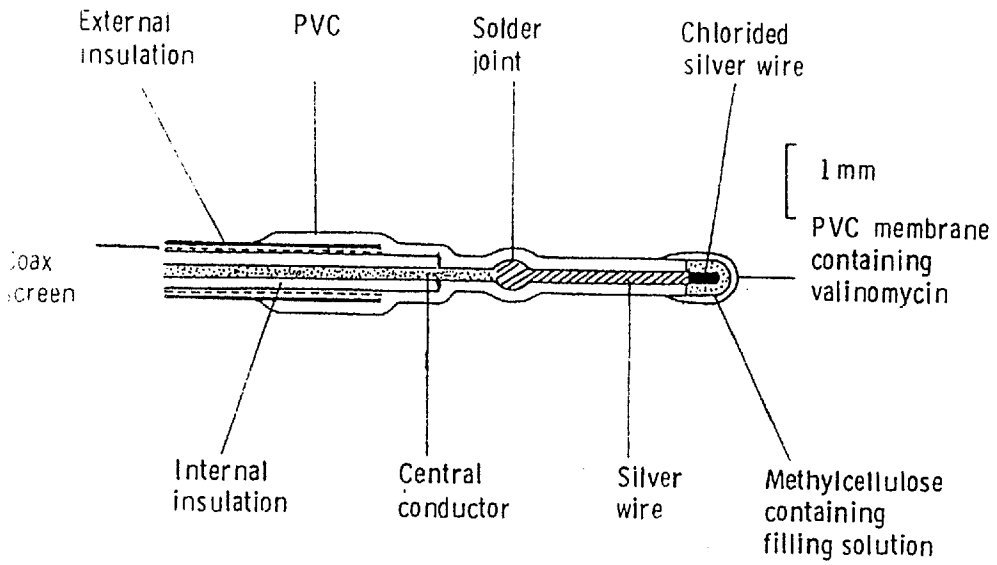


Figure 3.1 Construction of the K^+ electrode.

stabilisation period perfusion was stopped for 15 or 60 min followed by 30 min reperfusion or the heart was perfused with a solution gassed with 95% N_2,5% CO_2 in which the glucose had been replaced by mannitol for 10 min. Tension and extracellular K^+ concentration were recorded.

Needle type K^+ sensitive electrodes (Fig. 9.1) were constructed using methods similar to those previously described for measuring tissue pH (Cobbe and Poole-Wilson, 1980). A short length of 0.25 mm silver wire was bared, chlorided by electrolysis and then coated with a thin layer of polymethyl cellulose. This was allowed to absorb a solution of 125 mmol KCl saturated with silver chloride to act as an internal electrolyte. A potassium sensitive membrane was then formed over the tip by dipping in a solution of valinomycin (0.542%), tetrabenzene (18.0%), dioctyl adipate (54.2%) and polyvinyl chloride (27.258%) in tetrahydrofuran. The tip diameter of the electrodes was approximately 0.5 mm.

The electrodes responded to changes in K^+ concentration and temperature as predicted from the Nernst equation with a linear output of between 57.5 and 60.5 mV per decade change at 26°C and 0.3 mV per °C respectively. They had a 95% response time of less than 300 msec and were shown to be insensitive to changes in pO_2, pCO_2, pH, osmolality or the concentration of sodium, calcium and magnesium ions. The electrodes were calibrated before and after each experiment in solutions containing 140 mmol.litre^{-1} NaCl and known concentrations of KCL between 2 and 64 mmol.litre^{-1}. Measured activities were expressed relative to the concentrations of these standards.

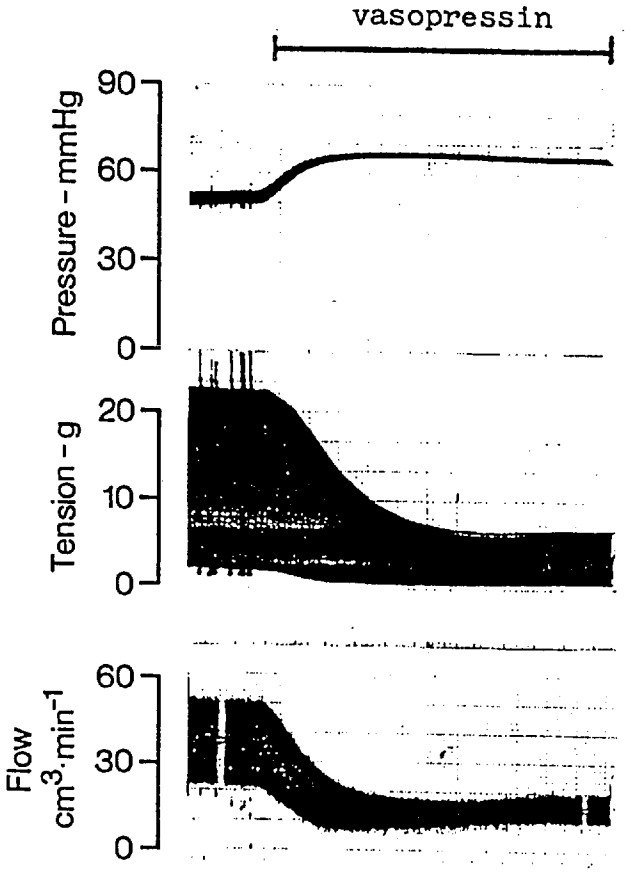


Figure 9.1 Example of the effect of vasopressin (0.5 I.U.litre^{-1}) on the perfusion pressure (upper panel), tension (middle panel) and coronary flow (lower panel) in the isolated rabbit heart.

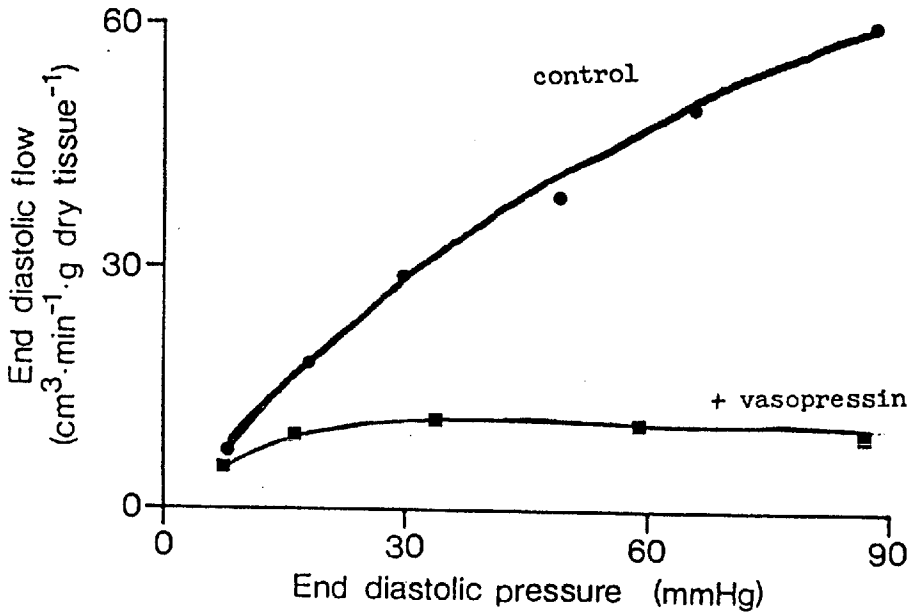


Figure 9.3 Example of the effect of vasopressin (0.5 I.U.litre^{-1}) on the relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated rabbit heart.

9.2.4 Materials

Adenosine was dissolved in distilled water to give a stock solution of 1.87×10^{-2} mol.litre^{-1}. Vasopressin was obtained in ampoules containing 20 I.U. per cm^3. This solution was diluted with distilled water to give a stock solution containing 0.5 I.U.per cm^3.

9.3 Results

9.3.1 Effect of Vasopressin

When vasopressin (0.5 I.U.litre^{-1}) was added to the perfusate there was an increase in perfusion pressure of 50.7 $\pm$ 9.5% and a decrease in diastolic flow of 52.4 $\pm$ 5.4% (n = 4). Developed tension fell to 53.5 $\pm$ 4.0% of that before the intervention and resting tension was reduced by 1.5 $\pm$ 0.3 g (Fig. 9.2).

The relationship between diastolic perfusion pressure and flow was linear before vasopressin was added ($r > 0.93, n = 4$). End-diastolic resistance was 1140 $\pm$ 170 mmHg.min.g.litre^{-1} and P_{ZF} was -2.6 $\pm$ 2.1 mmHg. In the presence of vasopressin little change in diastolic flow occurred as perfusion pressure was altered (Fig. 9.3) until the pressure was reduced below 25 mmHg.

9.3.2 Effect of Increased Perfusate K^+ Concentration

Increasing the K^+ concentration of the perfusate of the

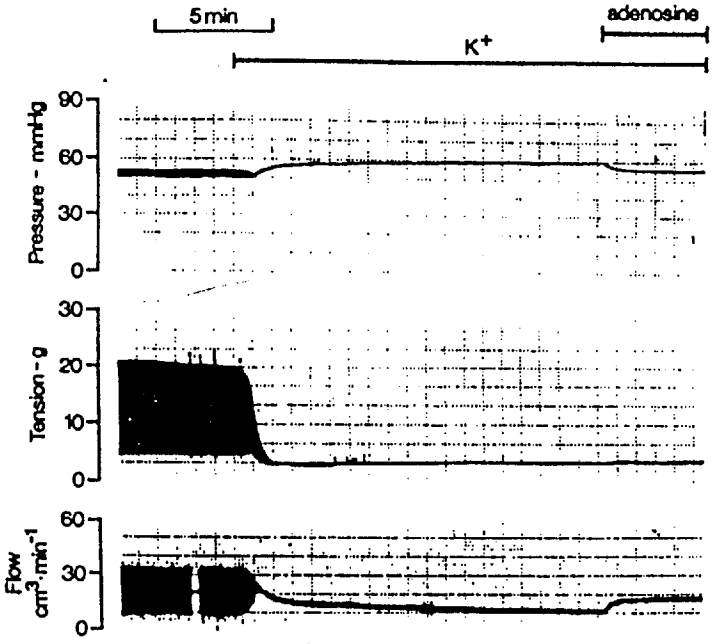


Figure 9.4 Example of the effect of increasing the perfusate K^+ concentration from 5.9 to 19.2 mmol.litre^{-1} on perfusion pressure (upper panel), tension (middle panel) and coronary flow (lower panel) in the isolated rabbit heart. This example also shows that the effect on perfusion pressure and flow can be partly reversed by administration of adenosine (1.87×10^{-5}mol.litre^{-1}).

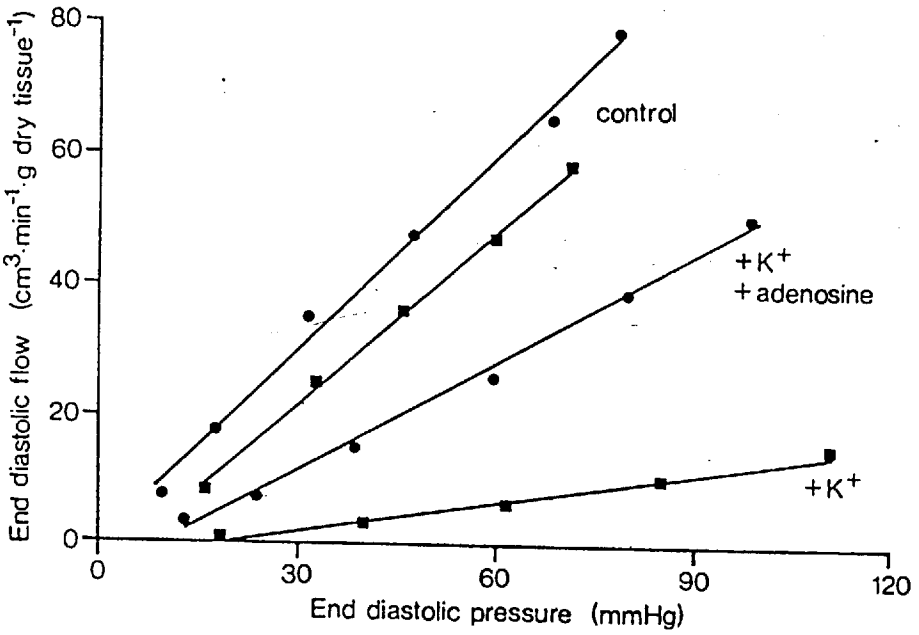


Figure 9.5 Example of the effect of increased perfusate $[K^+]$ on the relationship between end-diastolic pressure and end-diastolic flow in the isolated rabbit heart and the ability of adenosine (1.87×10^{-5} mol.litre^{-1}) to reverse this effect.

TABLE 9.1 EFFECT OF PERFUSION WITH A 'HIGH POTASSIUM' SOLUTION ON THE DIASTOLIC RESISTANCE TO FLOW IN THE ISOLATED RABBIT HEART

control		'High Potassium'		n
resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	resistance (mmHg.min.g.l^{-1})	P_{ZF} (mmHg)	
1570 ± 120	2.8 ± 4.6	7960 ± 1670†	15.1 ± 3.3	5
Adenosine (1.87 x 10^{-5}mol.litre^{-1}) added before $[K^+]$ increased				
520 ± 50*	5.4 ± 0.9	1220 ± 130†*	9.9 ± 0.7†	5
Adenosine (1.87 x 10^{-5}mol.litre^{-1}) added 15 min after $[K^+]$ increased				
1030 ± 200	2.7 ± 2.6	2680 ± 390†	13.5 ± 1.3†	5

† = significantly different ($p \leq 0.01$) from value before perfusate K^+ concentration increased
* = significantly different ($p \leq 0.01$) from equivalent value in the absence of adenosine

Langendorff preparation from 5.9 to 19.2 mmol.litre^{-1} resulted in an increase in perfusion pressure of 40.8 ± 5.3% and a decrease in flow of 75.3 ± 5.6% (n = 5). Developed tension fell to zero and no contracture was seen (Fig. 9.4). Resistance was increased by 336 ± 74% ($p < 0.05$ paired t-test)(Fig. 9.3, Table 9.1). P_{ZF} was not significantly changed (Table 9.1). If adenosine (1.87×10^{-5} mol.litre^{-1}) was given before the perfusate K^+ concentration was increased the increase in resistance observed was reduced to 135 ± 17% ($p < 0.01$ paired t-test)(Fig. 9.5, Table 9.1). If adenosine was given 15 min after the K^+ concentration had been increased the increase in resistance was reduced to 195 ± 53% ($p < 0.05$ paired t-test) (Fig. 9.4, Table 9.1). In the presence of adenosine (1.87×10^{-5} mol.litre^{-1}) increasing the K^+ concentration resulted in an increase in P_{ZF} of 4.5 ± 0.8 mmHg ($p < 0.01$ paired t-test)(Table 9.1).

9.3.3 Extracellular K^+ Concentration

Immediately after insertion into the myocardium the K^+ electrode recorded an extracellular K^+ concentration of between 15 and 25 mmol.litre^{-1}. By the end of the 20 min stabilisation period the K^+ concentration recorded by the electrode was equal to that in the perfusate. The initial high K^+ concentration was attributed to local tissue damage caused by the insertion of the electrode.

During a subsequent 90 min of aerobic perfusion measured extracellular K^+ concentration remained within 0.15 mmol.litre^{-1} of that in the perfusate.

In the experiments where perfusion was stopped (n = 10)

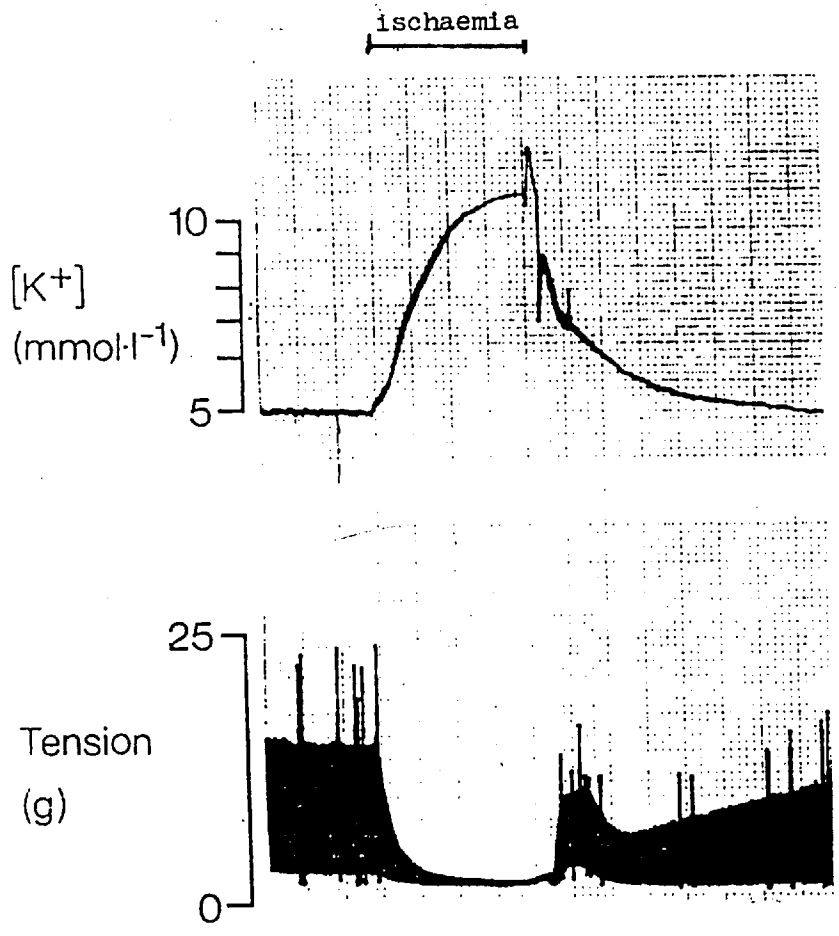


Figure 9.6 Example of the effect of 15 min total global ischaemia and subsequent reperfusion on the extracellular K^+ concentration in the isolated rabbit heart.

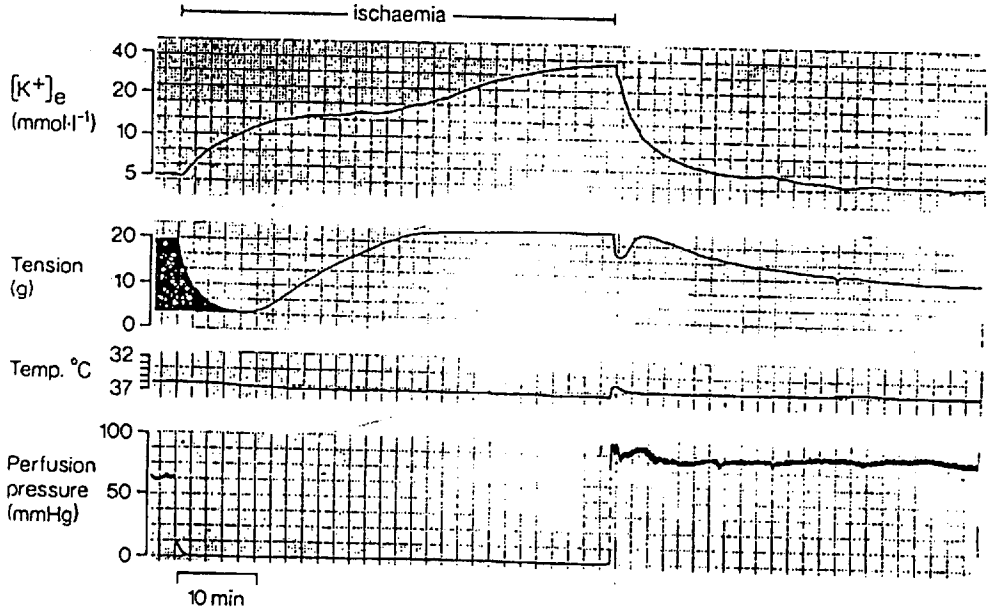


Figure 9.7 Example of the effect of 60 min total global ischaemia and reperfusion on the extracellular K^+ concentration in the isolated rabbit heart.

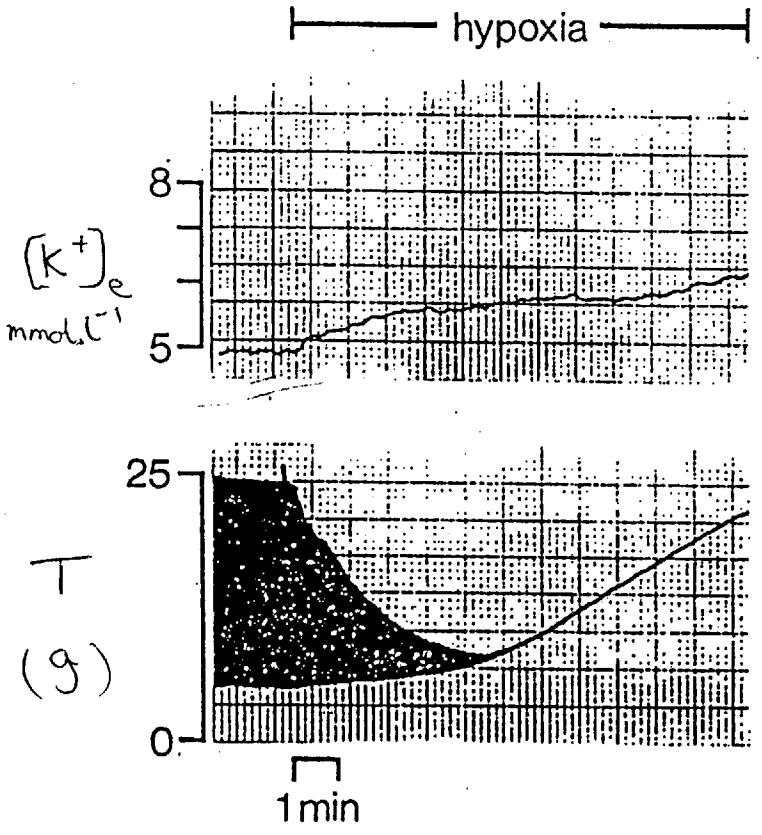


Figure 9.8 Example of the effect of hypoxia on the extracellular K^+ concentration in the isolated rabbit heart.

TABLE 9.2 EFFECT OF TOTAL GLOBAL ISCHAEMIA ON EXTRACELLULAR K^+ CONCENTRATION IN THE ISOLATED RABBIT HEART

Length of ischaemic period (min)	Extracellular $[K^+]$ mmol.litre^{-1}	n
10	$13.9 \pm 1.3^*$	10
20	$14.9 \pm 1.6^*$	5
30	$15.7 \pm 1.0^*$	5
40	$17.3 \pm 1.8^*$	5
60	$20.1 \pm 3.0^*$	5

* = significantly different ($p \leq 0.01$) from K^+ concentration of the perfusate

extracellular K^+ concentration rose within 15 s and was usually followed by a triphasic pattern of K^+ accumulation ,there was an initial rapid rise in the extracellular K^+ concentration which reached 13.9 $\pm$ 1.3 mmol.litre^{-1} after 10 min ischaemia (Fig. 9.6, Table 9.2). The extracellular K^+ concentration remained close to this level for the next 10 - 15 min before rising once more to reach 20.1 $\pm$ 3.0 mmol.litre^{-1} after 60 min ischaemia(Fig. 9.7, Table 9.2). On reperfusion the extracellular K^+ concentration fell rapidly (Fig. 9.6, 9.7) and had reached between 5 and 12 mmol.litre^{-1} after 30 min reperfusion.

Extracellular K^+ concentration rose within 30 s of the initiation of hypoxic perfusion and after 10 min had reached 6.1 $\pm$ 0.4 mmol.litre^{-1} (n = 5) (Fig. 9.8).

9.4 Discussion

9.4.1 Vasopressin and Coronary Constriction

Vasopressin (antidiuretic hormone) is an octapeptide which is secreted by the posterior lobe of the pituitary (du Vigneaud et al., 1954). Its major function in vivo is homeostatic control of the osmotic pressure of the body fluids but it also produces vasoconstriction. The pressor effect of vasopressin on the cardiovascular system was first reported by Oliver and Schafer (1895). Vasopressin may cause severe coronary constriction; when used clinically for treatment of diabetes incipidus cases of angina and even death have been reported (Slotnick and Teigland, 1951).

In the isolated rabbit heart vasopressin caused severe coronary

constriction and promoted an autoregulatory response. The decline in mechanical function after vasopressin was given indicates that the level at which flow was regulated was inadequate and that ischaemia was present. This suggests that the constriction induced by vasopressin overrides the mechanisms that control coronary resistance in response to myocardial demands possibly, as in coronary spasm, by constricting the major coronary arteries.

9.4.2 K^+ and Coronary Constriction

Small increases in plasma K^+ concentration cause vasodilation (Brace et al., 1974; Gellai and Detar, 1974; Murray and Sparks, 1978) and K^+ has been proposed as a mediator of the coronary dilation produced in response to increased myocardial work (Chapter 2). If coronary vessels are exposed to a K^+ concentration greater than 15 mmol.litre^{-1} however the vascular smooth muscle cell membrane becomes depolarised, calcium entry into the cell is augmented and contraction occurs (Bohr, 1964; Nakayama et al., 1978; Appendix 1). The increased K^+ concentration may also cause efflux of noradrenaline from nerve terminals which further enhances the contraction (Borda et al., 1977).

In the presence of 20 mmol.litre^{-1} K^+ the myocardium became quiescent. There was no alteration of myocardial resting tension so the increase in coronary resistance observed in the isolated rabbit heart must be solely due to an increase in vascular tone. This conclusion is supported by observation that adenosine, in a dose which has been shown to maximally dilate this preparation, was able to partly prevent or reverse the increase in coronary resistance

caused by increasing the K^+ content of the perfusate. The inability of adenosine to totally prevent the increase in resistance is probably a consequence of the change in membrane potential caused by raising the extracellular K^+.

9.4.3 Extracellular K^+ in Ischaemia

It has long been known that the K^+ concentration of coronary venous blood increases dramatically after coronary artery ligation (Dennis and Moore, 1938; Harris, 1966). Recent development of small K^+ sensitive electrodes has enabled the extracellular K^+ concentration within the myocardium to be measured. These electrodes have been used to record extracellular K^+ concentration in vivo after coronary ligation in the pig (Hill and Gettes, 1980; Hirche et al., 1980) and dog heart (Weigand et al., 1979) and in vitro in the interventricular septum of the rabbit (Weiss and Shine, 1982). The results from these studies are similar to those obtained in the isolated rabbit heart,i.e. an initial rise in extracellular K^+ concentration on initiation of ischaemia which reaches a plateau after 10 - 20 min followed by a secondary rise which corresponds to the development of myocardial contracture and the onset of irreversible damage. The primary rise in K^+ concentration during ischaemia is not caused by acidosis or catecholamine release but may be the result of substrate depletion (Weiss and Shine, 1982).

Continued perfusion during hypoxia limited extracellular K^+ accumulation. Although there was a small rise in K^+ concentration this was insufficient to cause coronary constriction and the increase in coronary resistance described during hypoxia (Chapter 6) could not

have been due to an increase in K^+ concentration.

The rapid decline in extracellular K^+ concentration observed on reperfusion after 15 or 60 min ischaemia was also seen by Weiss and Shine (1982) in the interventricular septum. During the ischaemic period extracellular K^+ concentration was sufficient to induce coronary constriction, however, as the vasoconstriction produced by K^+ is readily reversible (Appendix 1) and extracellular K^+ concentration declined to control levels within 10 min of reperfusion the increase in coronary resistance after ischaemia described in Chapter 6 could not have been caused by K^+ mediated constriction.

9.4.4 Vasoconstriction and Coronary Resistance

Constriction of the coronary vessels of the isolated rabbit heart caused a large increase in resistance to flow. The increase in diastolic coronary resistance produced by vasopressin or increased perfusate K^+ was greater than that seen after 15 or 60 min total global ischaemia (Chapter 6). It is therefore possible that the increase in resistance after ischaemia could have been produced by active vasoconstriction. Extracellular K^+ concentration increases during total global ischaemia and reaches concentrations equivalent to those which have been shown to produce vasoconstriction. However, K^+ cannot be the cause of increased coronary resistance on reperfusion after ischaemia because extracellular K^+ concentration decline to normal within 10 min of reperfusion whilst coronary resistance remains elevated.

CHAPTER 10

GENERAL DISCUSSION

10.1 The Experimental Preparation

The Langendorff preparation has been widely used to study the effects of pharmacological interventions on the myocardium (e.g. Langendorff, 1895). In the experiments described in this thesis I have attempted to use this preparation to assess the effects of interventions on the coronary vasculature and to investigate the effects of alterations in myocardial function on coronary resistance. The majority of studies of coronary function have been carried out in the open chested dog (e.g. Mosher et al., 1964; Rouleau et al., 1979; Sherman et al., 1980; Eng et al., 1981; Kirkeede et al., 1981). This model has the advantage of being suitable for chronic studies, has normal innervation and is perfused with blood. Coronary measurements are, however, altered by changes in systemic haemodynamics. The isolated perfused heart preparation permits rigorous control of extrinsic factors which influence cardiac perfusion and metabolism and, hence, provides a suitable preparation in which cardiac and coronary function can be studied in isolation.

The apparatus used to perfuse the isolated rabbit heart allowed control of heart rate and temperature and accurate measurement of true coronary perfusion pressure and coronary flow. The use of diastolic coronary perfusion values, which were free from myocardial influence,

enabled changes in flow due to alterations in coronary tone to be distinguished from those caused by alterations in myocardial function. Changes produced by altering the hydrostatic pressure or the flow rate were therefore consequences of the altered perfusion, not a result of alterations in myocardial function. When resistance changes occurred within the coronary circulation there was a change in the pressure loss within the perfusion system and hence neither perfusion pressure nor coronary flow could be kept precisely constant.

In vivo coronary flow increases with driving pressure at pressures below 70 mmHg and above 140 mmHg. Between these two pressures autoregulation occurs with little change in flow for a large alteration in pressure. The flow at which autoregulation occurs is determined by the substrate requirements of the heart. In the isolated rabbit heart the relationship between diastolic perfusion pressure and coronary flow was almost linear over the full range of perfusion pressures tested (Chapter 4). The lack of autoregulation and the high coronary flow in this preparation probably reflected the low oxygen carrying capacity of the perfusate, i.e. almost maximum vasodilation was required to supply enough oxygen to meet metabolic requirements. The coronary vasculature was, however, not maximally dilated; addition of adenosine (Chapter 4) or nifedipine (Chapter 7) to the perfusate or initiation of hypoxia (Chapter 6) caused a decrease in perfusion pressure and an increase in flow indicating that a vasodilatory reserve existed. The absence of a vasodilatory response with nitroglycerin (Chapter 4) indicated that the large coronary arteries were fully dilated and that the vasodilatory reserve was in the small arteries and arterioles preferentially dilated by adenosine (Schnaar and Sparks, 1972). The inability of dipyridamole

to potentiate vasodilation (Chapter 4) suggested that under control conditions coronary diameter was not regulated by adenosine.

10.2 Perfusion Parameters and Tension

Measurement of resting tension in the isolated perfused heart was affected by the perfusion parameters (Chapter 5). As the perfusion pressure was increased the aorta expanded and lengthened; the resting tension measured from the apex decreased. Increasing the perfusion pressure also increased ventricular wall thickness and changed heart shape by the "garden hose effect" (Salisbury et al., 1960; Arnold et al., 1968). This resulted in an increase in heart volume,hence decreasing resting tension measured from the apex of the heart. The observed relationship between perfusion pressure and resting tension was a result of the combination of these two effects. As alterations in resting tension affect developed tension (Frank, 1895; Starling, 1918) developed tension was also determined by perfusion parameters. If contractile function had been measured with a balloon within the left ventricle the effect of alterations in aortic length would have been eliminated. The "garden hose effect" would, however, still have influenced measured tension. Alterations in coronary resistance should therefore be taken into account when the effect of a drug on myocardial function is evaluated. The changes in myocardial function produced by changes in perfusion parameters must also be taken into account after ischaemia. Ischaemia causes an increase in resistance to flow through the coronary circulation (Chapter 6). If the isolated rabbit heart is reperfused at the same flow as before ischaemia the perfusion pressure is higher than that before ischaemia and resting

tension is therefore depressed (Chapter 6). If reperfused at the same perfusion pressure as before ischaemia flow is decreased and resting tension is therefore elevated (Chapter 6). The apparent degree of contracture observed is therefore dependent on the method of reperfusion and on the increase in coronary resistance. This should be taken into account when the ability of a drug to "protect" the myocardium from ischaemic damage is assessed. Many previous studies of myocardial ischaemia and the effects of drugs have ignored these factors. The results of such studies do not necessarily reflect changes in contracture or contractility of myocardial cells, and more caution is necessary in their interpretation.

10.3 Coronary Resistance and the Minimum Pressure Required for Flow

A series of values of end-diastolic perfusion pressures and flows were used to construct pressure-flow relationships. This method avoided the errors inherent in estimation of resistance from the ratio of pressure to flow at a single point (see Chapter 4). Linear regression was applied to this series of points, the correlation coefficient was always greater than 0.90. The reciprocal of the slope of the resultant line was used as an estimate of coronary resistance. The intercept with the pressure axis was used as an estimate of the minimum pressure required for flow (P_{ZF}).

In some experiments residual autoregulation was present, evidenced by slight curvature of the pressure-flow relationship and application of linear regression resulted in underestimation of P_{ZF}. When the vasculature was maximally dilated with adenosine

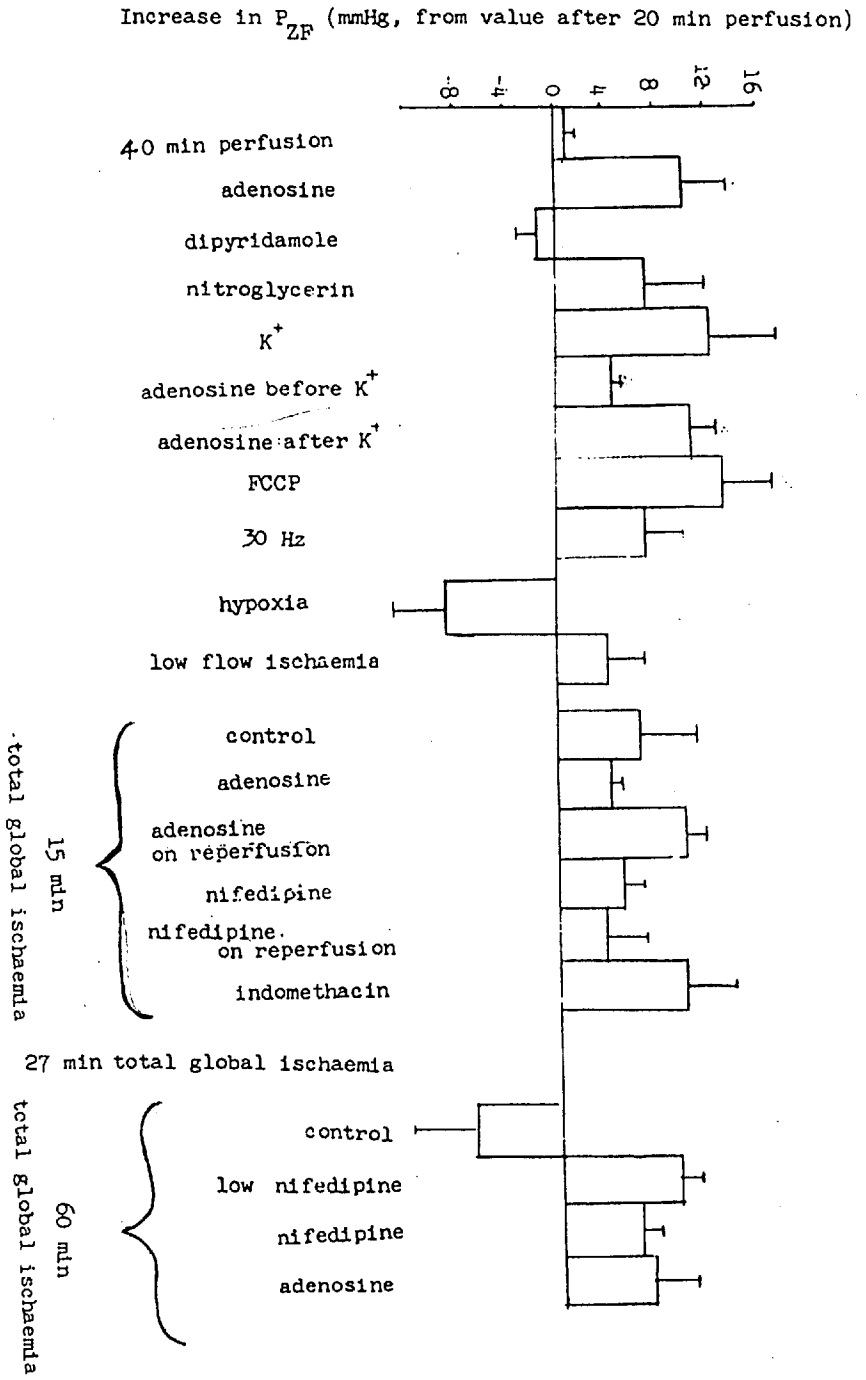


Figure 10.1 Interventions and the minimum pressure required for flow in the isolated rabbit heart. The minimum pressure required for flow was estimated by linear extrapolation of the relationship between end-diastolic pressure and end-diastolic flow to zero flow.

and the relationship was linear ($r > 0.99$) P_{ZF} was estimated to be 11.0 $\pm$ 3.9 mmHg.

Most interventions tended to increase P_{ZF} (Fig. 10.1) possibly because the relationship between pressure and flow became more linear. The increase in P_{ZF} was only significant in experiments where either adenosine, nifedipine or FCCP were present. In the former the increase in P_{ZF} was probably due to the abolition of residual autoregulation. In the latter the increase was probably indicative of an increase in intramyocardial pressure caused by myocardial contracture. When coronary resistance increases the slope of the pressure-flow curve is reduced and the error in measurement of P_{ZF} of a relationship which is slightly curved is exaggerated. This probably accounts for the apparent decrease in P_{ZF} after hypoxia and prolonged ischaemia (Fig. 10.1).

Resistance to flow increased with the length of perfusion (Chapter 4). The increase in resistance seen after a period of ischaemia, when the K^+concentration of the perfusate was increased (Chapter 9), when FCCP was administered, or when the heart was stimulated at 30 Hz (Chapter 8) was greater than that after the corrisponding perfusion period (Fig. 10.2). Administration of vasodilators such as adenosine or nifedipine reduced diastolic coronary resistance (Chapter 4, Chapter 7, Fig. 10.2).

Coronary resistance may be increased either as a consequence of contraction of the vascular smooth muscle or because the myocardium around the vessels has contracted restricting flow through them. The increase in diastolic coronary resistance produced by increasing the K^+ concentration of the perfusate occurred in the absence of an increase in resting tension (Fig. 10.3) and was partly prevented or

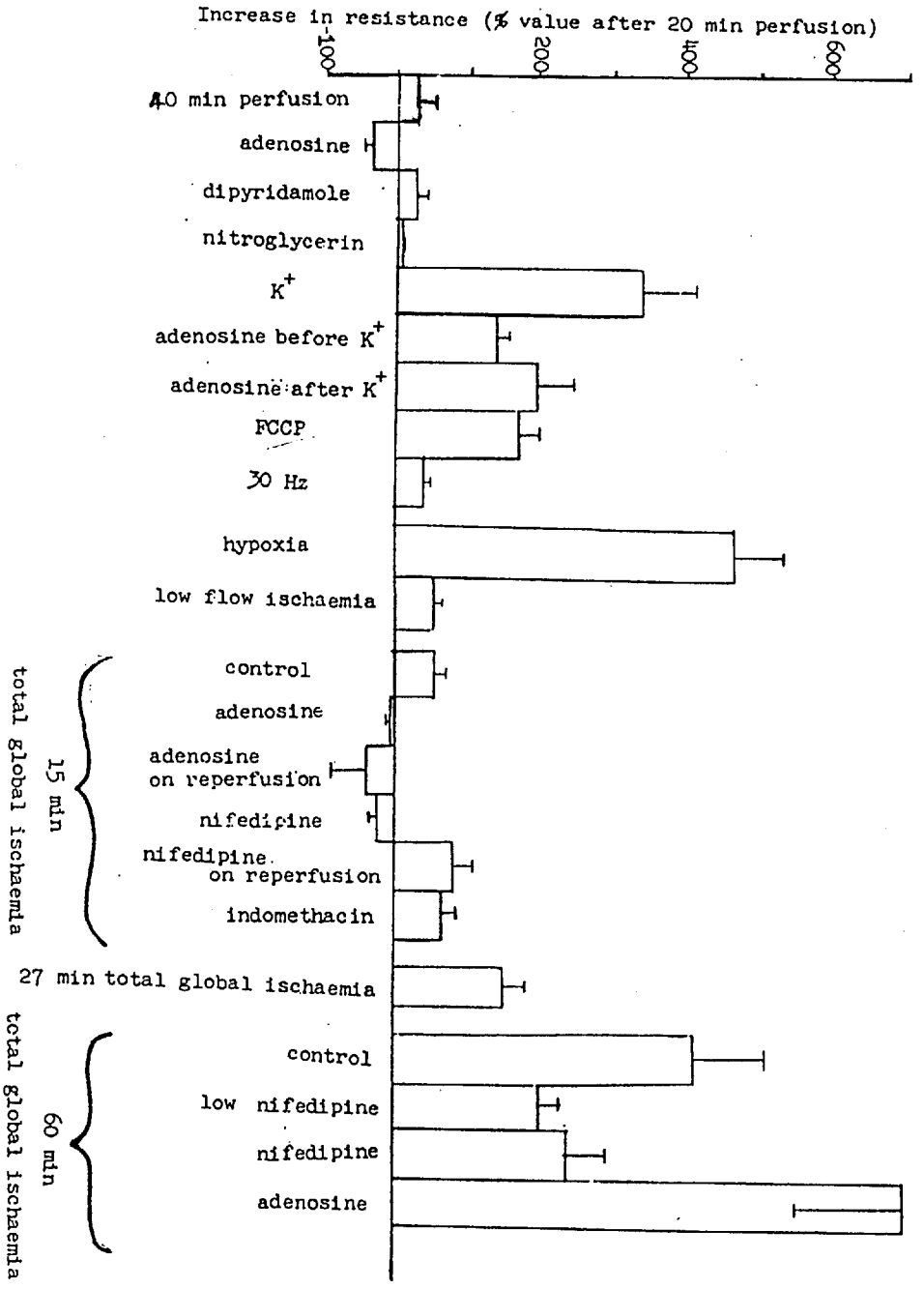


Figure 10.2 Interventions and the diastolic coronary resistance of the isolated rabbit heart. Resistance is taken to be the reciprocal of the slope of the relationship between end-diastolic pressure and end-diastolic flow.

reversed by adenosine (Fig. 10.2). This suggests that the increase in coronary resistance was due to active vasoconstriction. Stimulation at 30 Hz or administration of FCCP increased resting tension(Fig. 10.3). The resultant increase in diastolic coronary resistance was not reduced by adenosine (Chapter 8). The increase in coronary resistance was caused by the increase in intramyocardial forces.

10.4 Ischaemia and Coronary Perfusion

Resistance to flow in the coronary circulation increased during hypoxia and "low flow" ischaemia and on reperfusion after total global ischaemia (Chapter 6) (Fig. 10.2). After 15 min total global ischaemia resistance was initially reduced but flow rapidly declined and perfusion pressure increased so that by the end of the reperfusion period resistance was higher than before ischaemia (Fig. 10.3). After 60 min total global ischaemia no reactive vasodilation was seen and the increase in resistance was more severe(Fig. 10.3).

The increase in resistance after 15 min ischaemia occurred in the presence of little increase in resting tension and was partly prevented or reversed by adenosine (Chapter 6, Fig. 10.3). Contraction of the vascular smooth muscle was therefore involved in the increase in resistance. The gradual increase in resistance over the reperfusion period suggests that reperfusion promoted production of a vasoconstrictor or increased the sensitivity of the vasculature to an agent already present. Although K^+ is a potent vasoconstrictor and is present in the extracellular space during ischaemia in sufficient quantities to produce vasoconstriction (Chapter 9) the extracellular K^+ concentration declined rapidly on reperfusion

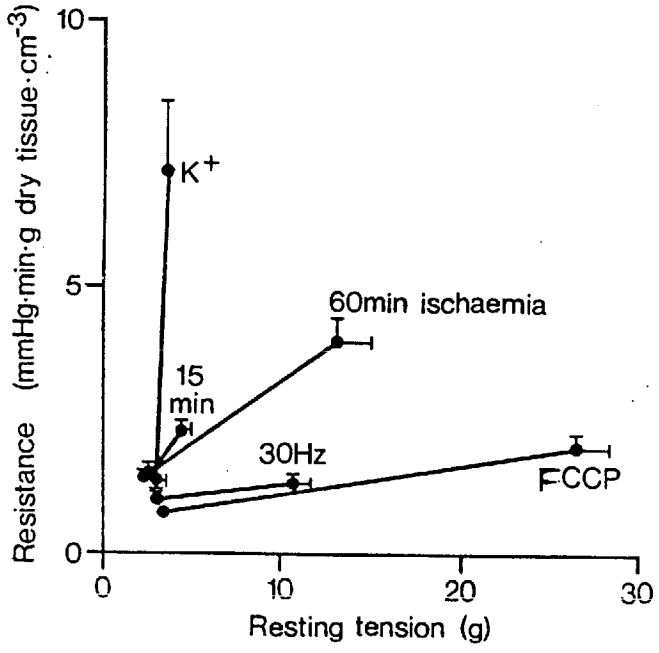


Figure 10.3 Effect of interventions on resting tension and end-diastolic coronary resistance of the isolated rabbit heart. Values plotted are the mean $\pm$ S.E. of 5 experiments before and after the intervention.

(Chapter 9) and K^+ is probably not involved in the post-ischaemic increase in resistance.

It has been suggested (Moncada and Vane, 1979) that in vivo a balance exists between the production of metabolites of arachidonic acid with vasodilatory actions and those with vasoconstriction actions. In the isolated rabbit heart administration of indomethacin slightly reduced coronary resistance suggesting that a constricting metabolite of arachidonic acid was produced under control conditions. The presence of indomethacin during ischaemia did not, however, prevent the post-ischaemic increase in coronary resistance. Constrictor metabolites of arachidonic acid were therefore unlikely to be involved.

The increase in diastolic coronary resistance during hypoxia correlated strongly with the development of contracture (Chapter 6). Measurements during "low flow" ischaemia suggested that whilst contracture contributed to the increase in resistance the primary cause was vasoconstriction (Chapter 6). There is also evidence that contracture contributed to the increase in resistance after 60 min ischaemia. After prolonged ischaemia the increase in resistance on reperfusion could not be prevented with adenosine (Chapter 7, Fig. 10.2), a mechanism other than vasoconstriction was therefore involved. The experiments with FCCP and increased stimulation frequency (Chapter 8) illustrate that contracture can cause an increase in coronary resistance (Fig. 3). The increase in coronary resistance produced by FCCP was, however, not as great as that observed after 60 min ischaemia, although the contracture developed was greater (Fig. 10.3). Thus, although contracture may contribute to the increase in resistance after prolonged ischaemia other factors

must be involved.

Reperfusion after prolonged ischaemia has been associated with oedema (Willerson et al., 1975; Ganote et al., 1977) and vascular damage (Kloner et al., 1980; Ku, 1982). Both of these factors could have contributed to the increase in resistance to flow.

In conclusion the increase in coronary resistance after short periods of ischaemia is primarily caused by active vasoconstriction. When ischaemia is more prolonged the increase in resistance to flow is enhanced by development of contracture and probably by myocardial oedema, endothelial cell swelling and vascular damage.

10.5 Coronary Perfusion and Myocardial Function After Ischaemia

Ischaemia caused a decline in myocardial contraction which was only partly restored on reperfusion. Even when the increase in coronary resistance after ischaemia was prevented with vasodilators there was no improvement in mechanical recovery (Chapter 7). Impaired mechanical function is thus not a simple consequence of inadequate reperfusion but of the damage caused by lack of substrates and build up of metabolites during the ischaemic period. Myocardial recovery cannot however occur as long as ischaemia is maintained. Thus, myocardial recovery requires reperfusion and improved reperfusion may be of long term benefit.

CHAPTER 11

CONCLUSIONS

The following conclusions can be drawn from the experiments described in this thesis:

1. The relationship between end-diastolic perfusion pressure and end-diastolic flow in the isolated rabbit heart is almost linear;

2. The coronary vascular bed is not maximally dilated; a small vasodilatory reserve exists within the small arteries and arterioles which can be exposed with adenosine or nifedipine;

3. Tension recorded from this preparation is dependent on the perfusion parameters;

4. Coronary resistance and resting tension are increased by a period of total global ischaemia. The severity of the effect increases with the length of the ischaemic period;

5. Coronary resistance and resting tension are also increased by low flow ischaemia and hypoxia;

6. The recorded increase in resting tension after total global

ischaemia is influenced by the method of reperfusion as a consequence of the relationship between the perfusion parameters and tension; contracture appears greater on reperfusion at constant perfusion pressure than at constant flow;

7. Increased coronary resistance is due to active vascular smooth muscle contraction, to an increase in extravascular forces or to increased resistance in the vascular bed as a result of oedema in the vascular wall;

8. The increase in resistance after 15 min total global ischaemia can be prevented or reversed with vasodilators and is therefore caused by contraction of the vascular smooth muscle;

9. Extracellular K^+ concentration rises rapidly within the first few minutes of total global ischaemia;

10. A similar increase in the K^+ concentration of perfusate causes an increase in coronary resistance and myocardial quiescence, with no change in resting tension. The increase in coronary resistance can be prevented by adenosine and is due to active vasoconstriction;

11. Extracellular K^+ concentration falls quickly on reperfusion and can not be responsible for the maintained increase in coronary resistance during this period;

12. Coronary vasoconstriction after ischaemia is not caused by increased production of cyclo-oxygenase products; indomethacin does not

prevent the increase in resistance;

13. The increase in resistance after 60 min total global ischaemia can not be prevented with vasodilators and is not due to vascular smooth muscle contraction;

14. Perfusion with FCCP causes myocardial contracture without directly constricting the coronary vessels. Coronary resistance rises due to an increase in extravascular forces;

15. Increasing the stimulation frequency also increases extravascular forces and causes coronary resistance to rise;

16. The increase in resistance in the prescence of FCCP is not as great as that after 60 min ischaemia, although the degree of contracture is equivalent. The increase in resistance after 60 min ischaemia cannot be totally due to the increase in extravascular forces caused by contracture;

17. The increase in resistance after short periods of ischaemia is predominantly due to vasoconstriction. After prolonged ischaemia increased extravascular forces due to myocardial contracture contribute to the increase in resistance but other factors must also be involved.

APPENDIX 1

RESPONSES OF EPICARDIAL CORONARY ARTERY SEGMENTS TO K^+ AND FCCP

A1.1 The Experimental Preparation

The left anterior descending artery of the rabbit heart was dissected and cleaned of surrounding tissue. Segments measuring 4 - 5 mm in length and 1 mm outer diameter were mounted horizontally on a tungsten wire which was connected with a rigid steel rod to a force transducer (Statham UC1). A second tungsten wire, attached to a 3-way micromanipulator by a steel rod, was inserted into the artery and the wires moved apart so that a preload of 1.5 g was applied to the artery. The artery was immersed in an organ bath containing physiological salt solution of similar composition to that used for perfusion of the Langendorff preparation. The solution was continually gassed with 95% O_2, 5% CO_2. The temperature was maintained at 36°C. Tension was recorded (recorder model MX412, Ormed Engineering, Welwyn Garden City, England). After an equilibration period of 90 min, during which artery underwent stress-relaxation, preload was adjusted to 1 g. The segment was then repeatedly exposed to 20 mmol.litre^{-1} K^+ (exposure for 10 min followed by 15 min recovery) until a stable response was obtained.

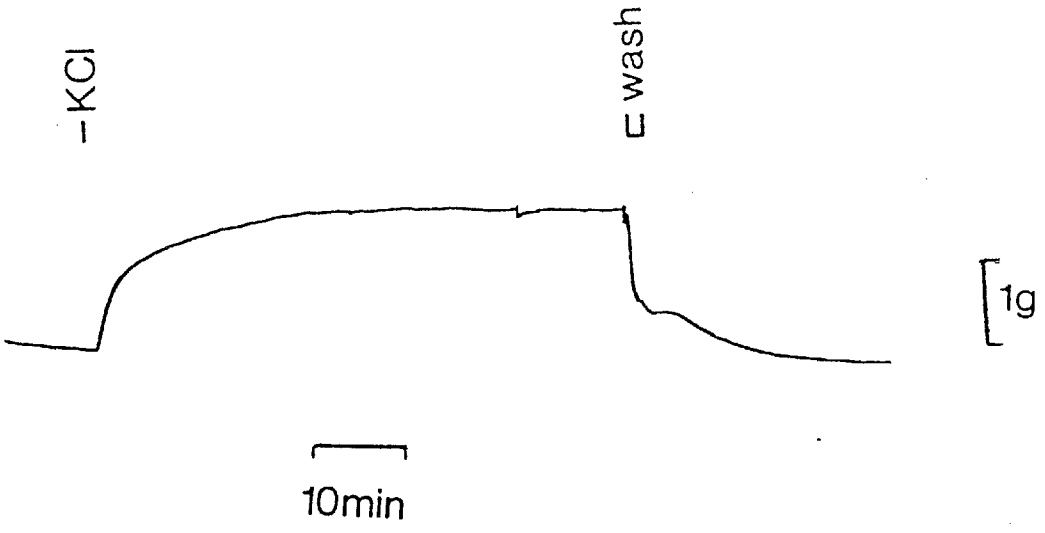


Figure A1.1 Example of the effect on an epicardial coronary artery segment of prolonged exposure (60 min) to K^+ (20 mmol.litre^{-1}). Note that tension increases when the K^+ concentration is increased and is maintained throughout exposure. When K^+ concentration is lowered to 5.9 mmol.litre^{-1} tension declines rapidly.

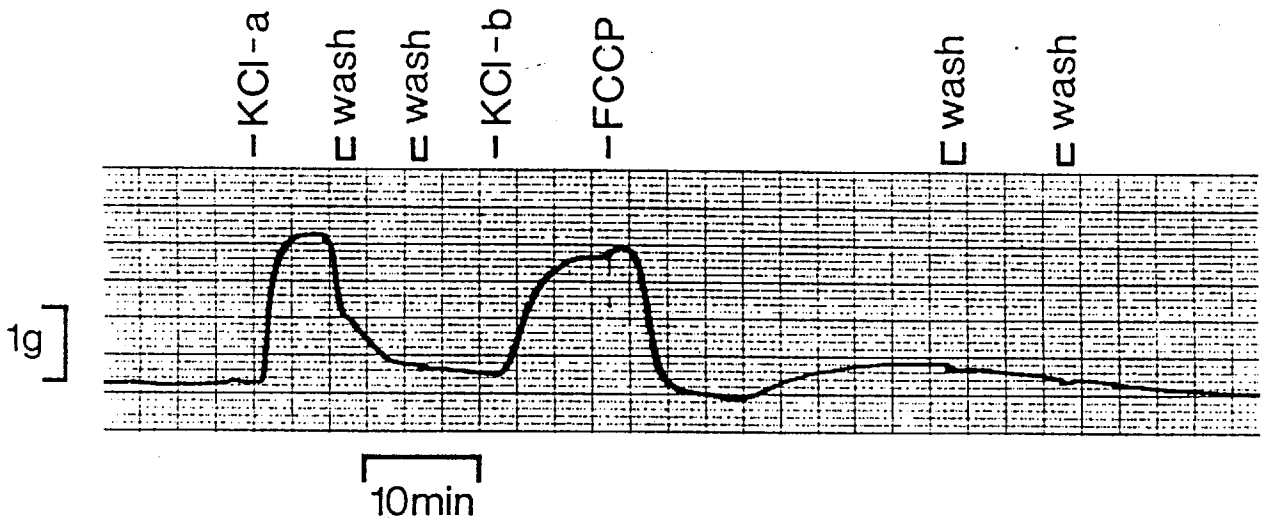


Figure A1.2 Effect of FCCP (1×10^{-6} mol.litre^{-1}) on an epicardial coronary artery segment. Response to 40 mmol.litre^{-1} K^+ was determined (a). The arterial segment was then exposed to 20 mmol.litre^{-1} K^+ (b) and the effect of FCCP (1×10^{-6} mol.litre^{-1}) on this contraction determined.

A1.2 Protocol

The K^+ concentration of the bathing fluid was increased by adding 0.4 cm^3 of a solution containing 2 $mol.litre^{-1}$ KCl. FCCP was dissolved in ethanol to give a stock solution containing 4×10^{-4} $mol.litre^{-1}$, 0.14 cm^3 of this solution was added to the bathing fluid.

A1.3 Responses to K^+ and FCCP

When the K^+ concentration of the bathing solution was increased to 20 $mmol.litre^{-1}$ by addition of KCl (2 $mol.litre^{-1}$) the arterial segment contracted. Tension developed was 2.08 ± 0.20 g ($n = 5$). This contraction was maintained as long as the K^+ concentration remained elevated (Fig. A1.1). When the K^+ concentration of the solution was returned to control arterial tone rapidly returned to values insignificantly different from those before the K^+ concentration was elevated, even after 60 min exposure to 20 mmol.litre K^+ (Fig. A1.1).

Addition of FCCP to give a bath concentration of 1×10^{-6} $mol.litre^{-1}$ resulted in no change in basal tension. In the presence of 20 $mmol.litre^{-1}$ K^+ this concentration of FCCP produced a $140.6 \pm 37.1\%$ ($n = 5$) relaxation (Fig. A1.2).

A1.4 Limitations of the Model

The epicardial coronary artery segment comes from a large conduit artery which plays little part in controlling the resistance of the

coronary bed. Most of the resistance occurs within the intramural arteries and arterioles (Malindzak, 1982). Large conduit arteries and small resistance vessels have been shown to differ in their response to a given stimulus (Fam and McGregor, 1968; Schnaar and Sparks, 1972). For the most part this difference is quantitative rather than qualitative and the responses of the model probably reflect those occurring within the coronary bed in the isolated heart.

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