

**STUDIES ON THE PATHOPHYSIOLOGY OF
HYPERTENSION IN THE ELDERLY**

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TABLE OF CONTENTS

TABLE OF CONTENTS	ii
ABSTRACT	vii
ACKNOWLEDGEMENTS	ix
STUDY DECLARATION	xi
ABBREVIATIONS	xiv
LIST OF FIGURES	xv
LIST OF TABLES	xvii
Chapter 1 INTRODUCTION	1
1.1 HISTORICAL BACKGROUND	2
1.2 EPIDEMIOLOGY OF HYPERTENSION	3
1.2.1 Hypertension and public health	3
1.2.2 Definition of hypertension	4
1.2.3 Relationship between blood pressure and ageing	5
1.2.4 Prevalence of hypertension	6
1.2.5 Hypertension and cardiovascular risk.....	8
1.3 BENEFITS OF TREATMENT OF HYPERTENSION IN THE ELDERLY	11
1.4 PATHOPHYSIOLOGY OF HYPERTENSION IN THE ELDERLY	13
1.4.1 Arterial haemodynamics and compliance.....	14
1.4.2 Renin-angiotensin system and the kidney.....	15
1.5 SYMPATHETIC NERVOUS SYSTEM	16
1.6 ARTERIAL BAROREFLEX SENSITIVITY	19
1.6.1 The arterial baroreflex	19
1.6.2 The arterial baroreflex and ageing	21
1.6.3 Relation between diminished baroreflex sensitivity and hypertension	24
1.6.4 Methods for the examination of arterial baroreflexes	25
1.6.5 Pharmacological methods	25
1.6.6 Objections to the pharmacological method	26
1.6.7 The use of the pharmacological method in studies of baroreflex sensitivity	28

1.6.8	<i>Effect of respiration</i>	30
1.6.9	<i>Reproducibility of the pharmacological methods</i>	31
1.6.10	<i>Pharmacological depressor methods</i>	32
1.6.11	<i>The neck chamber method</i>	34
1.6.12	<i>Reflexes from carotid and extracarotid baroreceptors</i>	36
1.6.13	<i>The Valsalva manoeuvre</i>	36
1.6.14	<i>Other techniques for the measurement of baroreflex sensitivity</i>	40
1.6.15	<i>Comparison and relation between methods</i>	42
1.6.16	<i>Arterial baroreceptor studies in the elderly</i>	43
1.6.17	<i>Other factors modulating the arterial baroreflex</i>	51
1.6.18	<i>Cardiopulmonary reflexes: effects of age and hypertension</i>	51
1.6.19	<i>The tilt test, and other tests of cardiovascular reflexes</i>	53
1.7	RESISTANCE VESSEL STRUCTURE AND FUNCTION	55
1.7.1	<i>Resistance vessel structure and hypertension</i>	56
1.7.2	<i>Function of the endothelium</i>	62
1.7.3	<i>Endothelial function in hypertension</i>	66
1.7.4	<i>Endothelial function and ageing</i>	69
1.8	AMBULATORY BLOOD PRESSURE	72
1.8.1	<i>Ambulatory blood pressure monitoring in the elderly</i>	74
1.8.2	<i>Validation of ambulatory blood pressure monitors in the elderly</i>	77
1.8.3	<i>Ambulatory blood pressure monitoring in the assessment of blood pressure variability in the elderly</i>	78
1.9	AIMS OF THE STUDY	79
 Chapter 2 CARDIOVASCULAR NEURAL CONTROL IN ELDERLY		
SUBJECTS		81
2.1	SUMMARY	82
2.2	BACKGROUND AND AIMS	83
2.3	MATERIALS AND METHODS	84
2.3.1	<i>Study subjects</i>	84
2.3.2	<i>Study protocol</i>	85
2.3.3	<i>Laboratory studies</i>	87

2.3.4	<i>Data acquisition</i>	91
2.3.5	<i>Data analysis</i>	92
2.3.6	<i>Statistical methods</i>	94
2.4	RESULTS	94
2.4.1	<i>Subject characteristics and clinic and ambulatory blood pressure</i>	94
2.4.2	<i>Laboratory cardiovascular testing</i>	102
2.4.3	<i>The Valsalva manoeuvre</i>	102
2.4.4	<i>Baroreceptor-cardiac reflex sensitivity testing</i>	106
2.4.5	<i>Baroreflex sensitivity and clinic and ambulatory blood pressure</i>	113
2.4.6	<i>Head-up tilting</i>	116
2.4.7	<i>Cold face stimulus</i>	120
2.5	DISCUSSION	123
2.5.1	<i>Blood pressure, heart rate and daytime blood pressure variability</i>	123
2.5.2	<i>Baroreceptor-cardiac reflex sensitivity</i>	125
2.5.3	<i>The baroreceptor-vascular reflex</i>	129
2.5.4	<i>Non-baroreceptor-mediated reflexes</i>	131
2.5.5	<i>Study limitations</i>	132
2.6	CONCLUSIONS	134
Chapter 3 ARTERIAL BLOOD PRESSURE AND RESISTANCE ARTERY		
STRUCTURE IN ELDERLY SUBJECTS		
3.1	SUMMARY	137
3.2	BACKGROUND AND AIMS	138
3.3	MATERIALS AND METHODS	138
3.3.1	<i>Study subjects and blood pressure measurement</i>	138
3.3.2	<i>Myograph protocol</i>	140
3.3.3	<i>Statistical methods</i>	143
3.4	RESULTS	144
3.4.1	<i>Subject characteristics and blood pressure</i>	144
3.4.2	<i>Resistance vessel structure</i>	145
3.5	DISCUSSION	152
3.5.1	<i>Vascular structure and blood pressure</i>	152

3.5.2 <i>Blood pressure and ageing</i>	155
3.5.3 <i>Study limitations</i>	157
3.6 CONCLUSIONS	158

**Chapter 4 ENDOTHELIAL AND VASCULAR SMOOTH MUSCLE CELL
FUNCTION IN RESISTANCE ARTERIES FROM ELDERLY SUBJECTS** 159

4.1 SUMMARY	160
4.2 BACKGROUND AND AIMS	161
4.3 MATERIALS AND METHODS	162
4.3.1 <i>Study subjects</i>	162
4.3.2 <i>Preparation of arteries</i>	163
4.3.3 <i>Myograph protocol</i>	164
4.3.4 <i>Statistical methods</i>	165
4.4 RESULTS	166
4.5 DISCUSSION	181
4.5.1 <i>Differences between hypertensive and normotensive subjects</i>	181
4.5.2 <i>Study limitations</i>	184
4.6 CONCLUSIONS	186

**Chapter 5 THE TILT TEST, VASCULAR STRUCTURE AND
BAROREFLEXES IN ELDERLY SUBJECTS** 188

5.1 SUMMARY	189
5.2 BACKGROUND AND AIMS	190
5.3 MATERIALS AND METHODS	191
5.3.1 <i>Study subjects</i>	191
5.3.2 <i>Study protocol</i>	191
5.3.3 <i>Statistical methods</i>	192
5.4 RESULTS	193
5.4.1 <i>Clinic blood pressure and vascular structure</i>	193
5.4.2 <i>Laboratory cardiovascular tests</i>	194
5.4.3 <i>Relation between vascular responses and vascular structure</i>	200
5.4.4 <i>Orthostatic responses and baroreceptor-cardiac reflex sensitivity</i>	203
5.5 DISCUSSION	207

5.5.1 <i>The baroreceptor-vascular reflex and vascular structure</i>	207
5.5.2 <i>Hypertension and orthostatic hypotension</i>	211
5.5.3 <i>Study limitations</i>	213
5.6 CONCLUSIONS	215
Chapter 6 DISCUSSION	216
6.1 SUMMARY OF PRINCIPAL STUDY FINDINGS	217
6.2 IMPLICATIONS OF THESE FINDINGS FOR THEORIES OF THE AETIOLOGY OF HYPERTENSION IN THE ELDERLY	220
6.3 IMPLICATIONS OF THESE FINDINGS FOR THE TREATMENT OF HYPERTENSION IN THE ELDERLY	229
6.4 PROSPECTS FOR FURTHER STUDIES IN HYPERTENSION IN THE ELDERLY	234
6.5 CONCLUSION	236
APPENDICES	237
VALIDATION OF THE FINAPRES DEVICE AND ITS USE IN LABORATORY CARDIOVASCULAR TESTS	238
PUBLICATIONS ARISING FROM THE WORK DESCRIBED IN THIS THESIS	241
REFERENCES	242

ABSTRACT

This thesis examines two of the most important issues in the pathophysiology of hypertension in the elderly, disordered cardiovascular neural control and raised peripheral vascular resistance, in clinical studies of hypertensive and normotensive subjects aged over sixty years.

Hypertension in elderly subjects is associated with reduced baroreceptor-cardiac reflex sensitivity irrespective of the method used to quantify the reflex. There is no difference in baroreceptor-cardiac reflex sensitivity between elderly subjects with combined systolic-diastolic hypertension and isolated systolic hypertension. Beyond the age of sixty years, baroreflex sensitivity is independently related to the level of systolic blood pressure but not to age. Subjects with isolated systolic hypertension have a greater fall in blood pressure with passive tilt than normotensives despite a greater rise in forearm vascular resistance.

Hypertension in elderly subjects is associated with an increased media:lumen ratio in subcutaneous resistance arteries which principally relates to the prevailing level of pulse pressure, particularly when measured by 24-hour ambulatory monitoring. However, there are no differences with hypertension in the contractile behaviour of vascular smooth muscle or in the endothelium-dependent and independent relaxation responses of such resistance arteries.

The sympathetic baroreceptor-vascular response is not correlated with the changes observed in the structure of the effector mechanism of the reflex, the resistance artery. However, blood pressure changes with orthostasis are negatively correlated with baroreceptor-cardiac reflex sensitivity, suggesting an aetiological link between hypertension and the postural hypotension with which it is often associated.

In conclusion, hypertension in the elderly is associated with reduced baroreflex sensitivity, an impaired blood pressure response to orthostasis and increased resistance artery media:lumen ratio, but no differences in endothelial or vascular smooth muscle function. These findings should help clarify some of the pathophysiological issues in hypertension in the elderly, and guide our approach to the treatment of this common and clinically important condition.

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My particular thanks go to the Sir Jules Thorn Charitable Trust for their funding of the project on pulse pressure and arterial structure in the elderly, which enabled me to undertake the period of full time research during which these studies were performed. Their support for research in an age group that does not usually attract financial support to the same extent as some others, is to be highly commended.

My sincerest thanks must go to 'the three Professors', John Potter, Bert Thurston, and John Swales, firstly for obtaining the grant that enabled this research, and subsequently for their vital and generous time, support, encouragement and guidance throughout these studies. It was a genuine pleasure to work with all three, and an invidious task to single any of them out for particular mention. However, it was Professor John Potter who initially encouraged me into this field of research and was responsible for much of the day-to-day supervision of the work, so it is to him I

owe my greatest vote of thanks. I can certainly claim personal responsibility for a substantial number of his grey hairs, but he showed great humour, patience and judgement whilst acquiring them.

My personal thanks must also go to my wife, Lindy and my sons, Matthew and Simon for their patient support and encouragement throughout the conduct of these studies, and to whom I dedicate this work.

APPOINTMENTS HELD DURING THE PREPARATION OF THIS THESIS

The work described in this thesis was conducted over a two and a half year period, during which time the author held the post of Clinical Research Fellow in the University Department of Medicine for the Elderly, Leicester (initially at Leicester General Hospital and subsequently at Glenfield General Hospital), under the supervision of Professor J F Potter, Professor H Thurston and Professor J D Swales. This work was supported by a project grant from the Sir Jules Thorn Charitable Trust for the study of the relation between arterial blood pressure and resistance artery structure in elderly subjects. The latter part of the work and final preparation of the manuscript were completed during the author's present post of Senior Registrar in Medicine to the Leicester Hospitals.

STUDY DECLARATION

This thesis consists of four experimental chapters:

1. Cardiovascular neural control in elderly subjects

The design, recruitment, organisation and conduct of this study were the sole responsibility of the author under the supervision of Professor J F Potter. I am grateful to Professor J D Swales, Professor H Thurston, Professor J F Potter and

Professor C M Castleden for permission to study their patients. I am also grateful to Dr T Smith, Dr J Oldring, Dr A Wilson and Dr M Rowe for their contributions to the recruitment of hypertensive subjects from general practice. I am grateful to Dr T G Robinson, Dr H Rakicka and Mr C Cave for their assistance during the experimental sessions for this study.

2. Arterial blood pressure and resistance artery structure in elderly subjects

This study was conceived and the project grant obtained by a joint application by Professor J D Swales, Professor H Thurston and Professor J F Potter of Leicester University. The subsequent recruitment, organisation and conduct of this study were the sole responsibility of the author. I am grateful to those colleagues mentioned above for permission to study their patients and their contributions to subject recruitment. All gluteal biopsies were performed by the author, but I am indebted to Ms P A C Watt for the dissection and mounting of resistance arteries in the myograph.

3. Endothelial and vascular smooth muscle cell function in resistance arteries from elderly subjects

The design, recruitment, organisation and conduct of this study were the sole responsibility of the author under the supervision of Professor H Thurston and Professor J D Swales. I am grateful to those colleagues mentioned above for their assistance and contributions to subject recruitment. I am indebted to Ms P A C Watt

for the dissection and mounting of resistance arteries in the myograph, and to Dr M Bennett for his assistance with the laboratory work. I am also grateful to Schering AG for the gift of the prostacyclin analogue Iloprost.

4. The tilt test, vascular structure and baroreflexes in elderly subjects

The design, recruitment, organisation and conduct of this study were the sole responsibility of the author. I am grateful to my colleagues mentioned in the above Chapters for their contributions to patient recruitment and the laboratory work.

All statistical analyses were performed by the author using the computer facilities in the Department of Medicine for the Elderly at the Glenfield Hospital.

ABBREVIATIONS

HT	hypertensive
NT	normotensive
BP	blood pressure
SBP	systolic blood pressure
ABPM	ambulatory blood pressure monitoring
CH	combined hypertension
ISH	isolated systolic hypertension
BRS	baroreflex sensitivity
PE	phenylephrine
SNP	sodium nitroprusside
V1	Valsalva manoeuvre (phase 4)
V2	Valsalva manoeuvre (overshoot)
CFS	cold face stimulus
FBF	forearm blood flow
FVR	forearm vascular resistance
T	active tension
σ	active media stress
ED ₅₀	agonist sensitivity (the dose required to produce 50% of the response)
NOARG	N ^G -nitro-L-arginine
NO	nitric oxide
EDRF	endothelium-derived relaxing factor

LIST OF FIGURES

	<i>Page</i>
Figure 1.1 Schematic diagram illustrating the main components of cardiovascular neural control	20
Figure 1.2 Simplified diagram illustrating the main components of the vascular L-arginine-nitric oxide pathway	64
Figure 2.1 Systolic blood pressure from 24-hour ambulatory blood pressure monitoring for the combined hypertensive, isolated systolic hypertensive and normotensive groups	98
Figure 2.2 Diastolic blood pressure from 24-hour ambulatory blood pressure monitoring for the combined hypertensive, isolated systolic hypertensive and normotensive groups	99
Figure 2.3 Heart rate from 24-hour ambulatory blood pressure monitoring for the combined hypertensive, isolated systolic hypertensive and normotensive groups	100
Figure 2.4 A typical recording of the cardiovascular response to the Valsalva manoeuvre in a study subject	105
Figure 2.5 A typical example of the response to a phenylephrine bolus injection in a study subject	105
Figure 2.6 Baroreceptor-cardiac reflex sensitivity by the different methods for the combined hypertensive, isolated systolic hypertensive and normotensive groups	111
Figure 2.7 Percentage changes in forearm blood flow with passive tilt for the combined hypertensive, isolated systolic hypertensive and normotensive groups	117
Figure 2.8 Changes in forearm vascular resistance with passive tilt for the combined hypertensive, isolated systolic hypertensive and normotensive groups	118
Figure 2.9 Changes in forearm vascular resistance with the cold face stimulus for the combined hypertensive, isolated systolic hypertensive and normotensive groups	122

Figure 3.1	<i>The Mulvany-Halpern myograph</i>	141
Figure 3.2	<i>Schematic diagram of a resistance artery mounted on two parallel 40 μm wires between the 'jaws' of the myograph</i>	142
Figure 3.3	<i>Relation between media:lumen ratio and 24-hour pulse pressure without and with adjustment for age</i>	150
Figure 4.1	<i>Dose-response curves for resistance arteries to noradrenaline</i> ..	169
Figure 4.2	<i>Dose-response curves for resistance arteries to noradrenaline in the presence of cocaine</i>	173
Figure 4.3	<i>Dose-response curves for resistance arteries to Angiotensin II</i> ..	174
Figure 4.4	<i>Dose-response curves for resistance arteries to acetylcholine</i> ...	175
Figure 4.5	<i>Dose-response curves for resistance arteries to bradykinin</i>	176
Figure 4.6	<i>Dose-response curves for resistance arteries to Iloprost</i>	177
Figure 4.7	<i>Dose-response curves for resistance arteries to sodium nitroprusside</i>	178
Figure 4.8	<i>Dose-response curves for resistance arteries to acetylcholine and bradykinin following incubation with N^G-nitro-L-arginine</i>	179
Figure 4.9	<i>Maximum relaxation responses to the various agents studied</i>	180
Figure 5.1	<i>Changes in forearm vascular resistance with the cold face stimulus for the hypertensive and normotensive groups</i>	199
Figure 5.2	<i>Relation between orthostatic systolic blood pressure fall and arterial baroreflex sensitivity</i>	205
Figure 6.1	<i>The relationship between baroreceptor-cardiac reflex sensitivity and age</i>	222

LIST OF TABLES

	<i>Page</i>
Table 1.1 <i>Baroreflex sensitivity in elderly and young hypertensive and normotensive subjects</i>	50
Table 2.1 <i>Demographic data for the three groups</i>	96
Table 2.2 <i>Clinic and ambulatory blood pressure data for the three groups</i> ..	97
Table 2.3 <i>Daytime ambulatory blood pressure variability for the three groups</i>	101
Table 2.4 <i>Blood pressure and heart rate responses to isometric handgrip for the three groups</i>	103
Table 2.5 <i>Baroreflex sensitivity data from the Valsalva manoeuvre for the three groups</i>	104
Table 2.6 <i>Baroreflex sensitivity by the phenylephrine pressor and sodium nitroprusside depressor methods for the three groups</i>	108
Table 2.7 <i>Baroreflex sensitivity by all methods for the three groups</i>	110
Table 2.8 <i>Univariate correlations (Pearson's <i>r</i>) between the various indices of baroreflex sensitivity</i>	112
Table 2.9 <i>Univariate correlations between indices of baroreflex sensitivity and clinic and ambulatory blood pressure</i>	114
Table 2.10 <i>Univariate correlations between indices of baroreflex sensitivity and daytime ambulatory blood pressure and heart rate variability</i>	115
Table 2.11 <i>Blood pressure and heart rate responses to orthostatic stress for the three groups</i>	119
Table 2.12 <i>Blood pressure and heart rate responses to the cold face stimulus for the three groups</i>	114
Table 3.1 <i>Blood pressure and heart rate for the whole study group and the hypertensive and normotensive subgroups</i>	146
Table 3.2 <i>Resistance artery structure for the whole study group and the hypertensive and normotensive subgroups</i>	147

Table 3.3 Correlation matrix for the relation between resistance artery structure and age, clinic and 24 hour blood pressure, heart rate, and daytime blood pressure variability	149
Table 4.1 Subject characteristics and morphology data for the vessels studied from hypertensive and normotensive subjects	167
Table 4.2 Maximum contractile responses and sensitivities for the resistance vessels studied from hypertensive and normotensive subjects	168
Table 4.3 Maximum relaxation responses and sensitivities for the resistance vessels studied from hypertensive and normotensive subjects	172
Table 5.1 Vascular morphology parameters for the study group as a whole, and for the hypertensive and normotensive groups	195
Table 5.2 Blood pressure and heart rate responses to isometric handgrip for the hypertensive and normotensive groups	196
Table 5.3 Blood pressure, heart rate, forearm blood flow and forearm vascular resistance responses to orthostatic stress for the hypertensive and normotensive groups	197
Table 5.4 Blood pressure, heart rate, forearm blood flow and forearm vascular resistance responses to the cold face stimulus for the hypertensive and normotensive groups	198
Table 5.5 Arterial baroreceptor-cardiac reflex sensitivity by the different methods for the hypertensive and normotensive groups	201
Table 5.6 Simple and partial correlation coefficients (correcting for baseline values) between media:lumen ratio and changes in blood pressure, heart rate, forearm blood flow and forearm vascular resistance responses to head-up tilt, the cold face stimulus and isometric exercise	202
Table 5.7 Univariate Pearson's correlation coefficients between the various measures of baroreflex sensitivity and orthostatic blood pressure changes with active standing and passive 60° head-up tilt	204

Chapter 1

Introduction

1.1 HISTORICAL BACKGROUND

Ancient Chinese medicine dating from many hundreds of years BC spoke of distinguishing the hardness of the pulse, which is influenced by the force of the heart and too much salt in the food, leading to dropsical swellings (oedema). Acupuncture or venesection were recommended in such cases. In the Western world, the Hippocratic school also recommended venesection for the treatment of apoplexy (stroke). Many centuries later, the first description of the biological phenomenon of blood pressure in modern times was given by the Reverend Stephen Hales, minister in Teddington, Middlesex, who published his experiments measuring the blood pressure of the horse in 1733 in his essay *Haemostatics*. Over a hundred years later, Bright (1836) described nephritis, proteinuria and dropsy associated with enlargement of the left ventricle and related this to an increased resistance to flow in small vessels of the circulation, but did not detect a link with blood pressure. Progress in this respect was hampered by the lack of an instrument for the reliable indirect measurement of the blood pressure, although Mahomed (1872) at Guy's Hospital had devised a primitive sphygmograph and described an association between hypertension, nephritis and cardiac hypertrophy [Swales, 1995a]. It was not until the invention of the first practical sphygmomanometer by Riva-Rocci in 1896, and the subsequent exploration of the auscultatory findings by Korotkoff (1905) that the true clinical significance of high blood pressure could be examined. At about the same time Janeway (1913) described the varied course of high blood pressure under the term 'hypertensive cardiovascular disease'.

Introduction

Although Mahomed had originally described patients in whom high blood pressure was not associated with renal disease, most of the research effort concentrated on a renal stimulus to hypertension. Oliver and Shafer (1894) had demonstrated the pressor effect of adrenal extracts leading to the synthesis of the first hormone adrenaline, and a similar line of enquiry was pursued with renal extracts by Tigerstedt at the turn of the century. This ultimately led to the discovery of renin, its product, angiotensin, and the renin-angiotensin system [Braun-Menendez & Page, 1958; Peart, 1991]. About this time that the first reports of the effective drug treatment of malignant hypertension were made, using ganglion-blocking drugs such as parenteral hexamethonium [Restall & Smirk, 1950; Harrington et al, 1959]. There followed the introduction of oral agents such as hydralazine and reserpine, and the thiazide diuretics, and these tablets were incorporated into the first studies to demonstrate the benefits of treating hypertension that was other than malignant [Veterans Administration Cooperative Study Group, 1967]. With the development of orally active treatments free of severe side-effects, the effective treatment of hypertension became a reality [Freis, 1990].

1.2 EPIDEMIOLOGY OF HYPERTENSION

1.2.1 Hypertension and public health

Hypertension is a substantial public health problem in all Westernised societies [Rose, 1985]. It is a major risk factor for coronary heart disease, the commonest

cause of death in such populations, and the main risk factor for cerebrovascular disease, a widespread cause of disability and death. As such, hypertension has profound resource implications for the provision of health care, both in terms of the need for large numbers to take lifelong medication, and in terms of the consumption of resources by victims of hypertension-related vascular disease [Swales, 1995b]. For example, patients with stroke occupy a fifth of all acute medical beds and a quarter of all long term hospital beds, consuming 5% of the British health budget [Allison, 1994]. Among the elderly, between 30 and 60% of all cardiovascular disease is related to hypertension to a greater or lesser extent [Wilson & Kannel, 1995]. The recognition, diagnosis and effective treatment of high blood pressure thus represent important public health priorities [HDFP Cooperative Group, 1979a].

1.2.2 Definition of hypertension

Such priorities require an understanding of the definition and prevalence of the disorder, together with an appreciation of the effects, both beneficial and otherwise, of treatment both for the individual and the wider population. Values for systolic and diastolic blood pressure are unimodally distributed in any population, so the population does not conveniently divide into distinct 'hypertensive' and 'normotensive' groups. Hypertension, therefore, is not a qualitative diagnosis, but a quantitative one [Pickering, 1973], with individuals with a blood pressure above an arbitrary threshold being labelled as 'sufferers', and those with blood pressure below (even if just below) being labelled as 'normal'. Indeed, epidemiological studies have failed to identify any anatomical, physiological or biochemical markers that serve to

Introduction

distinguish between normotensive and hypertensive subjects [Kannel & Stokes, 1985]. The preferred definition of high blood pressure is therefore a pragmatic one, defining hypertension as 'that level of blood pressure at which the benefits of intervention exceed those of inaction' [Rose, 1980]. At the present time, the level at which this principle obtains is generally regarded as a sustained systolic blood pressure >160 mmHg and/or a diastolic blood pressure of >90 mmHg, but these levels have changed in recent years and may well do so again in the light of future evidence [Sever et al, 1993; Levy, 1995]. Indeed, the latest report from the American Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure would define the lower limit of Stage I (mild) hypertension as a systolic blood pressure of 140 mmHg [NHBPEP, 1993]. It is also increasingly clear that such levels of blood pressure cannot be viewed in isolation, and an assessment of all modifiable cardiovascular risk factors (obesity, serum lipids, blood sugar, cigarette smoking, electrocardiographic findings) forms an essential part of the overall evaluation of any patient, suggesting a move towards an absolute-risk based individualised treatment [Jackson et al, 1993; Levy, 1995].

1.2.3 Relationship between blood pressure and ageing

A progressive rise in blood pressure with age is a feature of all Westernised societies [Rose, 1985]. There are well-described studies demonstrating the almost complete absence of such an age-related rise in blood pressure among African rural tribes [Shaper, 1969; Sever et al, 1980]. When non-industrialised populations migrate and adopt a more 'Western' lifestyle, blood pressure begins to rise with age

Introduction

and cases of hypertension emerge, suggesting a vital effect of environmental factors such as diet or circumstantial stress [Timio et al, 1988]. However, ageing has differential effects on the separate parameters of blood pressure. Whilst systolic blood pressure continues to rise throughout life, the rise in diastolic blood pressure levels off in the sixth decade, after which it tends to fall with increasing age. This is observed in both cross-sectional and in cohort studies, which allow for the phenomenon of the survival of subjects with lower blood pressure levels [Kannel & Dawber, 1974; Kannel, 1980]. The result of this is that the proportion of hypertensive subjects with elevation of only the systolic blood pressure (isolated systolic hypertension, usually defined as systolic blood pressure ≥ 160 mmHg with diastolic blood pressure < 90 or 95 mmHg) rises with increasing age, from approximately 6% of all hypertensives in the 35-44 year age group, to 52% in the 65-74 age group [US Department of Health, Education and Welfare, 1977]. Furthermore, while at any given age men tend to have higher diastolic blood pressures than women, the rise in systolic blood pressure with age is steeper in women [Kannel, 1980; Bulpitt, 1989; Staessen et al, 1990]. The result is that the proportion of elderly hypertensives with isolated systolic hypertension is higher among women than among men [Wilking et al, 1988; Amery et al, 1991].

1.2.4 Prevalence of hypertension

The prevalence of hypertension thus varies according to the population, the age group, and with the diagnostic thresholds that are used. The threshold for diastolic blood pressure used for the diagnosis of hypertension will have a dramatic effect on

Introduction

prevalence. In the Hypertension Detection and Follow-up Program [Hypertension Detection and Follow-up Program Cooperative Group, 1977], the prevalence of hypertension among 159 000 men aged 30-69 fell from 25.3% at diastolic blood pressure >90 mmHg, to 8.4% at diastolic blood pressure >100 mmHg. No similar data exist for systolic blood pressure, but the population distribution of systolic blood pressure would suggest a similar effect of different diagnostic thresholds.

Furthermore, studies in which a single casual reading has been taken have tended to overestimate the prevalence of hypertension. Levels of office blood pressure tend to stabilise after three readings on separate occasions [Armitage & Rose, 1966].

Prevalence rates for hypertension therefore vary depending on whether they are based on a single casual reading or on repeated readings over a number of visits.

Thus in one study the prevalence of hypertension in men and women aged 45-64 declined from 15.6% on the first visit to 5.5% after repeated measurement

[Hawthorne et al, 1974]. In the elderly, Colandrea and colleagues examined a group of 3245 subjects with a mean age of 69 years, and found the prevalence of isolated systolic hypertension to fall from 14% at first examination, to 3% after two further visits [Colandrea et al, 1970].

Data from the US Health Examination Survey of 1960-1962, before the widespread use of antihypertensive medication and based on the average of three readings taken on a single occasion, indicated a prevalence of hypertension in males (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg) as rising from 13.4% among ages 35-44, to 23.4% among ages 55-64, and 41.7% in the 75-79 age group. For females the prevalence rates were 8.3%, 30.4% and 46.0% respectively [Whelton, 1985]. From the same survey, prevalence rates for isolated systolic hypertension (systolic blood pressure ≥ 160

Introduction

mmHg with diastolic blood pressure <95 mmHg) in males were 0.8% among ages 35-44, 9.7% among ages 55-64, and 27.7% in age group 75-79. For females the prevalences were 0.8%, 12.3% and 32.9% respectively, and these figures are close to those from other epidemiological studies [Wing et al, 1982]. Amery et al (1991) report the prevalence of isolated systolic hypertension in Belgium to rise from 1.0% of men and 4.3% of women aged 50-59, to 22.3% of men and 19.8% of women aged more than 70 when blood pressure was measured in the subjects' own homes. As mentioned above, these rates fall sharply with repeated measurement, and a 'rule of thumb' for the prevalence of sustained hypertension would be to divide by 3 such prevalence figures obtained from single visits [Bulpitt, 1989]. However, on the basis of these prevalence rates, the population at risk from isolated systolic hypertension in the United States may be in excess of 3 million persons [Silagy & McNeil, 1992].

1.2.5 Hypertension and cardiovascular risk

The seminal epidemiological study of the relation between hypertension and cardiovascular risk is the Framingham Study [Kannel, 1978]. This long-term cohort study has followed a general population sample of over 5200 men and women aged 30-62 at entry to the study between 1948 and 1952, with published data for over 30 years of follow-up [Kannel, 1978]. This study has established a linear association between increasing blood pressure and increasing cardiovascular risk in both sexes and at all ages, including the elderly. In broad terms, overall mortality was doubled and cardiovascular mortality was tripled in subjects with hypertension when compared to normotensives. There is also a smooth, qualitative relationship

Introduction

between the incidence of cardiovascular disease and levels of blood pressure, with an approximate three-fold increased risk in hypertensive men and women, which again pertains equally strongly in the over 65 age group [Kannel & Stokes, 1985]. The risk of congestive cardiac failure among hypertensives is particularly high, being six times that of normotensives in the Framingham study [McKee et al, 1971; Kannel et al, 1972]. Hypertension is identified by the Framingham data as the pre-eminent risk factor for stroke, conferring a three-fold increased risk in those with definite hypertension [Kannel, 1970]. Similar findings emerged from the Chicago Stroke Study, based on a cohort of over 2700 subjects aged 65-74 years [Shekelle et al, 1974]. The picture in coronary artery disease is more complicated by the presence of other important co-factors, in particular serum cholesterol [Kannel et al, 1979; Gordon et al, 1981]. However, hypertension confers additional risk irrespective of the level of total or low density lipoprotein cholesterol, and interacts with other predictors such as cigarette smoking, obesity, glucose intolerance, high density lipoprotein cholesterol, and electrocardiographic left ventricular hypertrophy in accelerating atherosclerosis, the most important outcome of which is coronary artery disease but also peripheral vascular disease [Castelli et al, 1989; Wilson & Kannel, 1995].

Although historically more emphasis has been placed on diastolic blood pressure as a cardiovascular risk factor, this is probably unjustified [Rutan et al, 1989; Mann, 1992]. The epidemiological data indicate that the relation between blood pressure and cardiovascular risk is at least as strong for systolic pressure as it is for diastolic pressure [Kannel et al, 1970, 1972; Mann, 1992]. Indeed in the elderly, the diastolic

Introduction

component loses much of its effect, and systolic blood pressure becomes pre-eminent as a risk factor for both coronary artery disease and stroke [Amery et al, 1986; Taylor et al, 1991; Wilson & Kannel, 1995]. This has implications for the risk associated with isolated systolic hypertension. In the Framingham study, isolated systolic hypertension was associated with a nearly two-fold increased risk of death, and a 2.5-fold increased risk of cardiovascular events [Wilking, 1988]. These findings agree with similar assessments of risk with isolated systolic hypertension for mortality and coronary and cerebrovascular morbidity [Colandrea, 1970; Kannel et al, 1971; Shekelle et al, 1974; Garland et al, 1983]. There is also accumulating recent evidence of the importance of the pulse pressure as an independent risk factor, particularly for myocardial infarction [Darne et al, 1989; Madhavan et al, 1994; Scuteri et al, 1995], and a widening of the pulse pressure is a characteristic finding in elderly subjects. It is therefore plain that the rise in systolic pressure which often leads to isolated systolic hypertension is not a benign accompaniment of ageing as it was once regarded.

Two related issues require clarification. Firstly it has been suggested that isolated systolic hypertension may represent 'burned-out' diastolic hypertension [Brest & Haddad, 1978; Amery et al, 1989]. To take account of this, the Framingham study conducted an analysis of the risks of isolated systolic hypertension excluding subjects with a record of raised diastolic blood pressure at any point over the previous 20 years [Kannel et al, 1980; Wilking, 1988]. The cardiovascular risk associated with isolated systolic hypertension was still 3.5 times greater for men and 3.8 times greater for women compared to normotensives. Secondly, it is possible

Introduction

that systolic hypertension in the elderly merely reflects arterial rigidity and it is this rather than the blood pressure *per se* that confers the additional risk. The Framingham study would indicate that when arterial rigidity is assessed from the pulse wave configuration, systolic pressure acts independently of the degree of arterial damage in its role as a risk factor [Kannel et al, 1981].

1.3 BENEFITS OF TREATMENT OF HYPERTENSION IN THE ELDERLY

From the earliest introduction of effective pharmacological agents for the treatment of hypertension, the benefits of therapy in malignant hypertension were readily appreciated [Harrington et al, 1959; Sokolow & Perloff, 1960; Mohler & Freis, 1960]. The benefits of treatment of less severe forms of hypertension (those patients with diastolic blood pressures between 90 and 129 mmHg) were subsequently demonstrated by the Veterans Administration, Hypertension Detection and Follow-up Program, and Australian studies [VA Cooperative Study Group, 1967, 1970, 1972; HDFP Cooperative Group, 1979a&b, 1982; Management Committee, 1980]. These studies showed significant reductions in cardiovascular mortality (by about 41%) and cerebrovascular morbidity (by about 51%), with less definite benefits concerning coronary morbidity (reduced by about 15%) [Holzgreve & Middeke, 1993]. Although these studies tended to exclude older subjects both by having upper age limits (usually 69 years) and because entry criteria were based on diastolic blood pressure, subgroup analyses did suggest a benefit for treatment in older persons [VA Cooperative Study Group 1972; HDFP Cooperative Group,

Introduction

1979b, Management Committee, 1981]. The reluctance of physicians to extrapolate these results to the wider population of elderly hypertensives arose from the perception of the elderly as a group more likely to suffer adverse effects from antihypertensive treatments and doubts about the risk/benefit ratio of such treatments, particularly in subjects with limited life expectancy [Jackson et al, 1976; Anonymous, 1977]. These misgivings have been overcome in recent years, initially by reports of benefit in elderly patients with combined or diastolic hypertension [Amery et al, 1985; Coope & Warrender, 1986; MRC Working Party, 1992], and also by studies in isolated systolic hypertension [SHEP Cooperative Research Group, 1991]. More recent studies have indicated benefit in subjects aged up to 84 years [Dahlof et al, 1991]. Meta-analysis of all the recent studies of hypertension in the elderly have indicated that treatment reduced total mortality by 9%, cardiovascular mortality by 22%, cerebrovascular mortality by 33% and coronary mortality by 26% [Thijs et al, 1992a]. Although in some respects these relative risk reductions are similar or less than those achieved in middle-aged hypertensives, the increased prevalence of such events in the elderly and the common association with other cardiovascular risk factors mean that the absolute benefits of treatment in this age group are much larger [NHBPEP Working Group, 1994; Lever & Ramsay, 1995]. One result of this greater attributable risk is that a statistically significant reduction in coronary events has been observed in the elderly whereas in middle-aged hypertensives such a benefit has not always been demonstrated [SHEP Cooperative Research Group, 1991; Holzgreve & Middeke, 1993]. For example, in the study by the European Working Party on High Blood Pressure in the Elderly, antihypertensive treatment of 1000 patients for 1 year prevented 11 fatal cardiac events, 6 fatal

cerebrovascular events, 11 non-fatal cerebrovascular events and 8 cases of congestive cardiac failure [Fletcher et al, 1991]. It is evident that the elderly are in fact the age group in which most benefit can be obtained in terms of the prevention of death and disability by antihypertensive intervention [Lever & Ramsay, 1995].

1.4 PATHOPHYSIOLOGY OF HYPERTENSION IN THE ELDERLY

In view of the alterations of blood pressure with age previously mentioned, a discussion of the pathophysiology of hypertension in the elderly inevitably focuses on the causes of predominant elevation of the systolic blood pressure, which are different from those in the young [Applegate & Rutan, 1992]. The typical haemodynamic findings associated with an increased pulse pressure vary with age: in subjects younger than 40 years the usual findings are of a hyperdynamic circulation with increased heart rate and cardiac output, increased left ventricular ejection rate, and normal total peripheral resistance. In subjects over 65, heart rate and cardiac output tend to be reduced, and vascular resistance is increased [Adampoulous, 1975]. The natural history of mild hypertension parallels these observations, with a gradual progression from a hyperkinetic state to one with an elevated systemic resistance and normal or reduced cardiac output [Lund-Johansen, 1980, 1991]. Two of the classical findings associated with this progression are a reduction in arterial baroreflex sensitivity and a rise in peripheral vascular resistance, and the particular role of these factors in hypertension in the elderly provide the main focus of this thesis.

1.4.1 Arterial haemodynamics and compliance

The normal aorta and large elastic arteries modify the pressure fluctuations generated through the cardiac cycle to maintain a relatively constant blood flow for the perfusion of organs [Hallock & Benson, 1937; Koch-Weser, 1973]. Arterial wall changes associated with ageing include thickening of the media with hyaline degeneration and fracturing and loss of elastin, deposition of collagen matrix and calcium, and thickening and fibrosis of the intima [Safar & London, 1989; Byyny, 1995]. These changes lead to a loss of arterial compliance. Whereas in young subjects the aorta distends substantially between pressures of 50 and 200 mmHg, by the eighth decade this property is almost entirely lost, a process accelerated by hypertension [Koch-Weser, 1973; O'Rourke, 1970]. The large elastic arteries become dilated, thickened and stiff, with thickening and luminal encroachment in the small arteries [Chobanian, 1989; Boutouryie et al, 1992; Byyny, 1995]. At a given level of arterial compliance, the level of diastolic blood pressure is principally determined by the rate of blood flow through the small arteries, that is, by the peripheral resistance. Therefore it follows that at a given level of peripheral resistance, diastolic blood pressure falls with an increase in arterial rigidity [Safar, 1993]. Systolic blood pressure is also augmented by increased pressure wave reflections from the periphery [O'Rourke, 1985]. Loss of arterial compliance increases pulse wave velocity, and atherosclerosis and ageing result in reflection points closer to the heart. Reflected waves summate with the subsequent primary wave generated by ventricular ejection to create a higher systolic peak and

Introduction

increased pulse pressure [Murgo et al, 1980; Mann, 1992; Safar, 1993]. In their pure form, these alterations would lead to a rise in systolic blood pressure and a fall in diastolic blood pressure. However, the more usual observation of a normal or mildly raised diastolic blood pressure inevitably involves a parallel although smaller increase in peripheral resistance [Koch-Weser, 1973; Berger & Li, 1990].

These effects are accompanied by ageing changes in the heart, leading to concentric left ventricular hypertrophy and a loss of ventricular compliance [Fouad-Tarazi & Healy, 1989]. In elderly subjects with isolated systolic hypertension there is a higher prevalence of left ventricular hypertrophy when compared to age-matched normotensive controls, together with abnormal diastolic filling but preservation of systolic function and cardiac output [Pearson et al, 1991; Pasierski et al, 1991; Sagie et al, 1993]. This contrasts with the findings in elderly subjects with combined hypertension, in which there was lower cardiac output, stroke volume and left ventricular ejection rate [Messerli et al, 1983].

1.4.2 Renin-angiotensin system and the kidney

Ageing is accompanied by a progressive reduction in renal mass and glomerular filtration rate, with loss of functioning glomeruli. Renal blood flow also declines, but to a greater extent than the decline in glomeruli implying a fall in blood flow per unit of kidney volume, and this occurs together with redistribution of blood flow from the cortex to the medulla [Hollenberg et al, 1974; Messerli et al, 1983]. Loss of the juxtaglomerular apparatus and reduced numbers of nephrons may combine with

Introduction

reduced β -receptor responsiveness to produce the low-renin state observed in both elderly normotensives and hypertensives, with a diminished renin response to sodium loading [Weidmann et al, 1975; Swales, 1975; Crane & Harris 1976]. This runs counter to the hypothesis that low-renin hypertension is characterised by volume expansion, as elderly hypertensives have a reduced intravascular volume [Laragh et al, 1972; Messerli et al, 1983]. Angiotensin II levels and receptor affinity are unaffected by age or blood pressure, and plasma aldosterone is unchanged in elderly hypertensives [Nowarczynski et al, 1977; Weber et al, 1989; Duggan et al, 1992]. On balance, alterations in renal function and the renin-angiotensin-aldosterone system are unlikely to play a large part in the pathogenesis of hypertension in the elderly [Byyny, 1995].

1.5 SYMPATHETIC NERVOUS SYSTEM

The potential role of the sympathetic nervous system in the aetiology of hypertension in the elderly has been the subject of much research and study. There is evidence to suggest a role for sympathetic overactivity, perhaps in response to psychological or physical stress, in the origins of hypertension in genetically predisposed individuals [Brown & Macquin, 1981; Folkow, 1982; Abboud, 1982; Floras, 1992]. Plasma noradrenaline rises with age [Zeigler et al, 1976], and measurement of plasma noradrenaline in hypertensive and normotensive subjects has tended to show significantly higher levels only in younger subjects, aged less than about 40 years [Bertel et al, 1980; Goldstein et al, 1983; Goldstein, 1983a].

Introduction

Because the age-related increase in plasma noradrenaline is steeper in normotensives, this difference is lost in older subjects. There are well recognised difficulties with the assessment of sympathetic activity from the indirect measurement of plasma catecholamines, particularly from venous samples [Floras et al, 1986; Kjedsen et al, 1986] but raised plasma noradrenaline in older subjects appears to arise from increased spillover from sympathetic nerves (itself probably related to reduced neuronal reuptake) rather than reduced clearance of the hormone [Rubin et al, 1982; Christensen, 1982; Esler, 1995]. These results are consistent with studies of muscle sympathetic nerve activity, which is raised in young individuals with borderline hypertension [Anderson et al, 1989]. Muscle sympathetic nerve activity is also increased in normal elderly subjects, and responds normally to sympatho-excitatory stimuli, again suggesting an overall increase in sympathetic nervous system activity with age [Wallin et al, 1981; Ebert et al, 1992]. However, the cause-effect relationship between rising noradrenaline and/or increased sympathetic activity and the observed increase in blood pressure with age has not been firmly established. A correlation between blood pressure and plasma catecholamines is not confirmed in most studies, while some studies which include older subjects demonstrate a positive correlation between either plasma noradrenaline [Van Brummelen et al, 1981; Shimada et al, 1985], or adrenaline [Potter et al, 1993], and blood pressure. Other studies have demonstrated that sympathetic stimulation (such as upright posture and exercise) results in a greater rise in plasma noradrenaline in older subjects [Bertel et al, 1980; Sowers et al, 1983], while others have not [Rowlands et al, 1984; Jansen et al, 1989]. While sympathetic nerve firing and synaptic noradrenaline may be increased with ageing, this does not necessarily

Introduction

translate into greater adrenergic responses. Most studies have found that the cardiovascular response to sympathetic stimuli in the elderly is attenuated [Young et al, 1980; Lakatta, 1980; Esler et al, 1981, 1989; Sowers & Mohanty, 1989; Lucini et al, 1993; Esler, 1995]. The observation of a strong relation between increased levels of plasma noradrenaline and reduced aortic compliance suggests a role for the sympathetic nervous system in the pathogenesis of isolated systolic hypertension [Alicandri et al, 1981].

There is reduction of both the chronotropic and inotropic responses to β -adrenoceptor stimulation in the elderly [Lakatta et al, 1975; Vestal et al, 1979; Van Brummelen et al, 1981; Bowman et al, 1994]. While β -adrenoceptor numbers appear to be unchanged with age, isoprenaline-mediated stimulation of adenylate cyclase activity is diminished [Lowder et al, 1976; Abrass et al, 1982]. Recent work has suggested that the observed reduction of cardiac β -adrenoceptor sensitivity in the elderly is primarily due to an age-related reduction in cardiovascular reflex responses rather than a reduction in sensitivity *per se* [Jennings et al, 1981; Ford & James, 1994]. Peripheral vasodilatory responses to β_2 -adrenoceptor stimulation are also reduced with age [Van Brummelen et al, 1981]. It is postulated that such a reduction in β -adrenergic sensitivity is a result of receptor downregulation in response to chronically elevated sympathetic tone (manifested by raised plasma catecholamines) [Trimarco et al, 1983; McNeil & Silagy, 1990]. This increase in sympathetic outflow may itself be a consequence of reduced central inhibition from decreased high- and low-pressure baroreceptor sensitivity (see below) [Bertel et al, 1980; Goldstein, 1983b; Shimada et al, 1985; Karemaker et al, 1989; Mancia et al,

1990]. In contrast, there is no evidence of a reduction in peripheral α -adrenergic sensitivity with age [Amann et al, 1981; Elliott et al, 1982], and indeed some evidence of increased α -adrenoceptor-mediated vasoconstriction in essential hypertension [Egan et al, 1987]. This raises the possibility that the rise in blood pressure with age is related to a reduction in β -receptor-mediated vasodilatation leaving unopposed α -receptor-mediated vasoconstriction [Buhler et al, 1982; Weber et al, 1989].

1.6 ARTERIAL BAROREFLEX SENSITIVITY

1.6.1 *The arterial baroreflex*

The arterial baroreflex is the principal mechanism of short-term blood pressure regulation and homeostasis in the human and as such has been the focus of much interest regarding the possible role of the reflex in the pathogenesis of hypertension [Sleight, 1991]. Stimulation of arterial baroreceptors by a rise in mean arterial or pulse pressure provokes a number of reflex responses intended to reverse the initial change: a vagally-mediated bradycardia and reduction in stroke volume [Sarnoff et al, 1960; Casadei et al, 1992], a reduction in sympathetic-mediated chronotropy and inotropy, relaxation of veins and dilatation of resistance arteries [Goldstein & Kopin, 1990] (Figure 1.1). The response time of this reflex is sufficiently rapid that a rise in blood pressure can delay the onset of the next P wave, with the sympathetic effects occurring a few seconds later [Pickering & Davies, 1973; Eckberg, 1976, 1980a;

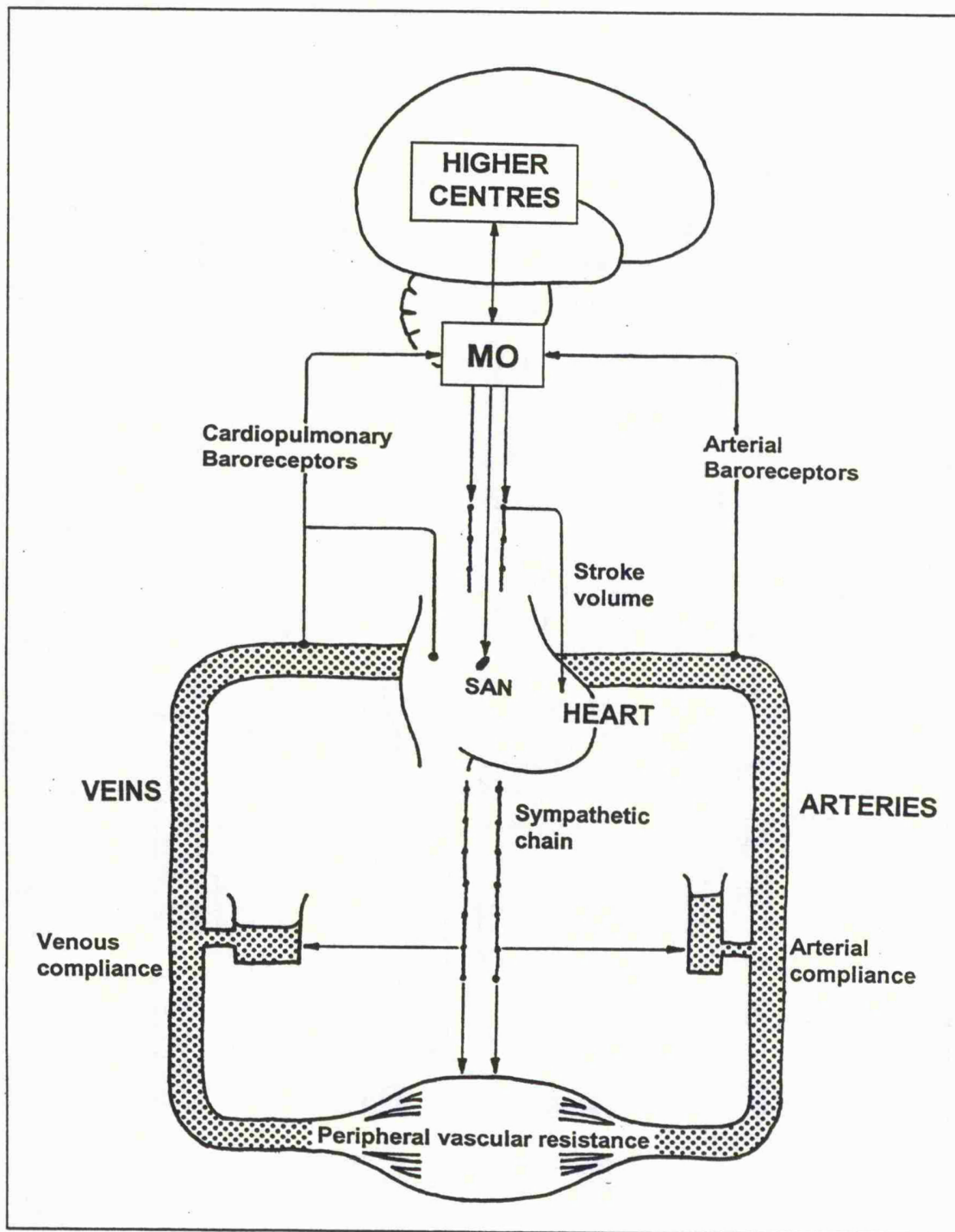


Figure 1.1 Schematic diagram illustrating the main components of cardiovascular neural control

MO: medulla oblongata; SAN: sino-atrial node

Borst & Karemaker, 1983].

Arterial mechanoreceptors are situated mainly in the carotid sinus and at branching points in the aortic arch. They are found at the junction of the medial and adventitial layers [Abraham, 1967; Rees et al, 1978]. Afferent fibres travel via the glossopharyngeal and vagus nerves to the nucleus of the tractus solitarius in the medulla oblongata, which acts as an important centre for the integration of baroreceptor and chemoreceptor reflexes and their modulation by higher centres (see Figure 1.1). Fibres from the nucleus tractus solitarius connect with the adjacent dorsal motor nucleus of the vagus and with the nucleus ambiguus, and to a lesser extent with the parabrachial nucleus, inferior olivary nucleus and the ventrolateral reticular formation [Ciriello et al, 1983]. The vagally-mediated component of the reflex involves α_2 -adrenoreceptors in the nucleus tractus solitarius [Goldstein & Kopin, 1990]. The sympathetic component depends on connection with adrenergic cells of the rostral ventrolateral medulla, and thus via the intermediolateral columns of the spinal cord to the thoracolumbar sympathetic outflow [Reis et al, 1984].

1.6.2 The arterial baroreflex and ageing

The function of the arterial baroreflex could thus be theoretically disrupted by ageing at a number of levels, both peripheral and central. Most obviously, this could occur in the afferent component of the reflex loop with changes affecting the baroreceptors in the arterial wall, with medial thickening and rigidity and the formation of atheromatous plaques in the intima. Experimentally, this has led to wide

Introduction

variations in the sensitivity of individual baroreceptors depending on their physical relation to arterial plaques [Angell-James, 1974]. Blood flow studies at the carotid bifurcation have indicated areas of increased turbulence in the carotid sinus which may predispose to endothelial damage and atheroma at this site [Heath et al, 1973; Zarins et al, 1983]. Degeneration of baroreceptor nerve fibres with ageing has been described [Abraham, 1967], and all these processes may lead to a decline in afferent baroreceptor function both with ageing and disease states.

Acute cerebrovascular disease can cause disruption of central baroreflex control apparently independent of afferent and efferent function, although this returns towards normal in the chronic phase [Appenzeller & Descarries, 1964; Korpelainen et al, 1994]. Furthermore, recent animal work on the central integration of baroreceptor afferents suggests that it may be affected by inhibition of the recently described potent vasodilator and neurotransmitter substance nitric oxide, resulting in a loss of the inhibitory effect of the baroreflex on sympathetic neural outflow [Scrogin et al, 1994; Sander et al, 1995]. This restraint of sympathetic efferent activity by the baroreflex may decline with age despite preservation of afferent baroreceptor activity [Hajduczkowicz et al, 1991; Chappell et al, 1995]. Vagal efferent reflex function has also been shown to decline with age, principally at the vagus-sinus node junction, with a progressive decline in respiratory sinus arrhythmia with age and a reduced increment in heart rate with atropine in the elderly [Dauchot & Gravenstein, 1971; Weiling et al, 1982]. The effect of age on the baroreceptor-vascular response is less well characterised, with some evidence of age-related impairment of reflex peripheral vasodilatation in response to baroreceptor stimulation (possibly as a result of

Introduction

structural alterations) [Lindblad, 1977], and others showing only a delay and not a reduction in the peripheral vascular response [Ferrari et al, 1989; Mancia et al, 1990]. Any such impairment will be the result of diminished end-organ responsiveness rather than a reduction in sympathetic outflow [Ebert et al, 1992]. The majority view is thus one of declining baroreflex function with increasing age even in healthy subjects. For instance, baroreceptor-cardiac reflex sensitivity in normal subjects aged 50-66 years is less than half that of subjects aged 19-29 [Gribbin et al, 1971; Lindblad, 1977; Shimada et al, 1985]. However, not all of the studies that have included an acceptably wide range of ages have demonstrated a significant relationship between baroreflex sensitivity and age [Parmer et al, 1992; Lage et al, 1993].

Diminished baroreflex sensitivity with ageing may also be manifested as increased blood pressure variability in the elderly. As the principal mechanism for the buffering of short-term blood pressure fluctuations through the baroreceptor-cardiac reflex, a functioning baroreflex is reflected in reduced blood pressure variability and increased heart rate variability [Watson et al, 1980; Conway et al, 1984; Mancia et al, 1986; Parati et al, 1992a, 1995a; Siché et al, 1993, 1995]. Although blood pressure variability over 24 hours is not different between young and older subjects, some workers have shown that short-term blood pressure variability (within half-hour segments) is significantly increased in older subjects, and heart rate variability decreased [Mancia et al, 1983]. Indeed age itself does not appear to increase blood pressure variability independent of the decline in baroreflex sensitivity [Mancia et al, 1986; Floras et al, 1988]. However, although diminished heart rate variability with

Introduction

ageing is a consistent finding, increased short-term blood pressure variability is not always found [Siché et al, 1993; Veerman et al, 1994]. It has been suggested that a lack of effective short-term blood pressure buffering may contribute to acute cerebrovascular disease in the elderly.

1.6.3 Relation between diminished baroreflex sensitivity and hypertension

The important role of the baroreflex in the regulation of blood pressure encouraged the view that sustained hypertension was of primarily 'neurogenic' origin. Complete or near-complete loss of the baroreflex results in a substantial increase in short-term blood pressure variability, but there remains a surprising lack of agreement as to whether this leads to sustained hypertension in the longer term [Cowley et al, 1973; Ito & Scher, 1979; Norman et al, 1981; Sleight, 1979, 1986, 1991; Karemaker et al, 1989]. Experience in humans is of course limited to case reports suggesting that hypertension may result from afferent baroreceptor damage, although between paroxysms of hypertension the blood pressure may be normal [Aksamit et al, 1987; Kuchel et al, 1987]. Sleight (1979) describes one case where increased blood pressure variability acutely following carotid body denervation was followed by sustained hypertension several years later. In experimental hypertension the baroreflex has been shown to be 'reset' to respond normally at a higher resting pressure [McCubbin et al, 1956; Munch et al, 1983]. Both carotid and aortic high pressure receptors can be reset very rapidly [Dorward et al, 1982; Burke et al, 1986], and this process is reversible [Salgado & Krieger, 1973]. The evidence now indicates that in established human hypertension the sensitivity of the

Introduction

baroreceptor-cardiac reflex is both reset at a higher pressure and simultaneously reduced in gain [Bristow et al, 1969; Gribbin et al, 1971], although not all agree that this effect is independent of age [Bevegard et al, 1977; Cowie & Rawles, 1989]. In borderline hypertension it has been observed that the reflex is reset before any reduction in gain [Eckberg, 1979]. One view is that this reduction in gain of the reflex is as a result of a diminished arterial baroreceptor sensitivity, rather than any blood pressure-dependent central or efferent dysfunction [Korner, 1989]. However, modulation of baroreflex sensitivity by central mechanisms has been demonstrated, for instance by arousal, exercise, or mental stress [Smyth et al, 1969; Sleight et al, 1978; Conway et al, 1983; Somers et al, 1987]. The presently available evidence indicates that the baroreceptor-vascular component of the reflex is only reset and not diminished in human hypertension [Mancia et al, 1990; Zanchetti & Mancia, 1991]. This may be the result of hypertension-related resistance vessel hypertrophy leading to an enhanced sympathetic response, serving to counteract any reduction in the sensitivity of this component of the baroreflex [Zanchetti, 1979; Folkow, 1982].

1.6.4 Methods for the examination of arterial baroreflexes

1.6.5 Pharmacological methods

There are two principal methods that have historically been used in human subjects to quantify baroreflex sensitivity from the reciprocal relation between changes in blood pressure and heart rate: pharmacological blood pressure alterations, and the neck chamber. The pharmacological method was first described

Introduction

by Smyth, Sleight and Pickering in 1969 for the quantification of the baroreceptor-cardiac reflex sensitivity, and involves the bolus injection of a pressor agent (angiotensin or phenylephrine) with continuous recording of the blood pressure (from an intra-arterial catheter) and heart rate response. During the period of blood pressure rise which follows 10-30 seconds after bolus injection, pulse interval progressively lengthens, and the regression coefficient for the relation of blood pressure to pulse interval is taken as an index of baroreceptor-cardiac reflex sensitivity. Systolic pressure is usually taken as the stimulus, but mean arterial pressure can also be used, and there is a slight tendency at lower values for baroreflex sensitivity to be underestimated when the systolic blood pressure is used [Gribbin et al, 1971]. Pulse pressure, although an acknowledged baroreceptor stimulus, often changes very little following pressor injection [Smyth et al, 1969]. The heart rate does not change before the blood pressure, and is unaffected if the rise in pressure is prevented, suggesting that the response is not a direct drug effect on the heart [Varma et al, 1960; Koch-Weser, 1964]. Additionally, atropine reduces or abolishes the bradycardic response and augments the pressor response to drug injection indicating that the reflex operates primarily via the vagus [Eckberg et al, 1971; Pickering et al, 1972a; Simon et al, 1977; Eckberg, 1980b; Casadei et al, 1992].

1.6.6 Objections to the pharmacological method

There are several acknowledged reservations regarding this method. Firstly, the method assumes a constant linear relation between blood pressure and heart rate

Introduction

across the full range of the response. The baroreflex can be considered to have a sigmoidal stimulus-response relationship, with threshold, approximately linear and saturation portions to the curve [Eckberg, 1980a; Korner, 1989]. The linear model is acceptable if one can be sure that the subject lies on that part of the curve [Eckberg, 1977]. Eckberg [1980a] showed that in young normotensives the systolic blood pressure tends to lie at the lower end of the linear portion, suggesting that at least in this group a linear model is acceptable for pressor responses. That this also pertains in hypertensive subjects is not proven, but is supported by the observation that the baroreceptor-cardiac response is less for a depressor stimulus than for an equivalent pressor stimulus [Pickering et al, 1972b].

Another possible objection to this method is that pressor agents may exert effects on other receptors such as cardiopulmonary receptors, or have direct effects on the arterial baroreceptors themselves. In one animal study, phenylephrine was shown to exert a direct effect on the smooth muscle of the carotid sinus, suggesting that direct interference by the drug is possible [Bergel et al, 1979]. However, the role of this effect in human subjects is unknown. Of course, pharmacological manipulations preclude the assessment of the baroreceptor-vascular response in humans because of their direct vascular effects, although more elaborate experimental preparations enable the use of drugs to measure reflex vascular responses in animal models of hypertension [Abboud, 1982].

1.6.7 The use of the pharmacological method in studies of baroreflex sensitivity

The published techniques using the pressor method for the assessment of the baroreceptor-cardiac reflex have varied to some extent. Early studies tended to use angiotensin as the pressor agent [Smyth et al, 1969; Bristow et al, 1969; Randall et al, 1976], but this has generally been replaced by the α -adrenoceptor agonist phenylephrine [Gribbin et al, 1971; Goldstein et al, 1982; Mancina et al, 1986]. Most studies have included an initial dose-ranging procedure to establish which dose of the agent produces a rise in blood pressure of 20-30 mmHg (usually between 50 and 200 μ g, but some have used up to 750 μ g) [Gribbin et al, 1971; Duke et al, 1976; Goldstein et al, 1982; Smith et al, 1987]; others have used a standard dose irrespective of the response [Conway et al, 1983; Mancina et al, 1986]. Also the resultant response has received differing mathematical treatment: regression analysis may include all beats from the time of bolus injection [Smyth et al, 1969; Bristow et al, 1969], only those beats following the onset of the pressure rise [Eckberg et al, 1971; Smith et al, 1987], or after an arbitrary delay of 10 beats [Simon et al, 1977]. This may affect the statistical significance of the regression and result in variation in the inclusion of responses; usually regressions with a value of $p < 0.05$ are included, and/or a correlation between blood pressure and pulse interval of $r > 0.65$ [Bristow et al, 1969; Takeshita et al, 1975; Palmero et al, 1981] or > 0.80 [Simon et al, 1977; Ferguson et al, 1985; Sullebarger et al, 1990]. Similarly, the regression relationship may be affected by the delay imposed between the beat-by-beat blood pressure and the subsequent pulse interval to which it is related. Some studies have used the immediately succeeding pulse interval [Duke et al, 1976;

Introduction

Ferguson et al, 1985], while others have imposed a one-beat delay [Smyth et al, 1969; Bristow et al, 1969; Goldstein et al, 1982], or a two-beat delay [Randall et al, 1976; Palmero et al, 1981], and others have taken that which in each case gives the highest correlation [Smith et al, 1987]. The choice of delay may be important because of the observation that subjects with less sensitive cardiac baroreflex responses tend to have a longer latency of the reflex and this would serve to exaggerate any differences in baroreflex sensitivity [Pickering et al, 1972b]. These mathematical stipulations create a problem with subjects in whom an adequate blood pressure rise provokes a poor pulse interval response; these subjects are doubtless of interest as they have very poor baroreflex responses, but their data are likely to be excluded by failing to satisfy the statistical criteria [Smyth et al, 1969]. The study of Goldstein (1982) did include one subject with idiopathic orthostatic hypotension whose calculated baroreflex sensitivity was 0.00, but the statistical criteria applied in this study are not specified. One way to obviate this problem is by the study of the later 'steady-state' responses rather than the immediate dynamic blood pressure 'ramp' response [Korner et al, 1974], or by the use of phenylephrine infusion rather than bolus injection [Sullebarger et al, 1990; Lage et al, 1993]. In one study phenylephrine infusion resulted in a lower value for baroreflex sensitivity than the bolus method [Sullebarger et al, 1990], but in another the value by the infusion method was greater [Ferguson et al, 1985]. Comparison of the 'steady-state' responses (the sustained reflex heart rate responses occurring during the later sustained blood pressure rise following phenylephrine injection) would allow the inclusion of subjects with only very small reflex changes [Korner et al, 1974; Mancia et al, 1977].

1.6.8 Effect of respiration

The influence of respiration on the calculation of baroreceptor-heart rate sensitivity is also a source of variation between studies. In the original description of the method, Smyth et al (1969) included beats only during expiration, and demonstrated that the calculation using both inspiratory and expiratory pulse intervals underestimated baroreflex sensitivity by about 10%. However, shortly afterwards the Oxford group abandoned the exclusion of the inspiratory portion of the respiratory cycle and included all beats [Bristow et al, 1971; Gribbin et al, 1971; Pickering et al, 1972a&b]. Other studies since then have used only expiratory beats [Eckberg et al, 1971; Simon et al, 1977; Watson et al, 1980; Goldstein et al, 1982], all beats [Smith et al, 1987], or used a smoothing technique throughout the respiratory cycle [Casadei et al, 1992]. In many studies the issue is not addressed, although the lack of mention of a pneumograph in the Methods section would imply that all beats are included [Mancia et al, 1977; Rowlands et al, 1984; Sumimoto et al, 1990; Parmer et al, 1992]. Eckberg and Orshan (1977) studied the respiratory-baroreceptor interaction and found in young normotensives an important influence of inspiratory vagal inhibition at low levels of baroreceptor stimulation (increases in carotid transmural pressure of approximately 19 mmHg) but no influence with a larger stimulus (approximately 38 mmHg). As most pressor stimuli are in the range 20-40 mmHg the influence of respiration may vary, but this issue has not been studied in hypertensive subjects or in the elderly, who manifest diminished heart rate variation with respiration [Weiling et al, 1982].

1.6.9 Reproducibility of the pharmacological methods

The reproducibility of the pressor drug method in the study of the baroreceptor-cardiac reflex has not been extensively studied. Gribbin et al (1969) restudied five hypertensive subjects after 1-15 months and found a coefficient of variation of 9%. Although the phenylephrine method has been widely used for the study of drug effects, no other study has addressed the issue of long-term reproducibility, so the powers of such comparative studies are not known [Mancia et al, 1982; McLeay et al, 1983; Harron, 1989]. Parati et al (1983) studied the blood pressure and heart rate responses to bolus injections of phenylephrine and sodium nitroprusside (as a depressor stimulus: see below) over a three-hour experimental session and found coefficients of variation for the baroreceptor-cardiac reflex of between 19 and 28%. Goldstein et al (1982) demonstrated considerable inter- and intra-individual variation in baroreflex sensitivity assessed by different methods and at the very least this indicates the need to calculate baroreflex sensitivity from the mean of a number of injections. Some studies, including that of Goldstein et al, are unclear on the number of injections used, while others have used the mean of two [Mancia et al, 1977] or as many as 34 [Smyth et al, 1969]. Most studies use the mean of at least three responses. Where infusions have been used, steady state data are usually obtained from only one infusion [Sullebarger et al, 1990; Muratani et al, 1990; Lage et al, 1993]. The possibility that intra-individual variation in baroreflex sensitivity is greater in the elderly remains but this has not been explored, although it may have methodological implications for studies in this age group.

1.6.10 Pharmacological depressor methods

The baroreceptor-heart rate reflex also operates when blood pressure varies in the opposite direction i.e. a fall in blood pressure is accompanied by a tachycardia. This tachycardia is also of reflex parasympathetic origin and is blocked by atropine [Pickering et al, 1972a], and the use of a pharmacological depressor agent (amyl nitrate inhalation, trinitroglycerin injection or sodium nitroprusside injection or infusion) has also been used to study the reflex. Pickering et al (1972b) compared the reflex responses to phenylephrine-induced pressor and amyl nitrate-induced depressor stimuli and made two observations: firstly that the baroreflex sensitivity calculated from a hypotensive stimulus is approximately 40% of that calculated from a hypertensive one, and secondly the latency of the response is greater for amyl nitrate than phenylephrine. With regard to this second observation, one has to consider the differing modes of administration (amyl nitrate inhalation vs. phenylephrine bolus injection), and that the depressor response received slightly different statistical treatment to the pressor response. Nonetheless, most other studies that have included reflex responses in both directions (including the use of trinitroglycerin or sodium nitroprusside bolus injection) have agreed with this finding [Goldstein et al, 1982; Goldstein, 1983b; Mancina et al, 1986]. The study by Parmer and colleagues (1992) found no difference in pressor and depressor reflexes using amyl nitrate inhalation in normotensive and hypertensive subjects, and, intriguingly, the study by Mancina and coworkers (1977) states in the text that there were no differences in baroreflex slopes between phenylephrine and trinitroglycerin blood

Introduction

pressure alterations but the data in an accompanying table demonstrate a significant difference, with a depressor response on average two-thirds that of the pressor response. The use of sodium nitroprusside infusion permits the assessment of later, steady-state responses, and the data from this method are more diverse. Lage et al (1993) found no relationship between age and baroreflex sensitivity as measured by phenylephrine and sodium nitroprusside infusions, but the results for the pressor and depressor stimuli were pooled thus preventing analysis of the separate directions of the reflex. Muratani et al (1990) found no differences with age in the heart rate response to sodium nitroprusside infusion in hypertensives, but a significant difference with age in the response to phenylephrine infusion. The results with sodium nitroprusside are consistent with those of McGarry et al (1983). This latter study is discussed in more detail in a later section.

Most of the reservations concerning the phenylephrine method apply similarly to the use of hypotensive agents, except that a depressor stimulus has been used much less frequently in the assessment of the baroreceptor-cardiac reflex. In particular, the consistent finding of a reduced cardioacceleration in response to hypotensive stimulus does suggest that the reflex is asymmetric about its set point, with the baroreceptor-cardiac reflex being more efficient at buffering rises than falls in blood pressure both in normotensives and hypertensives [Pickering et al, 1972b]. This is compatible with the resetting concept of baroreflexes, where the setpoint occupies the same position on the sigmoid stimulus-response curve albeit at a higher resting pressure, rather than moving to a different point on the same curve.

1.6.11 The neck chamber method

An alternative method for the assessment of carotid baroreceptor reflexes is the use of the neck chamber [Eckberg et al, 1975; Eckberg, 1980b]. The application of external suction to the region of the carotid sinus in the neck via an airtight chamber increases the transmural pressure in the carotid sinus and activates the baroreceptors. Conversely, externally applied pressure above atmospheric unloads the baroreceptors. The use of the neck chamber has been extensively studied by Eckberg, particularly the carotid baroreceptor-cardiac reflex component [Eckberg et al, 1992]. Short-lasting stimuli delivered via the chamber demonstrate a reflex latency of less than 0.5 seconds, with adaptation after approximately 2 seconds [Eckberg, 1976; 1977; 1980a]. Using the neck chamber technique, Eckberg (1979) demonstrated that in borderline hypertensives, the baroreflex was reset, but loss of gain did not occur until higher pressures were attained, offering support for a causal role for baroreceptor dysfunction in the genesis of hypertension.

The neck chamber technique has the advantage of permitting the measurement of the blood pressure responses to baroreceptor stimuli, something that cannot be done using vasoactive drugs [Zanchetti, 1979]. Using this technique, the Milan group have studied the baroreceptor-vascular reflex in normotensive and hypertensive subjects, and demonstrated no overall loss of sensitivity of that component of the reflex with hypertension [Mancia et al, 1978]. However, they did demonstrate that whilst for the normotensives the blood pressure rise with baroreceptor unloading was greater than the blood pressure fall with baroreceptor

Introduction

stimulation, in hypertensives the converse applied, with a greater blood pressure response to an increase in carotid transmural pressure. This suggests that the set point of the baroreceptor-vascular reflex has moved to another portion of the stimulus-response curve, and in hypertension the baroreflex becomes more efficient at buffering rises than falls in blood pressure. Interestingly, in the same study Mancia et al (1978) demonstrated similar changes to the pattern of heart rate responses i.e. a greater bradycardic response to an increased transmural pressure in hypertensives than normotensives, and a greater tachycardic response in the normotensives to simulated hypotension. As mentioned above, this is the converse of the findings for drug-induced responses in hypertension [Pickering et al, 1972b].

The neck chamber technique itself has a number of potential problems. Most notably, although the stimulus is delivered to the region of the carotid sinus, other arterial baroreceptors (principally aortic) will tend to mitigate the responses by reflex changes in the opposite direction. This may be responsible at least in part for the rapid decay of carotid sinus-cardiac responses within a few seconds [Eckberg, 1980b]. Additionally, the pressure changes in the chamber may be incompletely transmitted to the carotid sinus, causing variability in the extent to which carotid sinus transmural pressure is altered. This problem was addressed by Ludbrook et al (1977) who found that on average 86% of a positive pressure and 64% of a negative pressure were transmitted to the tissues adjacent to the carotid sinus. However, a further correction to take account of the induced changes in blood pressure is required before the actual change in carotid transmural pressure can be estimated [Mancia et al, 1978].

1.6.12 Reflexes from carotid and extracarotid baroreceptors

The use of the neck chamber does enable an estimate to be made of the relative roles of the carotid and aortic baroreceptor areas in the reflex regulation of heart rate and blood pressure. In normotensives, Mancina et al (1977) found the heart rate responses to changes in carotid transmural pressure to be approximately one third of the response to an equivalent drug-induced stimulus (both pressor and depressor stimuli were examined). However, in hypertensives they found that the heart rate responses to equivalent stimuli from the neck chamber and vasoactive drugs were not different [Mancina et al, 1978]. This led them to conclude that the aortic baroreceptor-cardiac reflexes were preferentially impaired in hypertension, with preservation of the carotid baroreceptor-cardiac reflex. These findings have been questioned [Sleight, 1979] and have not been replicated. Ferguson et al (1985) used the neck chamber to counteract drug-induced blood pressure changes in young normotensives, and found that the carotid baroreflex contributed approximately 40-70% of the heart rate response to steady-state blood pressure changes (phenylephrine infusion) and 30% of the dynamic heart rate response (phenylephrine bolus injection).

1.6.13 The Valsalva manoeuvre

The relationship between the rapid rise in blood pressure following the release of the Valsalva strain and the accompanying bradycardia has been used as an index of

Introduction

arterial baroreflex sensitivity [Pickering & Sleight, 1969]. The circulatory effects of the Valsalva manoeuvre are a complex mixture of mechanical and reflex effects [Korner et al, 1979; Eckberg, 1980b] that are traditionally divided into four phases [Hamilton et al, 1936]. Following release of the increased intrathoracic pressure sustained during the strain period (Phase 2), pooled blood in the venous system returns centrally and is ejected into an arterial system still intensely vasoconstricted. After an initial inhibition of the blood pressure response (Phase 3) probably mediated by low-pressure cardiopulmonary receptors, there follows a steep rise in blood pressure to peak at values above the resting pressure provoking arterial baroreceptor stimulation and a reflex bradycardia (Phase 4). This is vagally mediated as it is blocked by atropine [Korner et al, 1979; Eckberg, 1980b]. In an approach identical to that for drug-induced pressure alterations, the slope of the linear regression of pulse interval on blood pressure during phase 4 is taken as an index of the arterial baroreceptor-cardiac reflex sensitivity, and this method has been used in many studies of younger subjects [Pickering & Sleight, 1969; Palmero et al, 1981; Goldstein et al, 1982; Shimada et al, 1985; Smith et al, 1987; Horodyski et al, 1995]. However, this technique has virtually never been used to assess baroreflex sensitivity in the elderly; to date there is only one study of elderly subjects where baroreflex sensitivity has been measured using the Valsalva manoeuvre (see below)[Kawamoto et al, 1989].

As with the pharmacological methods of baroreflex sensitivity testing, there are important differences in the manner in which the Valsalva manoeuvre is performed and analysed. Apart from methodological variations in the duration and magnitude

Introduction

of the strain period [Eckberg, 1980b], the calculated value for baroreflex sensitivity obtained from phase 4 varies greatly according to the statistical treatment adopted. The most important difference is in the portion of phase 4 that is included in the linear regression analysis. Pickering and Sleight (1969) used only those beats in the later part of phase 4, during the overshoot when the systolic blood pressure exceeds the level before the manoeuvre. However, Goldstein and colleagues (1982) included all beats from the lowest blood pressure after the release of the Valsalva strain (the beginning of phase 4) to the peak value at the end of phase 4 and correlated this with the pulse interval with one beat delay, and also used a similar analysis to derive baroreflex sensitivity from phase 2. Horodyski (1995) also included all beats in phase 4 but imposed no delay. Others have used non-linear modeling of the relationship between pulse interval and blood pressure during phase 2 and phase 4 of the manoeuvre (McAloney et al, 1987). Shimada et al (1985, 1986) specified only that they used linear regression during the 'later period in phase 4', probably adopting a method similar to that of Palmero et al (1981), whom they cite, of excluding the initial two or three beats of phase 4 during which no appreciable prolongation of pulse interval occurs. This has the effect of steepening the blood pressure-pulse interval slope and producing a higher value for baroreflex sensitivity, but is highly subjective. The work of Palmero et al (1981) is widely cited as a method [Shimada et al, 1985, 1986; Smith et al, 1987; Rahman et al, 1991] and deserves further scrutiny. They studied nine healthy subjects, ten hypertensives and 25 patients with Chagas disease. A comparison was made with the phenylephrine method as described by Bristow et al (1969), with baroreflex sensitivity being determined from the response to a single bolus injection. They identified a highly

Introduction

significant correlation ($r = 0.91$) between the values for baroreflex sensitivity derived from one Valsalva manoeuvre (using a one-beat delay) and the phenylephrine methods, which is impressive given the variability in results obtained from one injection discussed above. Although they compared the two methods using an unsatisfactory technique [Bland & Altman, 1986], it is evident from their accompanying graph that the values at least lie close to the line of identity. They state that the calculation of baroreflex sensitivity from the whole of phase 4 gives a value about half that obtained by arbitrary exclusion of the first few beats. A similar comparison was made by Smith et al (1987) who found that using all beats from phase 4 tended to underestimate the phenylephrine-derived baroreflex sensitivity at higher values, while using only the overshoot tended to overestimate, although not to the 2.7-fold extent described by Pickering and Sleight (1969). Smith et al also demonstrated that the best relationship between blood pressure and pulse interval may require a variable delay: for calculations involving the whole of phase 4, the delay was usually two or three beats, and for the overshoot the best correlations were either with no delay or one beat only. It may be, however, that any attempt to closely relate baroreceptor-cardiac reflex sensitivity obtained by the Valsalva and phenylephrine methods is in vain, as there may be some differences in the mechanisms involved, and as Eckberg states, 'the simplicity of the Valsalva manoeuvre is illusory' [Eckberg, 1980b]. The validity of the Valsalva method has not been studied in a specifically elderly sample, and the only study to use this method in elderly hypertensives, that of Kawamoto and colleagues (1989), receives more detailed examination in a later section.

1.6.14 Other techniques for the measurement of baroreflex sensitivity

More recently two methods have been described for the assessment of baroreflex sensitivity that obviate the need for drug injections. The first of these involves the computer-scanning of long recordings of beat-to-beat blood pressure and pulse interval for spontaneously occurring sequences of three beats or more where a rise in blood pressure is accompanied by a lengthening of pulse interval, or a fall in blood pressure is associated with a shortening of pulse interval [Parati et al, 1988, 1992b, 1995a; Steptoe & Vögele, 1990; Hughson et al, 1993; Parlow et al, 1995]. This technique was first explored in animals [Bertinieri et al, 1988] where it was shown that these sequences represented approximately a quarter of all beats occurring during a three-hour continuous recording period, but that following sino-aortic denervation such sequences were almost entirely abolished. In man such sequences become steeper but less frequent at night, and are reduced both in number and in slope in hypertension, thus corresponding with the findings with the pharmacological measurement of the baroreceptor-heart rate reflex [Smyth et al, 1969; Gribbin et al, 1971; Parati et al, 1988, 1995a; Parlow et al, 1995]. One small study has also demonstrated a reduction with ageing in baroreflex sensitivity assessed by such sequence analysis [Parati et al, 1995a]. The second technique also involves the analysis of continuous beat-to-beat blood pressure and pulse interval recordings to break down the overall variability into its constituent frequencies using power spectral analysis [De Boer et al, 1985a&b, 1987]. This enables the linkage between the blood pressure and pulse interval signals to be

Introduction

quantified in terms of phase (the time shifts between signals) and coherence (the coefficient of determination between the signals within a specified frequency band). In animals, coherence between the two signals is lost following sino-aortic denervation in the mid-frequency range (around 0.1 Hz; sometimes called low-frequency), suggesting that the baroreflex is largely responsible for the coupling of signals in this region [Parati et al, 1992b]. Vagal blockade almost entirely abolishes the high frequency band (around 0.25 Hz) of pulse interval variability indicating that this component is principally of vagal origin [Akselrod et al, 1981; Pomeranz et al, 1985; Baselli et al, 1994]. It is possible to describe the gain of the transfer function between changes in blood pressure and changes in heart rate in the mid-frequency band (usually between 0.05 or 0.07 Hz and 0.15 Hz; expressed in msec/mmHg) in the frequency domain as equivalent to the regression coefficient used to quantify baroreflex sensitivity in the time domain [Robbe et al, 1987]. Another method computes the ratio 'alpha' between the spectral powers of blood pressure and pulse interval where the two signals are coherent (this is usually the case in the mid- and high-frequency bands in the intact animal and human) [Cerutti et al, 1987; Pagani et al, 1988; Hughson et al, 1993; Parati et al, 1992b, 1995b; Lucini et al, 1994]. Parati and colleagues (1992b) compared baroreflex gain (in the mid-frequency band) measured by this technique with that as assessed by sequence analysis in 24-hour intra-arterial recordings in ambulant human subjects and showed them to be similar. Moreover, a considerable degree of agreement is claimed between the spectral analysis-derived parameter alpha (in both the mid- and high-frequency bands) and baroreflex gain by the phenylephrine technique [Pagani et al, 1988]. In the study of Robbe et al (1987), they found that baroreflex sensitivity at rest assessed by spectral

analysis was very close to that obtained from phenylephrine injection in eight young normal subjects. Radaelli et al (1994) showed significantly reduced baroreflex sensitivity in young and middle-aged hypertensive subjects compared to normal subjects using power spectral analysis. These newer techniques thus show considerable promise in the evaluation of neural circulatory control, although several theoretical and methodological issues remain unresolved and the techniques have been rarely evaluated in the elderly [Takalo et al, 1994; Malliani et al, 1994; Veerman et al, 1994; Sleight et al, 1995; Parati et al, 1995b].

1.6.15 Comparison and relation between methods

Goldstein et al (1982) made a comparative study of several methods for the evaluation of baroreflex sensitivity: phenylephrine and nitroglycerin drug injection, the Valsalva manoeuvre, and neck chamber pressure and suction. In general the level of agreement between methods was low, and although it was improved when stimuli in the same direction were considered (e.g. phenylephrine injection and neck suction) the authors cautioned against the use of only one method in drawing conclusions about the overall functioning of the arterial baroreflex. As a general rule, the phenylephrine method is regarded as the 'gold standard', presumably because it was the first to be described rather than for any physiological reasons, against which other techniques are compared for agreement and accuracy. Thus other studies have sought to compare the phenylephrine method with baroreflex sensitivity from phase 4 of the Valsalva manoeuvre [Pickering & Sleight, 1969; Palmero et al, 1981; Smith et al, 1987], neck chamber techniques [Mancia et al, 1978], or sequence and

Introduction

spectral analyses [Robbe et al, 1987; Pagani et al, 1988; Parlow et al, 1995]. Some striking differences are apparent from the different studies: for example, Palmero et al (1981) found a highly significant correlation between baroreflex sensitivity from the phenylephrine method and from phase 4 of the Valsalva manoeuvre of 0.91 whereas in the study of Goldstein et al (1982) the correlation between the same parameters was 0.27. In the latter study the correlation between baroreflex sensitivity from the phenylephrine method and from nitroglycerin injection was only 0.18. The authors propose that the reasons for poor agreement between the various methods may be either that baroreflex sensitivity varies widely within and between subjects, or that the different techniques measure different aspects of baroreflex function. While this is certainly true for the carotid baroreceptor-blood pressure and -heart rate reflexes elicited by the neck chamber (see above), the reasons for such disparity between, for example, pressor and depressor drug injection are less clear. However, it is apparent that caution should be exercised in believing that the full range of baroreflex (or even just baroreceptor-heart rate) function or dysfunction can be described with the results from one method.

1.6.16 Arterial baroreceptor studies in the elderly

Although, as already mentioned, most but not all of the available evidence would indicate a progressive decline in baroreflex function with increasing age amongst younger subjects [Gribbin et al, 1971; Korner et al, 1974; Lindblad, 1977; Shimada et al, 1985; Parmer et al, 1992; Lage et al, 1993], there are comparatively few data on baroreflex sensitivity in the elderly (for these purposes the term will refer to subjects

Introduction

older than 60 years). Thus two studies of the effect of ageing on baroreflex function [Gribbin et al, 1971; Duke et al, 1976] have included respectively one and two patients older than 60 years. While these studies yield valuable information regarding the relation between ageing and baroreflex sensitivity, the results cannot necessarily be extrapolated to the elderly. There have been a small number of studies which have included some form of baroreflex assessment in the elderly and these will be considered in some detail as they highlight some important methodological differences and some startling statistical errors (Table 1.1) [McGarry et al, 1983; Rowlands et al, 1984; Jansen & Hoefnagels, 1989; Kawamoto et al, 1989; Sumimoto et al, 1990].

McGarry et al (1983) studied baroreflex function in elderly hypertensives by a unique method, often cited but never repeated. They studied a total of 27 patients in three groups: 7 young and middle-aged normotensives, 10 young and middle-aged hypertensives, and 10 elderly hypertensive subjects ranging in age from 66 to 80 years. Blood pressure and heart rate were recorded by conventional sphygmomanometry and ECG (supine and standing) during stepwise increases of a sodium nitroprusside infusion. No specific attempt was made to relate steady-state changes in blood pressure to changes in pulse interval in the supine position, but the available data indicate a baroreflex sensitivity of 2.4 msec/mmHg in younger normotensives, 1.0 msec/mmHg in younger hypertensives and 0.84 msec/mmHg in the elderly hypertensive subjects. For data obtained in the erect position, the slopes of the regression of change in pulse interval on fall in blood pressure were 5.3 msec/mmHg in the younger normotensives, 3.9 msec/mmHg in the younger hypertensives and

Introduction

2.0 msec/mmHg in the elderly hypertensive subjects. Whereas there was no difference between the young and old hypertensive groups in the supine position, there was no significant difference between the young normotensives and hypertensives in the erect position. This would suggest no effect of age in one position and no effect of hypertension in the other, creating a number of problems with the interpretation. The authors' demonstration that baroreflex sensitivity rises with standing is at variance with other data [Pickering et al, 1971; Steptoe & Vögele, 1990; Lucini et al, 1994], and this perhaps serves to emphasize that circulatory changes with standing cannot be viewed solely as descriptive of arterial baroreflex function, but also involve low-pressure cardiopulmonary receptors and other receptor areas [Eckberg, 1980b]. The absence of a group of elderly normotensive subjects prevents any analysis of the independent effects of blood pressure in an age-matched elderly group.

In the study of Rowlands et al (1984), a comparison was also made using three groups: 13 elderly hypertensive subjects (ranging in age between 60 and 90 years), 13 elderly normotensives, and an unspecified number of young hypertensives who were matched retrospectively for similar intra-arterial daytime mean blood pressure with the elderly hypertensive group. The number of young hypertensives that had to be studied in order to identify such a matched group is not given. Unfortunately, the subjects chosen were not typical of the populations they were purported to represent: four of the elderly hypertensives and five of the elderly normotensives were originally referred for unexplained falls, and three of the normotensives had originally been referred for evaluation of elevated blood pressure. The manner in

Introduction

which casual blood pressure or the period over which it was characterised are also not described (apart from the use of phase IV Korotkoff sounds) but an accompanying graph indicates casual blood pressures averaging (mean \pm standard deviation) $205 \pm 20 / 133 \pm 8$ for the elderly hypertensives, $155 \pm 25 / 90 \pm 15$ for the elderly normotensives and $160 \pm 10 / 103 \pm 8$ for the younger hypertensives. Evidently at least some of the 'normotensive' subjects did indeed have hypertension as we now understand it, although how sustained these blood pressures were is not clear.

The authors assessed baroreceptor-cardiac reflex sensitivity by the method of Smyth et al (1969), although the use of a pneumograph or exclusion of inspiratory beats, recording method for blood pressure and pulse interval, the number of phenylephrine injections and methods of analysis are not specified in any further detail. Baroreflex sensitivity in the elderly hypertensives was 2.05 ± 1.30 msec/mmHg, in the elderly 'normotensives' 4.44 ± 2.97 msec/mmHg and in the younger hypertensives 8.59 ± 4.76 msec/mmHg. The results for the elderly hypertensives are reported as significantly lower, but the authors erroneously used multiple Student's t-tests throughout the study for the comparison between the three groups, raising the risk of a type I error [Altman, 1991]. The data suggest that a non-parametric comparison such as the Kruskal-Wallis test would have been more appropriate.

In the study of Kawamoto et al (1989) already mentioned, baroreflex sensitivity was measured by the blood pressure and pulse interval responses during both

Introduction

phase 2 and phase 4 of the Valsalva manoeuvre [Goldstein et al, 1982]. In total they studied 72 subjects in three groups: 30 hypertensives of mean age 66 years, 30 normotensives of mean age 65 years, and 12 young normotensives of mean age 23 years. They found that baroreflex sensitivity from phase 2 of the Valsalva manoeuvre was not different between young and old normotensives, but was significantly lower in the elderly hypertensive group. By contrast, they found that baroreflex sensitivity from phase 4 was not different between the two elderly groups, but both were significantly lower than the young group. This again suggests no effect of age with one method, and no effect of hypertension with another. Unfortunately, these comparisons were once again made using multiple two-sample tests, this time the Mann-Whitney test, although bizarrely Student's t-test was also simultaneously used on the same data, which were evidently not normally distributed. The authors conclude on the basis of their data that hypertension has a marginal, if any, further influence on the baroreflex sensitivity in elderly subjects whose regulatory mechanisms are already substantially altered by advanced age.

In a study of the possible causes of post-prandial hypotension in elderly subjects, Jansen and Hoefnagels (1989) assessed baroreflex sensitivity in 10 young normotensives (mean age 26 years), 15 elderly normotensives (mean age 76 years) and 15 elderly hypertensive subjects (mean age 73 years) before and after a glucose load. They measured blood pressure and heart rate using the Finapres, a non-invasive instrument for the measurement of beat-to-beat blood pressure from the finger arteries (see Appendix I), although the exact method of data capture is not given. They used the phenylephrine and nitroglycerin bolus injection method

Introduction

described by Goldstein et al (1982), and found that baroreflex sensitivity by the phenylephrine pressor method was not different between elderly normotensives and hypertensives, but that by the nitroglycerin depressor method there was a significant reduction in the elderly hypertensive group when compared to the elderly normotensive group. Baroreflex sensitivity in both elderly groups, by both methods, was significantly lower than in the young normotensive group. Unfortunately, the data indicate that their study had insufficient power to detect a difference in baroreflex sensitivity between the elderly groups by the phenylephrine method, thus risking a type II error of rejecting the hypothesis of a difference when it could still be true.

Sumimoto et al (1990) studied both arterial compliance and arterial baroreflex sensitivity in 31 elderly subjects in three groups: 7 normotensives, 12 hypertensives (sustained diastolic blood pressure >95 mmHg since middle age) and 12 subjects with isolated systolic hypertension (systolic blood pressure > 160 mmHg with diastolic blood pressure < 90 mmHg of relatively recent onset). The groups were ostensibly matched but the subjects with isolated systolic hypertension were slightly, but significantly, older by 3.6 years. Furthermore, after seven days of hospital admission preceding the actual day of study, most of the hypertensive subjects (both diastolic and isolated systolic hypertension) had normal blood pressures. Baroreflex sensitivity was assessed by the method of Smyth et al, but again insufficient detail is included of the number of injections or the method of analysis of the blood pressure and heart rate responses. Baroreflex sensitivity was 5.7 ± 1.9 msec/mmHg in the normotensives, 2.4 ± 1.6 msec/mmHg in the hypertensives, and 2.0 ± 1.7

msec/mmHg in the subjects with isolated systolic hypertension. Analysis of variance with Duncan's multiple range test showed that baroreflex sensitivity was not different between the two types of hypertension, but both were significantly lower than the normotensives.

Table 1.1 summarises the results obtained from the studies described above. The available evidence is somewhat conflicting. Notwithstanding the methodological and statistical reservations outlined above, the data suggest, far from conclusively, a number of possibilities. By the phenylephrine pressor method, baroreflex sensitivity is either lower in elderly hypertensives than normotensives [Sumimoto et al, 1990], or not different [Jansen & Hoefnagels, 1989]. However, the latter study suggests that in response to a depressor stimulus, baroreflex sensitivity is lower in elderly hypertensives than normotensives, while the study of McGarry et al (also using a depressor stimulus) suggests that baroreflex sensitivity is not different between young and elderly hypertensives but a comparison cannot be made with elderly normotensives. Baroreflex sensitivity from phase 4 of the Valsalva manoeuvre does not appear to be different between elderly hypertensive and normotensive subjects [Kawamoto et al, 1989].

Study	n	Method	Baroreflex sensitivity (msec/mmHg)				Comments
			Elderly NT	Elderly HT	Elderly ISH	Young NT / Young HT	
McGarry et al (1983)	27	SNP depressor	-	0.84	-	2.4 / 1.0	Supine / Standing
Rowlands et al (1984)	26 + ?	Pressor	4.44 ± 2.97	2.05 ± 1.30	-	- / 8.59 ± 4.76	Mean ± SD; pressor agent not specified
Kawamoto et al (1989)	72	Valsalva Ph 2 Valsalva Ph 4	2.5 ± 0.6 2.3 ± 0.4	1.7 ± 0.4 2.7 ± 0.4	-	4.0 ± 1.1 / 8.7 ± 0.4	Mean ± SEM
Jansen et al (1989)	40	PE pressor NG depressor	8.0 ± 0.9 6.3 ± 0.9	5.8 ± 0.9 3.1 ± 0.4	-	30.4 ± 2.7 / 14.9 ± 2.2	Mean ± SEM
Sumimoto et al (1990)	31	PE pressor	5.7 ± 1.9	2.4 ± 1.6	2.0 ± 1.7	-	Mean ± SD; most subjects in fact NT

NT: normotensive; HT: hypertensive; ISH: isolated systolic hypertension; SNP: sodium nitroprusside; PE: phenylephrine; Ph 2/4: phase 2 or 4 of the Valsalva manoeuvre; NG: nitroglycerin

Table 1.1 Baroreflex sensitivity in elderly and young hypertensive and normotensive subjects

Introduction

1.6.17 Other factors modulating the arterial baroreflex

Thus far the discussion has been principally concerned with the effect of two factors on the baroreflex - age and blood pressure, although modulation of the baroreflex by other physiological factors (sleep, posture, exercise, mental stress and others) and genetic factors has been mentioned [Smyth et al, 1969; Bristow et al, 1971; Pickering et al, 1971; Sleight et al, 1978; Pagani et al, 1988; Parmer et al, 1992]. There is also much recent animal evidence indicating the modulation of peripheral baroreceptors by a variety of humoral agents related to hypertension, including noradrenaline [Munch et al, 1987], atrial natriuretic peptide [Jin et al, 1992], vasopressin [Schmid et al, 1985], aldosterone [Wang et al, 1991], prostaglandins [Chen et al, 1990; Xie et al, 1990], endothelin [Chapleau et al, 1992], aggregating platelets [Li et al, 1992] and oxygen free radicals [Chapleau et al, 1995]. While baroreceptors are activated by arterial wall stretch in the absence of endothelium, a modulating effect of the endothelium in transducing pressure changes into changes in nerve activity is increasingly apparent [Chapleau, 1991]. Endothelial dysfunction in chronic hypertension and atheroma with loss of sensitising influences may contribute to diminished baroreceptor sensitivity in these and other disease states, and with ageing [Xie et al, 1990].

1.6.18 Cardiopulmonary reflexes: effects of ageing and hypertension

Animal and human studies indicate that circulatory control and short-term blood pressure homeostasis depend not only on the arterial baroreflexes, but also on low

Introduction

pressure receptors situated in the heart and pulmonary circulation [Mark & Mancia, 1983]. These receptors respond to alterations of central venous pressure and cardiopulmonary blood volume via vagal afferent nerve fibres to alter sympathetic vasomotor tone [Abboud, 1979]. The reflex also affects plasma vasopressin levels and renin release from the kidney to modify blood volume in the longer term [Kiowski & Julius, 1978; Grassi et al, 1988a; Berdeaux et al, 1992]. The cardiopulmonary reflex is therefore important in the compensatory response to standing, and to simulated orthostatic stresses such as head-up tilt and the application to the lower body of a negative pressure to cause venous pooling in the lower limbs. However, other receptor areas may also be involved in the circulatory response to postural changes (vestibular and cerebellar receptors) [Doba & Reis, 1974; Eckberg, 1980b] and the main test for the selective unloading of cardiopulmonary receptors is the use of non-hypotensive lower body negative pressure (–5 to –15 mmHg) which avoids stimulation of arterial baroreceptors [Abboud, 1979; Thompson et al, 1991]. Alternatively, the low-pressure receptors can be stimulated by passive leg raising [Grassi et al, 1988b].

The effects of ageing and hypertension on the cardiopulmonary-vascular reflex are less well documented, but would appear to be broadly in parallel with those for the high-pressure arterial baroreflex. Thus the cardiopulmonary reflex is impaired by hypertension [Trimarco et al, 1986; Grassi et al, 1988b; Zanchetti & Mancia, 1991], and with increasing age [Ferrari et al, 1989; Cl eroux et al, 1989; Kuwajima et al, 1990]. There is evidence that the forearm vascular response to cardiopulmonary baroreceptor stimulation is augmented in borderline hypertension [Abboud, 1982;

Introduction

Mark & Kerber, 1982] but with a persistence of the hypertensive state a progressive impairment occurs, possibly through a reduction in the sensitivity of cardiac receptors with the development of reduced ventricular compliance [Trimarco et al, 1986; Grassi et al, 1988b]. In the study by Grassi et al (1988b), the authors demonstrated a significant improvement in the responsiveness of the cardiopulmonary reflex (both in terms of forearm vascular responses and plasma renin activity) when long-term anti-hypertensive drug therapy achieved regression of left ventricular hypertrophy. Normotensive athletes with left ventricular hypertrophy also appear to have reduced cardiopulmonary reflexes [Giannattasio et al, 1990]. Following cardiac transplantation there is substantial diminution of cardiopulmonary reflexes, again suggesting the predominant role of cardiac receptors in the reflex [Mohanthy et al, 1987]. Loss of left ventricular compliance with ageing may also be the origin of an age-related impairment of cardiopulmonary reflexes, even in normotensive subjects [Gerstenblith et al, 1977].

1.6.19 The tilt test, and other tests of cardiovascular reflexes

As mentioned above, the mechanisms behind the circulatory adaptation to postural change are complex. With active standing or passive head-up tilt, central venous and right atrial pressures fall, and there is a reduction in stroke volume and cardiac output of about 20% [Matalon & Farhi, 1979]. Carotid mean arterial pressure falls by about 15-20 mmHg, and aortic pressure by about 5 mmHg, so there is differentiated stimulation of arterial baroreceptors. Consequently efferent vagal activity falls, and sympathetic outflow rises together with heart rate [Esler, 1995]. It

Introduction

is likely that the initial heart rate response is modified by other receptors in addition to arterial baroreceptors [Doba & Reis, 1974; Eckberg, 1980b; Borst et al, 1982]. High pressure and cardiopulmonary reflexes act to raise plasma noradrenaline, peripheral vascular resistance and plasma renin. One study has suggested that arterial baroreflexes may predominantly increase splanchnic vasoconstriction, while cardiopulmonary reflexes act principally on the skeletal muscle and renal vasculature [Abboud et al, 1979]. Similar effects are seen with passive head-up tilt when compared to active standing apart from the initial alterations in heart rate and blood pressure which are provoked by voluntary effort [Borst et al, 1982, 1984].

With ageing several alterations occur in the response to postural change. There is a progressive decline in the heart rate response to tilting or standing [Dambrink & Weiling, 1987; London et al, 1987; Weiling et al, 1992; Tonkin & Wing, 1994; Yo et al, 1994], which is partly compensated by a greater rise in peripheral vascular resistance [London et al, 1987; Weiling et al, 1992]. Thus in the healthy elderly systolic blood pressure is usually little changed by postural change [Dambrink & Weiling, 1987; Hainsworth & Al-Shamma, 1988; Tonkin & Wing, 1994] and diastolic blood pressure may rise [Dambrink & Weiling, 1987; Hainsworth & Al-Shamma, 1988]. In hypertension, there also appears to be a diminished heart rate response to tilting, but London et al (1987) demonstrated that hypertensive subjects with already higher baseline peripheral vascular resistance responded to tilt with larger increments in peripheral resistance than normotensives, and suggested that this may be due to hypertension-related structural alterations in the resistance vasculature amplifying the sympathetic response [Folkow, 1973; Radaelli et al, 1994]. However,

Introduction

the study of Yo et al (1994) demonstrated that the sympathetic-vascular response to passive tilting was reduced in hypertension regardless of age.

The evidence of further impairment of cardiovascular reflex responses to tilting with hypertension in the elderly is mixed. Vardan et al (1994) found that subjects with isolated systolic hypertension showed a significant fall in systolic blood pressure with 45° head-up tilt, but Tonkin & Wing (1994) did not. Rowlands et al (1984) showed greater reductions in blood pressure with tilt in hypertensive than in normotensive elderly subjects, but the difference was not statistically significant. The results of the studies of Tonkin & Wing (1994) and Cléroux et al (1989) included a demonstration of no age-related differences in the cardiovascular response to non-baroreceptor mediated stresses such as isometric exercise and the cold pressor test. Rowlands et al (1984) also showed no difference in the responses to the cold pressor test and isometric exercise between elderly normotensives and hypertensives. Despite their differing findings, both Vardan et al (1994) and Tonkin & Wing (1994) concluded that there was no evidence of additional impairment of baroreceptor-mediated cardiovascular responses in subjects with isolated systolic hypertension beyond the effects of age.

1.7 RESISTANCE VESSEL STRUCTURE AND FUNCTION

The role of structural alterations in the resistance vasculature as an adaptive response to high blood pressure, and the implications for increased peripheral

Introduction

resistance, have been acknowledged for many years [Short, 1966; Folkow, 1973; 1995]. These structural alterations have already been referred to in the above text in terms of the effects they may have on sympathetically-mediated vascular responses. More recently, the significance of functional changes in the resistance vasculature, and in particular the role of the endothelium in modulating vascular smooth muscle tone, have received attention as possibly playing a part in the pathogenesis of hypertension, including hypertension in the elderly [Applegate & Rutan, 1992; Byyny, 1995]. This area of research has arisen mainly from the initial observations of Furchgott and Zawadzki (1980) of the crucial role of the endothelium in mediating the relaxation of blood vessels. The raised peripheral resistance that occurs with both ageing and hypertension may therefore have two, related origins in structural and functional alteration.

1.7.1 Resistance vessel structure and hypertension

The haemodynamics of established essential hypertension indicate a rise in peripheral vascular resistance even when the predominant alteration is a loss of large artery compliance, as in the elderly hypertensive subject [Koch-Weser, 1973; Berger & Li, 1990]. This increase in vascular resistance implies an effective narrowing of the pre-capillary circulation as capillary pressure in hypertensives and normotensives are approximately similar [Williams et al, 1990; Mulvany, 1994]. It remains to some extent controversial whether the main pressure drop occurs either in the small arteries (those with lumen diameter of approximately 150-300 μm) or the arterioles, and human evidence in this area is lacking [Davis et al, 1986; Mulvany &

Aalkjaer, 1990]. Some workers have suggested that small arteries are responsible for approximately 40% of the pre-capillary pressure drop, and arterioles contribute a further 30% [Bohlen, 1986; Schiffrin, 1992]. Other animal studies have indicated that the arterioles are the main region for the regulation of vascular resistance, at least in skeletal muscle, with micropuncture measurements of the pressure in feed arteries showing values up to 70-90% of central pressure, indicating that the main fall in pressure is distal to this point [Zweifach et al, 1981]. Such observations may vary according to the vascular bed that is studied, and progress has been hampered by methodological difficulties, not least that pressure measurement techniques exclude blood flow from the vessel in which they are being recorded [Heagerty & Izzard, 1995].

In human subjects, structural alteration of small arteries in the form of an increased wall thickness:lumen diameter ratio with hypertension has been confirmed by histological studies [Short, 1966], and is also suggested by the increased minimal vascular resistance of the forearm circulation of hypertensives when the vasculature is fully relaxed in reactive hyperaemia, and by the increased pressor responsiveness to infused agonists [Doyle & Black, 1955; Folkow et al, 1958; Conway, 1963; Mulvany, 1994]. Further confirmation of this relationship has recently been obtained by Agabiti Rosei and colleagues (1995) who demonstrated a good correlation between forearm minimal vascular resistance and media:lumen ratio of omental and subcutaneous small arteries in hypertensive and normotensive subjects. Studies such as this have been made possible by recent methodological advances enabling the *in vitro* study of the morphology of resistance vessels retrieved from omental or

Introduction

gluteal biopsies in human subjects [Mulvany & Halpern, 1977; Aalkjaer et al, 1986; Aalkjaer, 1993]. Such studies have demonstrated an increased wall:lumen ratio in human hypertension together with an increase in maximum constrictor responses to substances such as noradrenaline, which when corrected for the increased wall thickness ('active media stress') show no difference between hypertensive and normotensive subjects [Aalkjaer et al, 1987; Korsgaard et al, 1991; Schiffrin et al, 1992; Agabiti Rosei et al, 1995].

Such structural adaptations do not rule out a role for arterial and arteriolar rarefaction in hypertension, with a possible reduction in the absolute number of vessels in parallel also contributing to increased peripheral resistance [Schiffrin, 1992; Shore & Tooke, 1994]. There are reports of vascular rarefaction in the vascular beds of some hypertensive animals, and arteriolar density in the conjunctivae of human hypertensives has been shown to be reduced, although not early in the course of hypertension [Harper et al, 1978; Sullivan et al, 1983; Vicaut, 1992].

The nature of the structural alterations in the resistance bed appears to vary between experimental animal hypertension and human hypertension. In the spontaneously hypertensive rat (SHR) luminal encroachment results from both rearrangement of existing smooth muscle cells (remodeling) and vascular growth [Mulvany et al, 1978; Heagerty et al, 1993]. The evidence from human studies indicates that although luminal diameter is reduced and media:lumen ratio increased in hypertension, media cross-sectional area remains approximately the same,

Introduction

suggesting that remodeling predominates and there is very little, if any, cellular hypertrophy or hyperplasia [Short, 1966; Korsgaard et al, 1991; Schiffrin, 1992; Heagerty et al, 1993; Cooper et al, 1993].

The main stimulus to these adaptive changes has traditionally been regarded as the prevailing mean arterial pressure, based among other things on the observation of parallel increases in forearm vascular resistance and mean arterial pressure [Folkow et al, 1958]. However, recent evidence from the microscopic study of resistance vessels from various sites in experimental hypertension suggests it may be the pulse pressure that is more closely related to vascular alteration than the mean blood pressure [Baumbach, 1991]. In one study, Christensen (1991) treated SHR with a variety of antihypertensive agents (the vasodilator hydralazine, the beta-blocker metoprolol, the calcium blocker isradipine and the angiotensin-converting enzyme (ACE) inhibitors perindopril and captopril) and observed that structural regression in the mesenteric arteries of the SHR was greatest in those animals with the greatest reductions in pulse pressure with treatment. These tended to be the animals given either one of the ACE inhibitors or hydralazine. His data showed a highly significant correlation between on-treatment pulse pressure (measured intra-arterially over 24 hours) and media:lumen ratio of $r = 0.64$. The metoprolol-treated rats showed reductions in systolic and diastolic blood pressure but no change in pulse pressure, and these animals showed no reduction in media:lumen ratio by comparison with untreated SHR controls. The analysis used in this study has attracted criticism for failing to distinguish independent blood pressure effects from the effect of treatment group [Harrap & Forbes, 1993] and the methodology is

Introduction

intriguing in that metoprolol had no effect in the rat on heart rate, suggesting that this group was perhaps inadequately treated. In another study, Hajdu and colleagues (1990) studied the effects of treatment with hydralazine and captopril on the structure of cerebral arterioles in the stroke-prone SHR. Again both drugs had similar effects in reducing pulse pressure while hydralazine was less effective in reducing mean arterial pressure, yet both drugs were equally effective in preventing hypertrophy of the cerebral arterioles. Other work from the same group [Baumbach et al, 1991, 1994; Baumbach & Heistad, 1991] has shown a similar effect in preventing medial hypertrophy in the stroke-prone SHR with carotid clipping, which normalises cerebral artery pulse pressure but not mean arterial pressure. Similarly Bund et al (1991) have shown that when the femoral artery of the SHR was partially constricted by a ligature, structure of distal resistance vessels was normalised by comparison with the contralateral limb fed by an unstricted artery. Other coarctation studies have resulted in a reduction in the DNA and collagen content in the distal aortic wall [Bomberger et al, 1980]. When vascular smooth muscle cells are grown in tissue culture and subjected to cyclical stretching, they display greater DNA synthesis and rate of growth than cells grown under static conditions [Leung et al, 1976; Nakayama et al, 1986]. These studies raise interesting questions on the relation between the different parameters of blood pressure and vascular structural adaptation, and suggest that the cyclical stresses exerted by the pulse pressure may have an important influence on the vascular alterations that accompany hypertension.

These findings may have particular relevance to the elderly in view of the effects of large arterial stiffness in raising systolic and pulse pressure (see above) and its

Introduction

consequences for the resistance bed. Currently in humans the only available data testing this relationship are from a younger pooled group of 57 hypertensive and 70 normotensive subjects published only in abstract form, which do not suggest a strong relation between small artery structure and pulse pressure [Cooper et al, 1993]. The correlation between media:lumen ratio and pulse pressure was $r = 0.21$, while that for mean arterial pressure was $r = 0.49$. However, the relevance of the findings of Cooper et al to the elderly can be questioned. In their study pulse pressure was measured casually, and the period of observation and the number of readings made is not given. What is more, although in young subjects pulse pressure in peripheral arteries tends to be higher than in central arteries, increasing arterial rigidity means that this difference declines with age so that above the age of 50 years pulse pressure tends to be similar in central and peripheral arteries [Safar & Laurent, 1993; O'Rourke, 1990]. Therefore whereas in younger subjects brachial blood pressure may be a poor indicator of the pressure operating in the small arteries, in the elderly the two values will be closer. Given the changing blood pressure profile with increasing age previously discussed, the effects of a widened pulse pressure on cardiovascular structural adaptation both in the form of left ventricular hypertrophy and in the distal small arteries needs to be considered in this age group. A correlation between elevated pulse pressure and left ventricular hypertrophy has been demonstrated [Pannier et al, 1989], and it may be that the role of the pulsatile component of the blood pressure in hypertensive target organ effects has been neglected in favour of the steady component, the mean arterial pressure. These findings need to be interpreted in the context of recent epidemiological evidence that the pulse pressure is a greater risk factor than mean arterial pressure

for future cardiovascular events including myocardial infarction [Vadhavan et al, 1994] and stroke [Darne et al, 1989; Scuteri et al, 1995].

1.7.2 Function of the endothelium

The endothelium is a single layer of cells on the luminal side of all blood vessels. It is now recognised as vital to the regulation of vascular smooth muscle tone, growth, platelet function and coagulation and cellular chemotaxis [Lüscher, 1994]. The endothelium converts angiotensin I to angiotensin II, inactivates circulating bradykinin, noradrenaline and serotonin, and releases factors that inhibit platelet aggregation and both relax and contract vascular smooth muscle [Tolins et al, 1991]. Furchgott and Zawadzki (1980) were the first to demonstrate that the potent vasorelaxant effect of acetylcholine on arterial rings was lost if the endothelium was removed, and proposed that the endothelium released a substance, originally termed endothelium-derived relaxing factor (EDRF) and later shown to be nitric oxide (NO) or a closely related nitrosothiol, that in turn acted on the vascular smooth muscle to produce relaxation [Palmer et al, 1987, 1988; Furchgott, 1990]. The action of EDRF/NO in producing vascular smooth muscle relaxation through the activation of soluble guanylate cyclase (sGC) to produce cyclic 3',5'-guanosine monophosphate (cGMP) is similar to the long-observed vasodilating action of the 'nitrovasodilators' such as sodium nitroprusside and nitroglycerin and led to its identification as nitric oxide [Vane et al, 1990]. EDRF/NO also increases cGMP in platelets thereby inhibiting platelet adhesion and aggregation [Lüscher & Dubey, 1995]. Nitric oxide is synthesized from L-arginine by the enzyme NO synthase and its production is blocked

Introduction

by analogues of L-arginine such as L-N^G-monomethyl-L-arginine (L-NMMA), L-N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NOARG) [Rees et al, 1989a; Woolfson & Poston, 1990; Bennett et al, 1992] (Figure 1.2). The observation of a rise in blood pressure and a reduction in regional blood flow with the *in vivo* infusion of L-NMMA in young human subjects would indicate a tonic role for the L-arginine-nitric oxide pathway in the maintenance of a baseline vasodilation, and inhibition or dysfunction of this pathway may produce sustained hypertension as seen in animal models [Vallance et al, 1989; Rees et al, 1989b; Johnson & Freeman, 1992; Panza et al, 1993a; Haynes et al, 1993; Lüscher, 1994; Sander et al, 1995; Castellano et al, 1995]. Whilst acetylcholine is the classical endothelium-dependent vasorelaxant, its physiological role *in vivo* is still uncertain. What is known is that carbachol, a specific M₂-muscarinic agonist, evokes responses of similar magnitude to acetylcholine, indicating the presence of such receptors on endothelial cells. Bradykinin also produces endothelium-dependent relaxation via the release of EDRF/NO, acting on receptors of the B₂ subtype, but acts by a different G protein signal transduction pathway [Flavahan, 1992].

There is evidence, however, that not all endothelium-dependent vasodilation is produced by EDRF/NO. In porcine coronary arteries, L-NMMA only slightly inhibits the relaxation response to bradykinin, suggesting the release of another relaxing factor distinct from nitric oxide [Bény & Brunet, 1988; Richard et al, 1990]. This property may reflect the presence of an endothelium-derived hyperpolarising factor activating adenosine triphosphate (ATP)-sensitive potassium channels in smooth

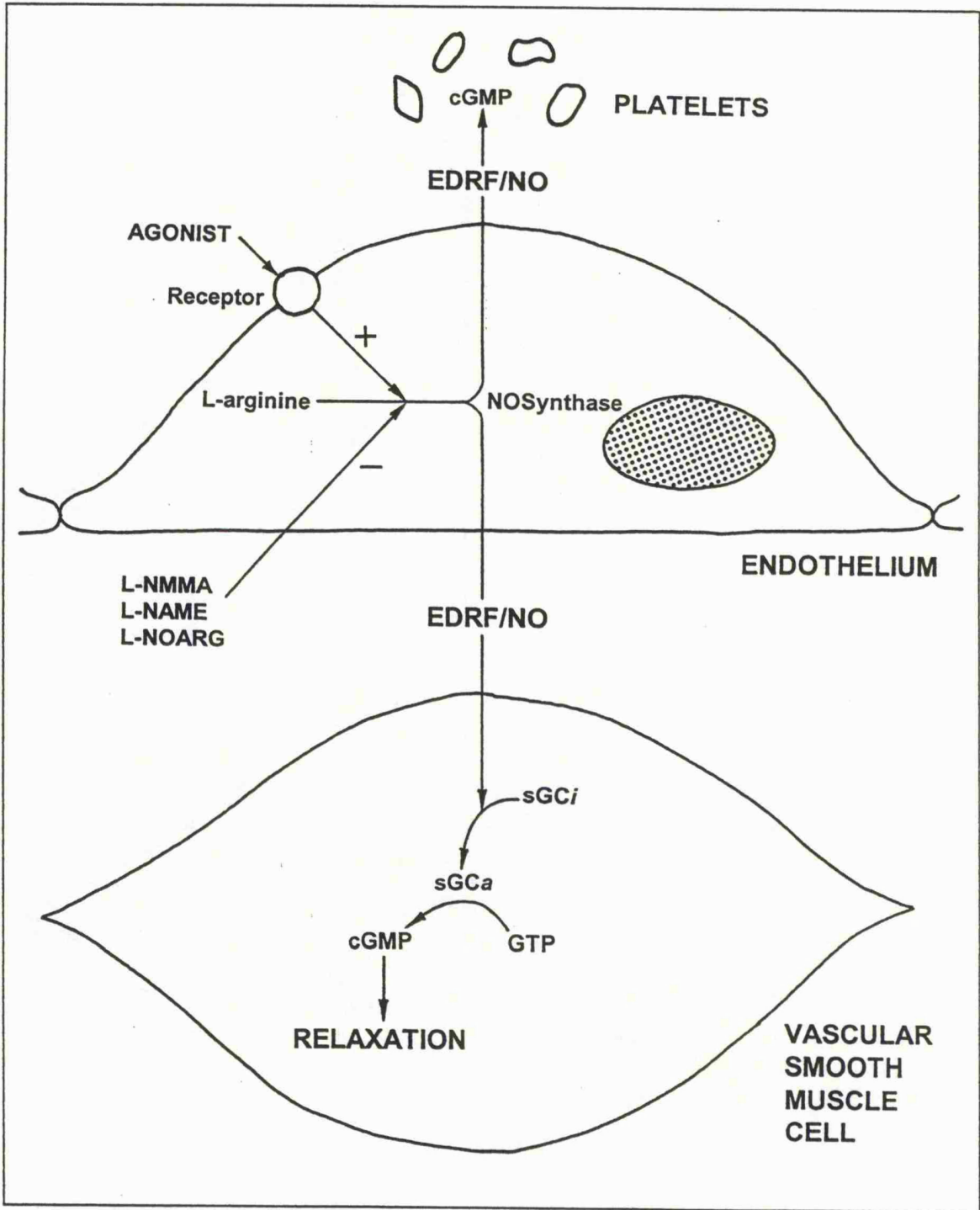


Figure 1.2 Simplified diagram illustrating the main components of the vascular L-arginine-nitric oxide pathway

EDRF/NO: endothelium-derived relaxing factor/nitric oxide; L-NMMA: L-N^G-monomethyl-L-arginine; L-NAME: L-N^G-nitro-L-arginine methyl ester; L-NOARG: N^G-nitro-L-arginine; sGC: soluble guanylate cyclase (*i*: inactive, *a*: active); GTP: guanosine triphosphate; cGMP: cyclic 3',5'-guanosine monophosphate.

Introduction

muscle, with a parallel role for non-nitric oxide mediated endothelium-dependent relaxation [Feletou & Vanhoutte, 1988; Standen et al, 1989; Lüscher & Dubey, 1995]. In recent human studies, Deng et al (1995) have demonstrated that only 30% of the relaxation induced by acetylcholine is mediated via EDRF/NO, with the remainder resulting from release of another factor, possibly endothelium-derived hyperpolarising factor. Furthermore, prostacyclin, the main product of the endothelial cyclooxygenase pathway which opposes the aggregating influence of thromboxane A₂ in platelets [Radomski et al, 1987], acts directly on vascular smooth muscle to increase cyclic 3',5'-adenosine monophosphate (cAMP) and produce relaxation, thereby acting by an alternative second messenger to the L-arginine-nitric oxide pathway [Moncada & Vane, 1979]. Prostacyclin also potentiates the action of EDRF/NO and stimulates its release from endothelial cells [Lüscher & Vanhoutte, 1990]. Bradykinin may also stimulate cyclooxygenase causing the production of prostacyclin, and in some vessels this is the major mechanism behind bradykinin-induced relaxations [Cherry et al, 1982] but this is not thought to be the case in humans [Richard et al, 1990; Yang et al, 1991; Lüscher & Dubey, 1995].

Since the identification of EDRF/NO, it has become clear that the endothelium also produces substances with vasoconstricting properties. Among these are the prostaglandins thromboxane A₂ and PGH₂, superoxide anions O₂⁻, locally active angiotensin II, and the 21-amino-acid peptide endothelin [Yanagisawa et al, 1988; Kutasic & Vanhoutte, 1989; Kato et al, 1990; Tolins et al, 1991]. Unlike EDRF/NO, detectable levels of circulating endothelin can be found in humans, suggesting systemic effects, with possibly increased levels in essential hypertension [Kohno et

al, 1990]. This also suggests a dynamic balance between endothelium-derived relaxing and contracting factors in the regulation of vascular tone and growth.

Vascular smooth muscle cell contraction also occurs in response to noradrenaline release from adrenergic nerves activating α -receptors, although in ring preparations extraluminally-applied noradrenaline may reach endothelial cells to cause an endothelium-dependent relaxation [Tesfarmariam & Halpern, 1988]. In a number of arteries and species, endothelial removal or blockade of NO synthase with NOARG potentiates the response to α -agonists [Lüscher & Vanhoutte, 1990; Bennett et al, 1992]. Preventing noradrenaline reuptake by the blockade of presynaptic α_2 -receptors with cocaine should increase synaptic noradrenaline concentrations with a concomitant increase in sensitivity. Angiotensin II also provokes vascular smooth muscle contraction, although animal experiments suggest that this effect is mitigated by stimulation of endothelial prostacyclin synthetase which blunts the direct action of the peptide [Lüscher & Vanhoutte, 1990]. Angiotensin II may be generated *in vivo* from circulating angiotensin I or locally by the vascular renin-angiotensin system.

1.7.3 Endothelial function in hypertension

This topic has been excellently reviewed by Lüscher and Vanhoutte (1990). In brief, several alterations of endothelial structure and function have been observed in hypertension. Endothelial cells are subjected to abnormal forces of stretch and shear stress, and no longer provide the smooth 'pavement' appearance of normotensive vessels, but bulge unevenly into the lumen with increased fibrin and

Introduction

cells in the subintimal space. In the SHR, endothelium-dependent relaxation of vascular smooth muscle by acetylcholine is impaired, and at higher concentrations such vessels develop contractions [Lüscher & Vanhoutte, 1986]. Such contractions are prevented by the cyclooxygenase inhibitor indomethacin, suggesting that the primary abnormality is not an impaired release of EDRF/NO, but simultaneous release of a cyclooxygenase dependent contracting factor. What is more, bioassay experiments suggest that the perfused SHR aorta produces comparable amounts of EDRF/NO [Lüscher et al, 1986]. In human hypertension, impaired endothelium-dependent relaxation to acetylcholine (usually assessed by the changes in forearm blood flow following brachial artery drug infusion) has been demonstrated in most but not all studies, with normal smooth muscle function confirmed by unimpaired relaxation to sodium nitroprusside [Panza et al, 1990, 1993a; Cockcroft et al, 1994; Taddei et al, 1995; van Zwieten et al, 1995]. Similar findings have been made in studies of isolated human resistance artery preparations [Falloon & Heagerty, 1994]. Whether these abnormalities are a primary cause or secondary effect of hypertension remains to be established [Schiffrin, 1992], but of relevance is the recent finding of impaired endothelium-dependent forearm vasodilatation in normotensive subjects with a family history of hypertension [Taddei et al, 1992].

A reduction in the basal release of EDRF/NO in essential hypertension is suggested by the finding of reduced forearm vasoconstriction with brachial artery infusion of the L-arginine analogue L-NMMA [Vallance et al, 1989; Calver et al, 1992]. Furthermore, the diminished response to infused acetylcholine in hypertension is not reversed by L-arginine [Panza et al, 1993b], suggesting a

Introduction

primary defect of the L-arginine-NO pathway, particularly as in humans, unlike the SHR, endothelium-dependent relaxations are not restored by inhibition of cyclooxygenase-dependent contracting factors by indomethacin (see above).

Studies of endothelin in human hypertension have produced differing results, with some showing no increase in plasma levels and others an increase [Lüscher et al, 1992a]. Plasma levels may be a poor indicator of local effects of the peptide, as most endothelin is not released into the lumen. Vascular responsiveness to endothelin also varies with the arterial site and the experimental preparation [Lüscher et al, 1992a], so the role of circulating and local endothelin, particularly at the very low concentrations recorded, remains to be clarified.

Enhanced vascular smooth muscle sensitivity to vasoconstrictor agonists has been perceived for some time as contributing to increased peripheral resistance [Abboud, 1982]. This smooth muscle defect has not been apparent in recent human resistance artery studies which have shown either unchanged or depressed excitation-contraction coupling with hypertension, with the increased force development being almost entirely accounted for by the alterations in structure [Aalkjaer et al, 1986, 1987]. The study of Aalkjaer et al, 1987, did suggest increased neuronal noradrenaline reuptake with hypertension (as indicated by a greater increase in noradrenaline sensitivity with cocaine in hypertensive subjects).

1.7.4 Endothelial function and ageing

There are data obtained from a variety of methods in both experimental and human studies regarding the influence of ageing on endothelial function and the interaction of this factor with hypertension. Tschudi et al (1991) found no difference in either serotonin concentration-dependent contractions or endothelium-dependent and independent relaxations in coronary arteries from SHR aged 16 and 32 weeks. However, they were unable to exclude the possibility that endothelial dysfunction occurred at a very old age in the SHR model of hypertension [Lüscher & Dubey, 1995]. Hongo et al (1988) demonstrated impairment of acetylcholine endothelium-dependent relaxation with both ageing and hypertension in carotid arteries from Wistar-Kyoto rats and SHR. Mayhan and colleagues (1990) also found impaired endothelium-dependent relaxation (to both acetylcholine and bradykinin) in cerebral arterioles in aged Wistar rats, with no difference in nitroglycerin-induced endothelium-independent relaxation with age. Similarly, sodium nitroprusside-induced vasodilatation was unaffected by age or hypertension in aortae from young and old Wistar-Kyoto rats and SHR in the study of Koga et al (1989). This study demonstrated augmented cyclooxygenase and thromboxane A₂-dependent contractions at higher doses of acetylcholine in the older animals, but the significance of these acetylcholine-related contractions to human hypertension is not yet clear. In angiographically-normal human coronary arteries, Yasue et al (1990) have demonstrated that in younger subjects intracoronary injection of acetylcholine is accompanied by significant vasodilatation, whereas in older subjects (ranging in age from 31-68 years in this study) there was a constrictor response. A further

Introduction

group of subjects with demonstrable atherosclerosis also showed vasoconstriction to acetylcholine, which has been reported by other workers [Ludmer et al, 1986; Werns et al, 1989]. There were no differences with age in the response to nitroglycerin, whereas Egashira et al (1993) have shown an additional age-related decline in endothelium-independent relaxation in angiographically normal coronary arteries. There are methodological problems in making comparisons between all these studies, particularly in the patient groups selected and in the doses of drugs used.

Alternative methods for the study of endothelial function include the measurement of forearm blood flow responses to brachial artery drug infusion, or the assessment of brachial artery diameter by ultrasound. Using this latter method, Celermajer and colleagues (1994) described an age-related decline in endothelium-dependent arterial vasodilatation that was delayed in women, possibly due to protective effects of oestrogens, while there was no similar decline in endothelium-independent responses. This study showed that by the age of 65 years all subjects demonstrated endothelial dysfunction, and that by this age the women had 'caught up' with the men. Recently Taddei et al (1995) studied forearm blood flow responses to brachial artery drug infusion in normocholesterolaemic normotensive subjects and essential hypertensive patients in an age range from 20 to 78 years. They found a significant fall with age in the maximal vasodilating response to acetylcholine in both normotensive and hypertensive subjects, and also a less pronounced but statistically significant decline with age in the response to sodium nitroprusside. However, they made no direct comparisons between normotensive and hypertensive subjects within each age group.

Introduction

There are well recognised problems with the interpretation of all the above data on ageing and endothelial function. The application of data obtained from one species to another is always a potential difficulty, as is the comparison between studies at different arterial sites [van Zwieten et al, 1995]. Certainly the predilection for atheroma in the coronary rather than the brachial circulation makes generalisations about the whole arterial tree difficult, although a close relationship between the two sites is possible [Uehata et al, 1993]. In humans, studies of the coronary circulation have naturally been restricted to subjects with clinical coronary artery disease, even if angiographically normal. These studies also risk confounding by other factors that may modulate endothelial function such as hypercholesterolaemia [Vita et al, 1990; Zeiher et al, 1993]. Notwithstanding these reservations, a reasonable interim position would suggest that ageing in humans is associated with impairment of endothelium-dependent relaxation with relative preservation of endothelium-independent responses [Lüscher et al, 1992b]. The work of Taddei et al (1995) would suggest an independent effect of age on endothelial function in both normotensive and hypertensive subjects [Salvetti et al, 1995]. However, there are no published data on the separate effect of hypertension in an elderly population already affected by endothelial dysfunction, and no data on the role of alternatives to the L-arginine-NO pathway in the vasodilating responses of human resistance arteries. Thus the contribution of endothelial dysfunction to the overall picture of hypertension in the elderly is difficult to assess on the current evidence.

1.8 AMBULATORY BLOOD PRESSURE

Technical advances over the last 30 years have enabled the measurement of blood pressure outside the clinic setting using portable recorders. The first of these, the Remler recorder, was used to demonstrate that blood pressure measured under ambulatory conditions correlated more closely with target organ damage, and ultimately prognosis, than casual measurements [Sokolow et al, 1966; Perloff et al, 1983; Pickering, 1990]. Such recorders, technically refined over time, are now widely available and provide information on blood pressure over prolonged (usually 24 hour) periods by automatic intermittent recording by either an auscultatory or oscillometric technique. An alternative method for the measurement of continuous beat-to-beat blood pressure by cannulation of the brachial artery provides more information but its invasive nature means it is likely to remain only a research tool [Bevan et al, 1969; Mancia et al, 1993].

Ambulatory blood pressure monitoring (ABPM) has a number of important advantages over clinic blood pressure measurement:

1. The increased number of recording taken over a 24-hour period (usually between 48 and 72 readings) greatly increases the reproducibility of ambulatory blood pressure measurement [Thijs et al, 1992b; Fotherby & Potter, 1993; Engfeldt et al, 1994; Mansoor et al, 1994; James et al, 1995]. Together with a reduction in or abolition of the placebo effect [Dupont et al, 1987; Mutti et al, 1991], this among other things enables studies of antihypertensive interventions using considerably

smaller number of subjects [Conway et al, 1988].

2. ABPM identifies a proportion of subjects judged to be hypertensive on the strength of their clinic readings whose blood pressure outside the clinic setting is consistently normal - so-called 'white coat' hypertension [Harshfield et al, 1982; Pickering et al, 1988].

3. ABPM provides data on blood pressure variability which is not available from clinic readings [Parati et al, 1992a; Mancia et al, 1993]. Recordings made with readings taken at 15 minute intervals yield measures of variability (the standard deviation of all the readings) that are close to those obtained from continuous intra-arterial monitoring [Di Rienzo et al, 1983]. In the context of baroreceptor sensitivity, this observation may be useful in studying the inverse relationship between baroreflex sensitivity and blood pressure variability (see above). Furthermore, blood pressure variability may also be independently related to target organ damage in hypertension [Parati et al, 1987; Palatini et al, 1992; Frattola et al, 1993].

4. Twenty-four hour recordings can provide clinically useful information about blood pressure during the entire period, particularly night-time blood pressure, which is not available either from clinic readings or shorter monitoring periods. A diminished or absent decline in blood pressure at night is more frequent in cases of secondary hypertension [Baumgart et al, 1989; Stewart & Padfield, 1992; Middeke & Schrader, 1994], and blunting of the normal circadian pattern of blood pressure may have pathological implications (see below).

1.8.1 Ambulatory blood pressure monitoring in the elderly

Several of the features of ambulatory blood pressure monitoring described above are of particular value in elderly subjects. Firstly, ABPM permits the more accurate diagnosis of hypertension in the elderly when compared to assessments based solely on clinic measurements. Inaccuracies in clinic blood pressure measurement may be greater in elderly subjects because of the observed increases in blood pressure variability and reactivity with age [Mancia et al, 1983]. Shimada et al (1990) performed ABPM during clinic visits in elderly hypertensives and found average rises of 17 mmHg in systolic and 7 mmHg in diastolic blood pressure while patients were being examined by the doctor. Thus, in a study of 81 elderly subjects with clinic systolic blood pressure ≥ 160 mmHg and diastolic blood pressure ≤ 90 mmHg and 39 clinic normotensives, Ruddy et al (1988) found that 42% of the hypertensives had daytime ambulatory blood pressure less than the 90th centile of the normotensive group. Other workers have also found substantial discrepancies particularly between systolic blood pressure measured in the clinic and by ambulatory techniques [Silagy et al, 1990; Prager et al, 1991; Cox et al, 1991a&b; Thijs et al, 1992b]. In the European study of older patients with isolated systolic hypertension (SYST-EUR), the average discrepancy between clinic and daytime ambulatory systolic blood pressure was 21 mmHg although diastolic blood pressure was similar by the two methods [Thijs et al, 1992b].

The accurate description of blood pressure over longer periods also allows a better assessment of the effects of antihypertensive drug treatment in the elderly.

Introduction

This has been an area of particular concern given the extra hazards of overtreatment in this age group [Jackson et al, 1976; Traub et al, 1976; Jansen et al, 1986]. In view of the circadian variation in onset of ischaemic cardiovascular disease, nocturnal blood pressure measurement may be particularly relevant [Marshall, 1977; Muller et al, 1985]. The possibility that ischaemic events are precipitated in susceptible persons by the blood pressure falling below a critical level has been proposed to explain the J-shaped relationship between mortality and diastolic blood pressure reduction seen in some studies [Cruikshank et al, 1987; Floras, 1988; Alderman et al, 1989; Madhavan et al, 1994], and this has been one of the reasons behind a reluctance to treat isolated systolic hypertension in the elderly. Nevertheless, the Systolic Hypertension in the Elderly Program (SHEP) demonstrated significant reductions in both stroke and myocardial infarction by treating a group of elderly subjects with isolated systolic hypertension with an initial *clinic* diastolic blood pressure of 77 mmHg and a final pressure of 68 mmHg [SHEP Cooperative Research Group, 1991; Pickering, 1993]. A causal link between low nocturnal blood pressure and cardiovascular mortality and morbidity remains to be confirmed.

Analysis of 24 hour recordings provides further information about the circadian rhythm in blood pressure, with a fall in blood pressure at night of 20% or more [Millar-Craig et al, 1978]. This pattern is replicated in most hypertensives albeit at a higher overall level [Mancia et al, 1983], but subjects with a loss or blunting of this normal blood pressure decline at night may be at additional cardiovascular risk. Left ventricular mass is greater in hypertensive subjects with a higher nighttime blood

Introduction

pressure and a reduced day-night blood pressure difference [Verdecchia et al, 1990; Palatini et al, 1992; Kuwajima et al, 1992; James et al, 1994] and elderly subjects with loss of the normal nocturnal blood pressure fall have a higher risk of hypertensive end-organ damage such as cerebrovascular disease and stroke [Kobrin et al, 1984; O'Brien et al, 1988; Toghi et al, 1991; Shimada et al, 1992]. The general view is of a progressive reduction in the fall in blood pressure at night with increasing age, mainly through a gradual increase in nocturnal blood pressure rather than any alteration in diurnal blood pressure [Khoury et al, 1992; James et al, 1994; Imai & Abe, 1994; Fotherby & Potter, 1995a].

A rise in recorded blood pressure at night may be a reflection of poorer sleep quality and duration in elderly subjects. Sleep disturbance is the most commonly reported side-effect of ABPM, and its effect on nocturnal blood pressure and the circadian blood pressure pattern remains controversial and may be age-dependent. Electroencephalogram (EEG) recordings do register arousal in response to cuff inflation, but there is disagreement as to whether this causes alteration in recorded blood pressure. Davies and colleagues (1994) found some sharp increases in systolic blood pressure with automatic cuff inflations in young normotensive subjects, but Schwan & Eriksson (1992) and Degaute et al (1992) detected EEG arousal but no effect on blood pressure. Villani et al (1992) found no alteration in nocturnal blood pressure with ABPM by comparison with continuous intra-arterial recordings. None of these studies included elderly subjects, so it is not yet possible to draw firm conclusions regarding the possible attenuation of nocturnal blood pressure in this age group.

1.8.2 Validation of ambulatory blood pressure monitors in the elderly

The clinical significance attached to the recording of ambulatory blood pressure presupposes that such measurements are made accurately. Conventional blood pressure measurement with the standard sphygmomanometer is acknowledged to have particular problems in the elderly [O'Callaghan et al, 1983] and it is possible that the same is true for automated recordings. Miller and colleagues (1992) compared simultaneous measurements with the Accutracker II ambulatory blood pressure system and conventional sphygmomanometry in 103 subjects ranging in age from 23 to 92 years, and demonstrated that the inaccuracies in measurement of systolic blood pressure with the automatic recorder increased with increasing age and level of systolic blood pressure. Pannarale et al (1993) found a similar effect of increasing age and systolic blood pressure when studying the SpaceLabs 90207 oscillometric monitoring device in 118 subjects between 17 and 94 years of age. Fotherby et al (1995b) found in a specifically elderly population of mean age 76 years that systolic measurement error increased with the level of systolic blood pressure, and diastolic error increased with increasing age. O'Brien et al (1991, 1993a) stratified their subjects by tertile of blood pressure and also found that the accuracy of most automatic devices (both oscillometric and auscultatory) declined in the upper tertile (clinic blood pressure $\geq 160/100$ mmHg). Naturally the greatest use of ambulatory blood pressure recording in assessing diagnosis or efficacy of treatment is in subjects with blood pressure in this upper range. Guidelines for the

Introduction

evaluation of ambulatory blood pressure recorders have been modified to address the related issues of validation in all age groups and at all levels of blood pressure [O'Brien et al, 1990, 1993b].

1.8.3 ABPM in the assessment of blood pressure variability in the elderly

As mentioned above, it is possible to obtain information regarding blood pressure variability from intermittent recordings taken with ambulatory monitors [Parati et al, 1992a; Mancia et al, 1993]. Blood pressure variability can be assessed with ABPM from the standard deviation of daytime blood pressure, and correction made for the level of blood pressure with the coefficient of variation (the standard deviation as a percentage of the mean). In younger subjects the blood pressure variability depends to a large extent on the level of blood pressure, so the coefficient of variation is not different between hypertensive and normotensive subjects [Watson et al, 1980; Mancia et al, 1983]. In the elderly, Muneta et al (1991) made a comparison of daytime blood pressure and heart rate variabilities in untreated hospitalised subjects with essential hypertension (18 subjects), isolated systolic hypertension (19 subjects) and normal blood pressure (16 subjects). They found a significant increase in the coefficients of variation for systolic and diastolic blood pressure in the subjects with isolated systolic hypertension, suggesting a relative increase in blood pressure variability compared to the other two groups. However, their data were insufficient in that ambulatory recordings were made only every 30 minutes, which yields variability measures that are poorly descriptive compared with data obtained from continuous intra-arterial monitoring [Di Rienzo et al, 1983]. Furthermore, although their results

Introduction

agree with those of Rowlands et al (1984) who showed no difference in variability between elderly hypertensives and normotensives with 24-hour intra-arterial monitoring, they conflict with those of Ruddy et al (1988) who found no difference in blood pressure variability between elderly subjects with isolated systolic hypertension and normotensives (with ABPM recordings at 15 minute intervals). Thus the evidence of increased blood pressure variability in elderly hypertensive subjects is at best scant, and the relationship between 24-hour ambulatory blood pressure variability and baroreflex sensitivity in this age group has not been thoroughly examined, despite the inverse relation between the two confirmed in younger subjects [Watson et al, 1980; Mancia et al, 1986]. In view of the likely clinical significance of blood pressure variability as an independent determinant of hypertensive target organ damage [Pessina et al, 1985; Parati et al, 1987; Palatini et al, 1992; Frattola et al, 1993; Meredith et al, 1995] this is another aspect of hypertension in the elderly that urgently requires attention.

1.9 AIMS OF THE STUDY

In the above text, the author has reviewed the currently available evidence for two of the most important pathophysiological changes that are held to contribute to the genesis of hypertension in the elderly: disordered cardiovascular neural control and increased peripheral vascular resistance. In so doing, the review has indicated areas where our knowledge of these changes is inadequate: the available evidence on baroreflex sensitivity in the elderly is imperfect and leaves an unclear and

Introduction

sometimes contradictory picture, and the structural and functional characteristics of the resistance vasculature are not described in this age group. The studies described in this thesis therefore have been designed to test the following main hypotheses:

1. That there are differences in cardiovascular neural control between elderly hypertensive and normotensive subjects, including differences between elderly subjects with combined hypertension and isolated systolic hypertension;
2. That there are differences in resistance artery structure between elderly hypertensive and normotensive subjects, and that pulse pressure is a significant determinant of that structure;
3. That there are differences between elderly hypertensive and normotensive subjects in the contractile and relaxant behaviour of resistance arteries;
4. That the baroreceptor-vascular response is related to alterations in resistance artery structure.

Chapter 2

Cardiovascular neural control in elderly subjects

2.1 SUMMARY

This study was designed to examine cardiovascular neural control in elderly subjects, and in particular differences between elderly persons with combined hypertension, isolated systolic hypertension and normal blood pressure. In 54 subjects of mean age 70 years (range 60 - 81), the baroreceptor-cardiac reflex sensitivity was quantified from the pulse interval and blood pressure responses to the Valsalva manoeuvre, bolus phenylephrine injections and sodium nitroprusside infusion. Blood pressure, heart rate and forearm blood flow and vascular resistance were also assessed with passive 60° head-up tilt and the cold face stimulus.

The study findings demonstrated a reduced baroreceptor-cardiac reflex sensitivity with hypertension in elderly persons, but no difference between subjects with combined systolic-diastolic hypertension and isolated systolic hypertension matched for similar levels of systolic blood pressure. These observations were consistent across all methods of baroreflex sensitivity testing. Indeed in this age group, baroreflex sensitivity was related only to the level of systolic blood pressure and not to diastolic blood pressure or age. The study also demonstrated an independent relation between diminished baroreflex sensitivity, increased twenty-four hour blood pressure variability and reduced heart rate variability.

Hypertensive subjects exhibited a reduced heart rate response to head-up tilting. Elderly subjects with isolated systolic hypertension also demonstrated an enhanced

increment in forearm vascular resistance to tilt, but this was insufficient to prevent a greater fall in blood pressure in those subjects compared to those with normal blood pressure. These abnormalities of cardiovascular neural control in hypertension in the elderly indicate a consistent reduction in baroreceptor-cardiac reflex sensitivity which may be responsible for an attenuated cardiac response to orthostasis and which, despite an augmented baroreceptor-vascular response possibly related to resistance artery adaptation, leads to a greater fall in blood pressure in hypertensive subjects.

2.2 BACKGROUND and AIMS

The neurogenic theory of hypertension has featured prominently in theories of the pathophysiology of hypertension at all ages, including the elderly, since the first descriptions of the role of the baroreflex in blood pressure homeostasis [McCubbin et al, 1956]. This has led to the view that in hypertension in the elderly there is reduction of baroreflex sensitivity with a concomitant reduction in the inhibition of sympathetic outflow, leading to peripheral vasoconstriction and raised peripheral vascular resistance. The view has also been that in isolated systolic hypertension there is a further reduction of baroreflex sensitivity attributable to failure of the afferent portion of the reflex, itself attributed to more severe arteriosclerosis in the large arteries of such subjects. However, as discussed in detail in Chapter 1, an

examination of the evidence for these views in elderly persons reveals it to be scant and in some cases contradictory.

The aims of this study were therefore:

1. to resolve the disparities in evidence regarding baroreflex sensitivity in elderly subjects and in hypertension, including isolated systolic hypertension;
2. to do this by studying baroreflex sensitivity by several of the previously described methods including the response to both an elevation and a reduction in blood pressure;
3. to examine differences between elderly subjects with and without hypertension in the response to baroreflex and non-baroreflex mediated stimuli resulting in increased peripheral vascular resistance.

2.3 MATERIALS and METHODS

2.3.1 Study subjects

This study involved 54 elderly subjects (age {mean \pm SEM} 69.6 \pm 0.7 years) of whom 32 were hypertensive and 22 normotensive. Hypertensive subjects were

Cardiovascular neural control

recruited from among outpatient attenders at two large teaching hospitals and through a liaison with several large local general practices. Normotensive subjects were recruited from among the spouses and friends of the hypertensive subjects, and from the respondents to a local newspaper advertisement. All subjects gave written informed consent to participate in the study, which received local ethical committee approval. All subjects were active and ambulant, and living independently in the community. All subjects had a normal physical examination with no evidence of end-organ damage, all were in sinus rhythm with a normal electrocardiogram, and all had normal routine biochemistry including renal function. Subjects with a history of other cardiovascular disease including atrial fibrillation, other disorders associated with autonomic dysfunction or other major illness were excluded. No subject in the normotensive group had any history of hypertension or pre-eclampsia. Any subject taking medication with cardiovascular or autonomic effects was excluded. The majority of subjects (n=34) had never previously received antihypertensive treatment, but in those currently on treatment (n=20) when recruited to the study, antihypertensive medication was progressively withdrawn leaving a minimum one month drug-free period before the blood pressure values used for the study were commenced.

2.3.2 Study protocol

Subjects attended the morning research clinic on three separate occasions at least one week apart. Subjects' height, weight and body mass index (BMI; weight

divided by square of height, kg/m^2) were recorded. Supine blood pressure (Korotkoff phase V) and heart rate were recorded three times on each visit after at least five minutes rest, using a standard mercury sphygmomanometer and a cuff of appropriate size. Subjects were allocated to one of three groups according to the final average of the nine blood pressure readings obtained over the three clinic visits. Group 1 comprised 16 subjects with sustained 'combined' hypertension (CH; systolic blood pressure >160 mmHg and diastolic >90 mmHg) on clinic readings, Group 2 a further 16 subjects of similar age with sustained isolated systolic hypertension (ISH; systolic blood pressure >160 mmHg with diastolic <90 mmHg), and Group 3 consisted of 22 age-matched normotensive subjects (NT; clinic blood pressure of systolic <160 mmHg and diastolic <90 mmHg). Groups 1 and 2 were also matched for similar levels of systolic blood pressure. None of the subjects in Group 2 had a past history of raised diastolic blood pressure, therefore as far as possible excluding subjects with 'burnt out' combined hypertension [Amery et al, 1989].

On their third visit to the research clinic, subjects were fitted with a SpaceLabs 90207 ambulatory blood pressure monitor for a 24 hour recording. This was fitted to the non-dominant arm, using a cuff of appropriate size. Before beginning the recording the monitor was validated in each subject by the sequential same-arm comparison with values obtained by sphygmomanometry, readings having to agree to within 5 mmHg. The monitor was programmed to take readings at 15 minute intervals during a notional 'daytime' period of 7 am to 10 pm, and at 30 minute intervals during a 'night-time' period of 10 pm to 7 am [Scientific Committee, 1990]. On completion of the 24 hour recording, subjects returned for removal of the monitor

which was downloaded via a SpaceLabs interface to an IBM-compatible personal computer. Data were automatically edited to exclude unphysiological readings e.g. where diastolic blood pressure exceeded systolic blood pressure, but no other editing was undertaken. Recordings with less than 80% of possible readings were repeated to obtain a usable record.

2.3.3 Laboratory studies

Within two weeks subjects attended the cardiovascular laboratory at 8 am for a morning session, having emptied the bladder and following a light breakfast, and having refrained from smoking, alcohol and caffeine-containing products for at least twelve hours. Subjects wore light clothing and the laboratory temperature was thermostatically controlled between 20 and 22°C. Subjects rested supine for a minimum of thirty minutes after the insertion of a cannula into a dorsal hand vein. This was kept patent by periodic flushing with 2 mls 0.9% saline. The subject was fitted with chest leads for recording of the continuous surface electrocardiogram (model CR7, Cardiac Recorders Ltd, London, UK), and the appropriate-sized finger cuff of the Finapres 2300 non-invasive beat-to-beat blood pressure recording device (Ohmeda Monitoring Systems, Englewood, Colorado, USA) was fitted to the middle finger or thumb of the non-dominant hand, which rested throughout on an adjustable support at the level of the heart. The Finapres measures blood pressure in the digital arteries by the volume-clamp method of Peñáz [Peñáz, 1973]. The finger cuff is fitted with an infrared photoplethysmograph which measures the arterial blood

Cardiovascular neural control

volume in the finger and constantly adjusts the cuff pressure to keep the arterial wall unloaded. Cuff pressure thus parallels digital arterial pressure and the Finapres provides a continuous output of beat-to-beat blood pressure that closely follows brachial intrarterial blood pressure in comparative testing with an offset that remains fairly constant [Imholz et al, 1988, 1990; Parati et al, 1989] (see Appendix I: *Validation of the non-invasive Finapres device for cardiovascular testing*).

Following achievement of a satisfactory blood pressure signal from the Finapres and the stabilisation of blood pressure at the same level for at least ten minutes, subjects then performed the following tests of cardiovascular autonomic function [Ewing & Clarke, 1982]:

[i] the variation in R-R interval with deep breathing at 6 breaths/minute for one minute;

[ii] the blood pressure and heart rate response to isometric handgrip, with the subject maintaining a grip of 30% of maximum for up to five minutes;

[iii] the cardiovascular responses to the Valsalva manoeuvre. After several practices, subjects blew into a modified sphygmomanometer to maintain a pressure of 40 mmHg for 15 seconds whilst seated. The manometer contained a small air leak so that subjects had to maintain a constant respiratory effort. The manoeuvre was repeated three times and the average response taken.

Baroreflex sensitivity testing was then performed by the following pharmacological methods:

[iv] the blood pressure and pulse interval response to phenylephrine injection

[Smyth et al, 1969; Sleight, 1979]. An initial bolus dose of 50 μg phenylephrine was progressively increased in 50 μg steps as necessary (up to a maximum of 200 μg) to achieve a peak blood pressure rise of 20-40 mmHg, and the effective dose was repeated to obtain a minimum of three adequate responses. Bolus injections of 0.9% saline were interspersed at random between the drug injections and the subject was blinded to the nature of each injection;

[v] the blood pressure and pulse interval response to a graded sodium nitroprusside infusion [Sullebarger et al, 1990; Muratani et al, 1990; Lage et al, 1993]. The infusion was commenced at 0.25 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and increased (by 0.25 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ each minute) until a fall in blood pressure of at least 20 mmHg was observed.

Following this, the cardiovascular responses to head-up tilt were recorded. After familiarising subjects with the tilting procedure, they were manually tilted rapidly to 60° (the manoeuvre taking about 3-5 seconds) while lightly strapped to a hydraulic tilt table fitted with foot support (Akron Medical Products, Ipswich, UK) and then held in that position for 3 minutes. This manoeuvre was repeated a further two times with at least five minutes in between and the average of the three responses taken. During tilt blood pressure and pulse interval were recorded in the same manner as previously, and forearm blood flow and vascular resistance were measured with a mercury-in-silastic strain gauge plethysmograph (QMC Medical Physics, Nottingham, UK). This measures forearm blood flow by the venous occlusion method originally described by Whitney (1953). After measurement of the circumference of the forearm (with a tape measure) at a point approximately 5 cm from the olecranon, a

Cardiovascular neural control

cuff was applied to the wrist of the arm contralateral to that attached to the Finapres. This cuff was inflated to a pressure approximately 60 mmHg above systolic blood pressure to exclude blood flow to the hand, while another cuff was applied to the upper arm on the same side and rapidly inflated to 40 mmHg for 8 seconds and deflated for 7 seconds to temporarily occlude the venous return. The arm was raised slightly above the heart to permit passive emptying of the limb. A mercury-in-silastic strain gauge of suitable length attached to a Wheatstone bridge was looped around the forearm and connected to a chart recorder to record volume changes in the forearm over time. Forearm blood flow (FBF) was recorded for one minute (four inflation-deflation cycles) at baseline and then during the three minutes of tilt. After a further period of supine rest, subjects underwent the cold face stimulus, where the blood pressure, heart rate and FBF responses to the application of a refrigerated gel pack (2–4°C) to the subject's forehead for 45 seconds were recorded [Khurana et al, 1980; Anderson et al, 1988; Heath & Downey, 1990]. Subjects wore protective glasses to avoid the stimulation of the oculo-cardiac reflex. The average of two responses was taken. A suitable interval of at least five minutes was observed between all the above tests to permit the recovery of baseline values for blood pressure and heart rate. During all the above manoeuvres the hand and forearm attached to the Finapres was supported on an adjustable shelf to maintain it at heart level and minimise any hydrostatic effects, and during head-up tilting the contralateral arm attached to the FBF equipment was also raised on an adjustable support to retain the angle to the horizontal during tilt.

2.3.4 Data acquisition

The analogue outputs from the Finapres device and the simultaneous ECG signal were routed to a dedicated personal computer fitted with an analogue-to-digital converter sampling at 200 Hz per channel. A third channel recorded pressure from a transducer and amplifier connected to the sphygmomanometer used for the Valsalva manoeuvre. Specially written software allowed the recording, calibration and editing of the digitised signal and the derivation for later off-line analysis of beat-to-beat data for systolic, mean arterial and diastolic pressure, together with the pulse interval from the Finapres and ECG signals and the Valsalva pressure.

The FBF equipment amplified the signal from the mercury-in-silastic strain gauge and produced an output on a polygraph (WR7400, Graphtec Corp., Japan). The slope of the trace on the recorder paper was proportional to the volume change in the limb, according to the following equation:

$$\text{FBF} = \frac{200 \cdot p}{C \cdot a} \cdot S \cdot \text{Tan}\theta$$

where C is the circumference of the limb, S the chart speed (set to 120 cm.min⁻¹), and θ the slope of the polygraph trace. p/a is the ratio produced by calibration of the strain gauge off-limb on a wooden former where a stretch equivalent to a change in limb circumference of p produces a pen deflection of a . FBF is expressed as ml.min⁻¹.100 mls of forearm tissue⁻¹, and the ratio of FBF to the

mean arterial pressure prevailing during that cycle (taken from the simultaneous Finapres recording) is calculated and expressed in arbitrary units representing forearm vascular resistance (FVR).

2.3.5 Data analysis

The data from the three Valsalva manoeuvres were first analysed to obtain the mean Valsalva ratio, i.e. the ratio of the shortest R-R interval during the strain part of the manoeuvre to the longest R-R interval occurring after release [Ewing & Clarke, 1982]. The blood pressure and pulse interval data were then analysed to derive an index for baroreflex sensitivity from Phase 4 of the manoeuvre according to the method of Smith et al (1987: *see Methods for the examination of the arterial baroreflexes: the Valsalva manoeuvre, Chapter 1*). Two figures were derived: BRS_{V1} describes the baroreflex sensitivity from the linear regression of pulse interval on blood pressure for the whole of Phase 4 (from the lowest systolic blood pressure recorded immediately after release of the strain to the peak value observed several seconds later) with the 'lag' (in beats) between stimulus (blood pressure) and response (pulse interval) that achieved the highest correlation, and BRS_{V2} describes the results of a similar analysis confined to that portion of Phase 4 where arterial pressure exceeded that before the manoeuvre, that is, the pressure overshoot.

The baroreflex sensitivity was derived from the phenylephrine injection technique also by two methods: the first involved the linear regression of pulse interval on blood

Cardiovascular neural control

pressure for the immediately preceding beat for the 'ramp' portion of the blood pressure response including both inspiratory and expiratory beats, i.e. from the first beat where a sustained rise in blood pressure was observed (usually 15-25 seconds after injection) to the maximum value (ramp BRS_{PE}) [Smyth et al, 1969]. The second method took account of the observation that subjects with a very low baroreflex sensitivity would have a non-significant correlation between pulse interval and blood pressure for the ramp response, and instead compared baseline values recorded over the 10–15 secs immediately prior to drug injection with values obtained during the later, sustained part of the blood pressure response, the 'steady-state' method (steady-state BRS_{PE}) [Korner et al, 1974]. Values for ramp BRS_{PE} and steady-state BRS_{PE} were obtained for each of the (minimum three) effective phenylephrine doses and averaged to derive a final value for each individual. Baroreflex sensitivity was derived from the sodium nitroprusside depressor response by the same methods; this yielded values for each individual for ramp BRS_{SNP} and steady-state BRS_{SNP} .

Baseline values for FBF prior to tilting and the cold face stimulus (CFS) were obtained from the mean of the four curves in the minute immediately before the stimulus. FBF and FVR values at 15, 30, 60, 90, 120 and 180 seconds following tilt were considered. The mean of the three values obtained in each subject at each of those time points during the three tilts was taken as the final value. If for technical reasons a single curve at any time point was uninterpretable, the value for the next adjacent curve was taken. Similarly the two values were averaged for 15, 30, and 45 seconds following application of the CFS.

2.3.6 Statistical methods

Data are expressed as mean \pm standard error of the mean (SEM). Student's two-tailed paired t-test was used for within-subject comparisons. Differences between groups for the baroreflex sensitivity data were examined using analysis of variance (ANOVA) with Tukey's pairwise correction for multiple comparisons. For the tilt and CFS data, statistical analysis was performed using the General Linear Models procedure (to allow for unbalanced data i.e. differing group sizes) for repeated measures ANOVA, with group and time point as factors. Summary statistics (SBP, DBP and heart rate change, and time to maximum change) were also studied. Pearson's correlation coefficient and least-squares regression analysis were used to examine the linear association between continuous variables, and simultaneous effects of a number of variables studied using multiple linear regression. A p value of < 0.05 was regarded as indicating statistical significance.

2.4 RESULTS

2.4.1 Subject characteristics and clinic and ambulatory blood pressure

Of the 54 subjects in the study, 16 had combined systolic-diastolic hypertension (Group 1, CH), a further 16 had sustained isolated systolic hypertension (Group 2, ISH), and 22 were normotensive (Group 3, NT). Age, weight, height, and BMI were

not different between the three groups (Table 2.1). Table 2.2 shows the blood pressure data for both clinic and ambulatory readings for the three groups. The two hypertensive groups were matched for similar levels of clinic systolic blood pressure, but diastolic blood pressure was highly significantly different between the ISH and CH groups. On 24-hour ambulatory recording the two hypertensive groups had similar levels of systolic blood pressure (Table 2.2 and Figure 2.1), but 24-hour diastolic blood pressure, although consistently slightly lower in the ISH group than in the CH group, was not significantly so (95% confidence interval for the difference between ISH and CH groups -2 to 12 mmHg; Figure 2.2). A significantly higher heart rate was seen in the CH group both in the clinic and on ambulatory recording (both during the day and at night) than in the other two groups (Figure 2.3).

Table 2.3 shows the data obtained on daytime blood pressure variability from the 24 hour ambulatory blood pressure monitoring. Data are shown only for the daytime (07:00 to 22:00) as it was only during this period that readings were made at 15 minute intervals; intervals longer than this (such as the 30 minute intervals used in the present study during the night-time 22:00 to 07:00) do not yield sufficiently accurate data regarding blood pressure variability [Di Rienzo et al, 1983]. Although the absolute level of daytime systolic and diastolic blood pressure variability (expressed by the standard deviation {SD} of all the readings taken during that time) was greater in the hypertensive groups 1 and 2 by comparison with the normotensive group 3, when correction was made for the level of blood pressure by calculation of the coefficient of variation (CV; the SD as a proportion of the mean) there were no differences between the groups.

Cardiovascular neural control

	Group 1 (CH)	Group 2 (ISH)	Group 3 (NT)
n	16	16	22
Male:Female	7:9	7:9	11:11
Age (years)	68 ± 1	70 ± 1	70 ± 1
Weight (kg)	72.7 ± 2.6	73.9 ± 2.5	68.9 ± 2.7
Height (cm)	165 ± 2	167 ± 2	168 ± 2
BMI (kg/m ²)	26.7 ± 0.7	26.6 ± 0.7	24.4 ± 0.7

BMI: body mass index

Table 2.1 Demographic data for the three groups

Cardiovascular neural control

		Group 1 (CH)	Group 2 (ISH)	Group 3 (NT)
Clinic	SBP	184 ± 4**	180 ± 3††	138 ± 2
	DBP	102 ± 2**	85 ± 1††‡‡	76 ± 1
	HR	77 ± 2**	66 ± 2‡‡	66 ± 2
24 hour	SBP	150 ± 3**	153 ± 2††	128 ± 2
	DBP	89 ± 2**	84 ± 2†	75 ± 2
	HR	79 ± 2**	71 ± 2‡	70 ± 2
Daytime	SBP	154 ± 3**	157 ± 2††	132 ± 2
	DBP	92 ± 2**	87 ± 2†	78 ± 2
	HR	82 ± 2*	73 ± 2‡	74 ± 1
Night-time	SBP	139 ± 3**	142 ± 3††	117 ± 3
	DBP	80 ± 2**	74 ± 2†	67 ± 2
	HR	70 ± 2**	63 ± 2‡	61 ± 2

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; all blood pressures in mmHg
 * $p < 0.05$, ** $p < 0.01$ CH v NT; † $p < 0.05$, †† $p < 0.01$ ISH v NT; ‡ $p < 0.05$, ‡‡ $p < 0.01$ ISH v CH
 (ANOVA with Tukey's correction for multiple comparisons).

Table 2.2 Clinic and ambulatory blood pressure data for the three groups

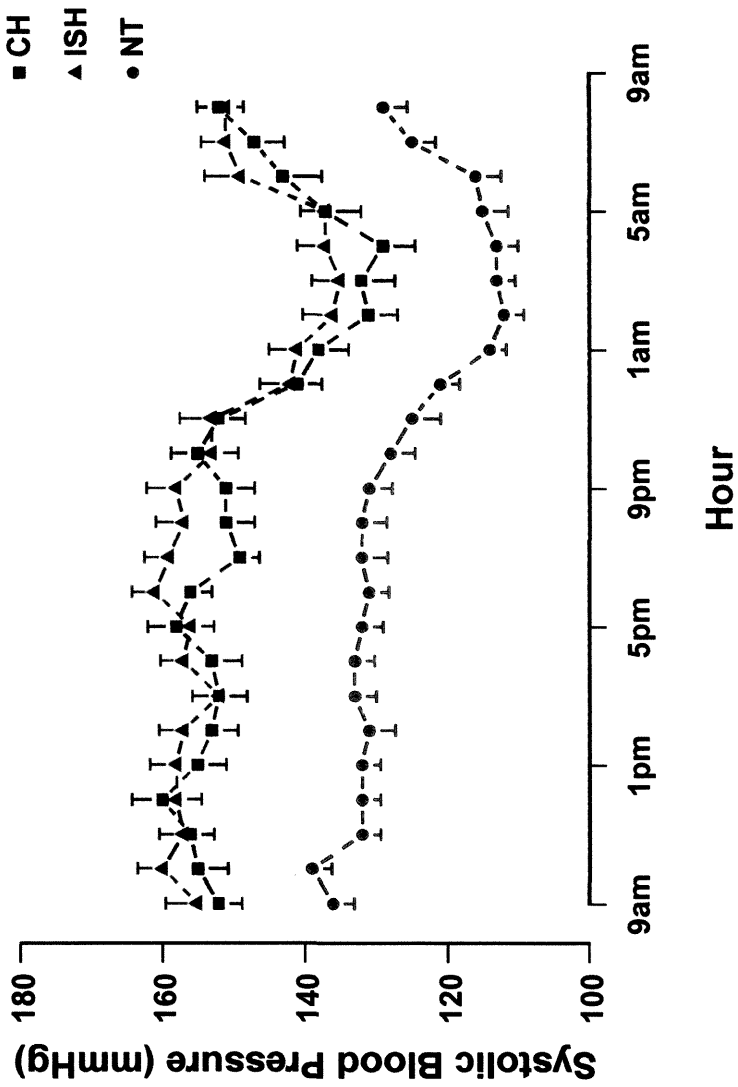


Figure 2.1 Systolic blood pressure from 24-hour ambulatory blood pressure monitoring for the combined hypertensive (CH), isolated systolic hypertensive (ISH) and normotensive (NT) groups

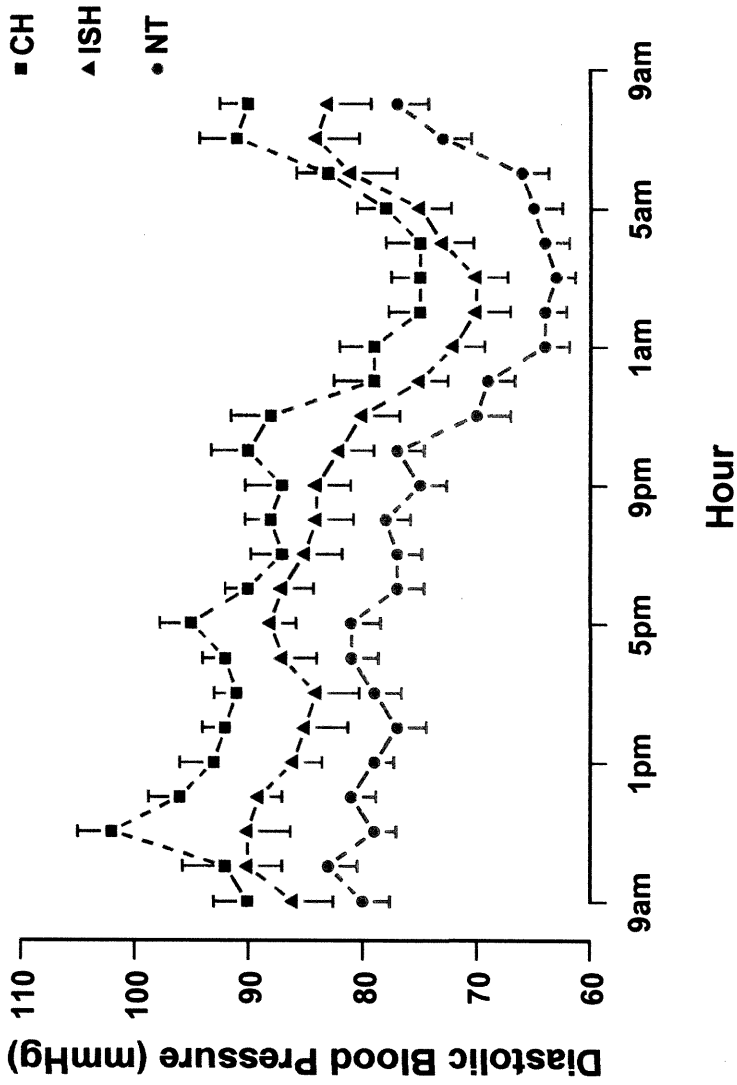


Figure 2.2 Diastolic blood pressure from 24-hour ambulatory blood pressure monitoring for the combined hypertensive (CH), isolated systolic hypertensive (ISH) and normotensive (NT) groups

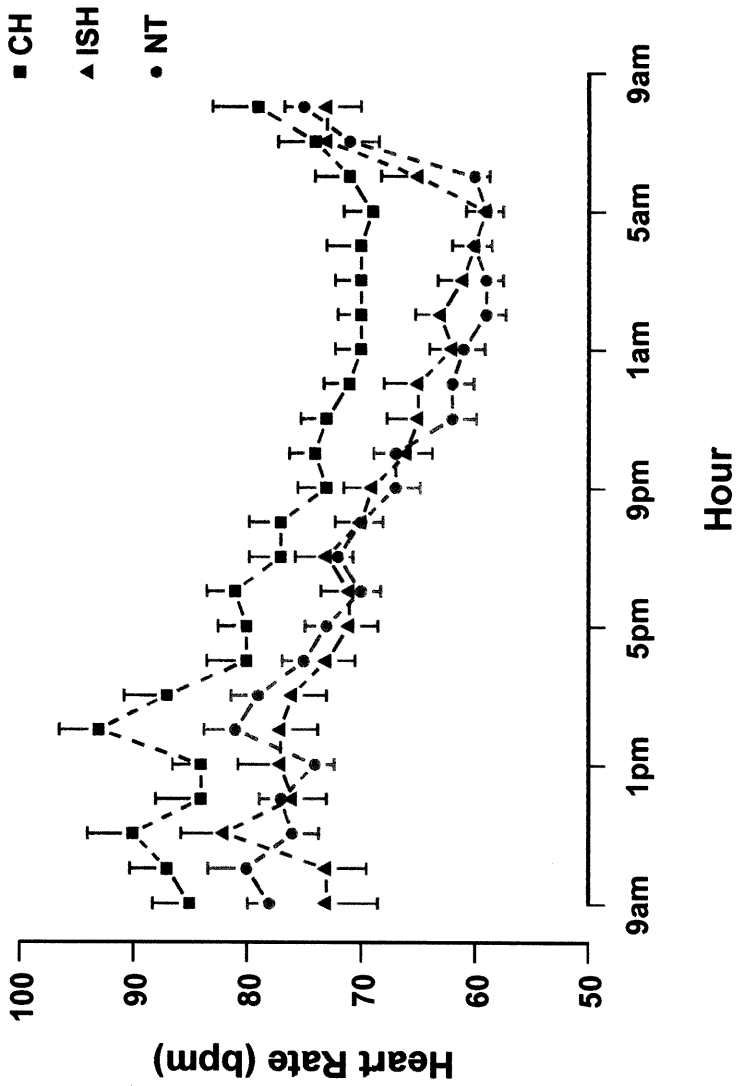


Figure 2.3 Heart rate from 24-hour ambulatory blood pressure monitoring for the combined hypertensive (CH), isolated systolic hypertensive (ISH) and normotensive (NT) groups

Cardiovascular neural control

		Group 1 (CH)	Group 2 (ISH)	Group 3 (NT)	<i>p</i> (ANOVA)
SBP	SD (mmHg)	14.6 ± 0.7	14.5 ± 0.6	11.6 ± 0.6	0.001
	CV (%)	9.5 ± 0.5	9.2 ± 0.4	8.8 ± 0.4	0.51
DBP	SD (mmHg)	11.6 ± 0.5	11.1 ± 0.8	8.7 ± 0.5	0.002
	CV (%)	12.7 ± 0.6	12.9 ± 0.9	11.3 ± 0.7	0.26
HR	SD (mmHg)	10.9 ± 1.0	10.5 ± 1.2	10.6 ± 0.7	0.95
	CV (%)	13.3 ± 1.2	14.7 ± 1.8	14.5 ± 1.0	0.73

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; SD: standard deviation of all readings taken during the daytime (07:00 to 22:00); CV: coefficient of variation

Table 2.3 Daytime ambulatory blood pressure variability for the three groups

2.4.2 Laboratory cardiovascular testing

Heart rate (R-R) variability for the three groups was not significantly different (Group 1, 10.0 ± 1.1 bpm; Group 2, 9.7 ± 1.2 bpm; Group 3, 12.1 ± 1.5 bpm; $p > 0.4$). Similarly, the blood pressure and heart rate responses to isometric handgrip were not significantly different between the three groups (Table 2.4), although baseline blood pressure was significantly higher in the two hypertensive groups and baseline heart rate was again higher in the combined hypertensives.

2.4.3 The Valsalva manoeuvre

The mean Valsalva ratio for the entire study group was 1.38 ± 0.03 . There was no difference between the three groups in the Valsalva ratio (Table 2.5) and the Valsalva ratio was not related to age ($r = 0.07$; $p > 0.2$). However, the Valsalva ratio was significantly related to systolic blood pressure independent of diastolic blood pressure and heart rate (regression equation: Valsalva ratio = $1.91 - 0.003$ SBP + 0.001 DBP - 0.001 HR; $p = 0.04$).

A typical recording of the cardiovascular response to the Valsalva manoeuvre is shown in Figure 2.4. Baroreflex sensitivity from the whole of phase 4 of the manoeuvre (BRS_{V_1}) was significantly lower in the two hypertensive groups 1 and 2 compared to the normotensive group 3 (Table 2.5). The optimum lag time for the correlation of pulse interval on systolic blood pressure was not different between the

Cardiovascular neural control

		Group 1 (CH)	Group 2 (ISH)	Group 3 (NT)
Baseline	SBP	198 ± 4**	183 ± 7††	144 ± 4
	DBP	98 ± 3	86 ± 3‡	84 ± 3
	HR	77 ± 2**	66 ± 2‡‡	66 ± 2
Peak	SBP	237 ± 5**	237 ± 10††	191 ± 6
	DBP	123 ± 3*	110 ± 4	108 ± 5
	HR	89 ± 3	78 ± 3‡	81 ± 3
Change	SBP	41 ± 4	51 ± 6	48 ± 4
	DBP	25 ± 2	24 ± 4	24 ± 3
	HR	14 ± 3	12 ± 2	15 ± 3
Time to peak (secs)	SBP	206 ± 21	191 ± 25	237 ± 14
	DBP	184 ± 24	193 ± 22	225 ± 14
	HR	212 ± 21	191 ± 22	198 ± 18

SBP: systolic blood pressure(mmHg); DBP: diastolic blood pressure (mmHg); HR: heart rate (beats per minute)
 * $p < 0.05$, ** $p < 0.01$ CH v NT; † $p < 0.05$, †† $p < 0.01$ ISH v NT; ‡ $p < 0.05$, ‡‡ $p < 0.01$ ISH v CH
 (ANOVA with Tukey's correction for multiple comparisons).

Table 2.4 Blood pressure and heart rate responses to isometric handgrip for the three groups

Cardiovascular neural control

	Group 1 (CH)	Group 2 (ISH)	Group 3 (NT)
Valsalva Ratio	1.37 ± 0.06	1.30 ± 0.06	1.44 ± 0.04
V1 BRS_{V1} (msec/mmHg)	1.9 ± 0.3**	2.8 ± 0.5†	4.4 ± 0.4
BP change (mmHg)	98 ± 6**	81 ± 8	66 ± 5
Lag (beats)	3.3 ± 0.3	2.8 ± 0.4	2.7 ± 0.3
Optimum r	0.90 ± 0.01	0.86 ± 0.02	0.89 ± 0.01
V2 BRS_{V2} (msec/mmHg)	3.9 ± 0.5**	5.6 ± 1.0†	8.8 ± 1.0
BP change (mmHg)	37 ± 3	33 ± 3	28 ± 3
Lag (beats)	1.5 ± 0.3	1.6 ± 0.3	1.5 ± 0.3
Optimum r	0.90 ± 0.01	0.87 ± 0.02	0.89 ± 0.01

BRS: baroreflex sensitivity; BP: blood pressure; for definitions of V1 and V2 see text
** p < 0.05, ** p < 0.01 CH v NT; † p < 0.05, †† p < 0.01 ISH v NT; ‡ p < 0.05, ‡‡ p < 0.01 ISH v CH*
(ANOVA with Tukey's correction for multiple comparisons).

Table 2.5 Baroreflex sensitivity data from the Valsalva manoeuvre for the three groups

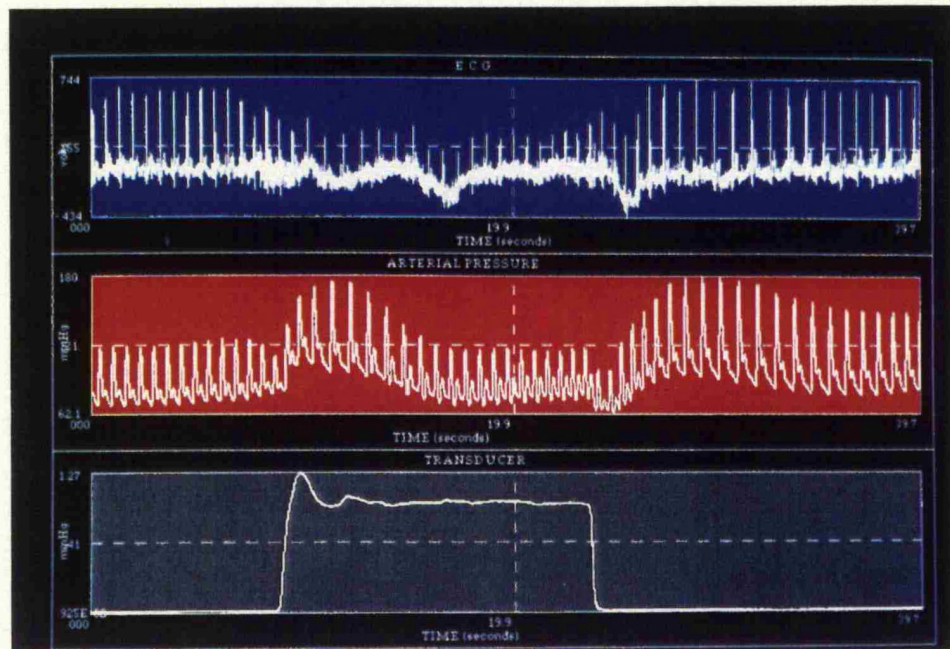


Figure 2.4 A typical recording of the cardiovascular response to the Valsalva manoeuvre in a study subject

Top blue panel: electrocardiogram; middle red panel: blood pressure; bottom grey panel: mouth pressure

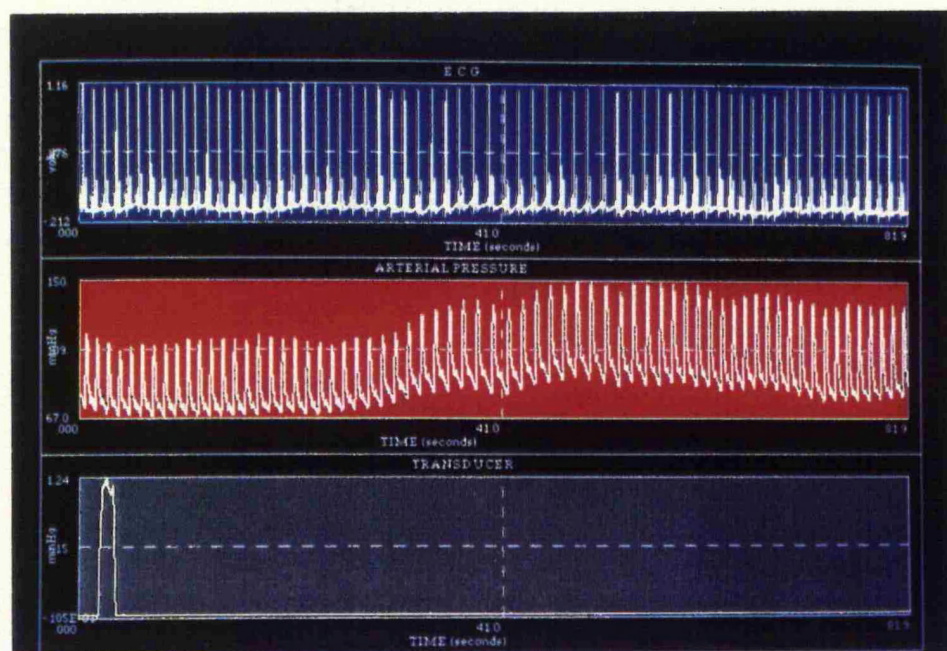


Figure 2.5 A typical example of the response to a phenylephrine bolus injection in a study subject

Top blue panel: electrocardiogram; middle red panel: blood pressure; bottom grey panel: event marker - bolus injection given at the marked point. Recording period shown has been shortened for clarity

three groups, nor was the value for the correlation coefficient. However, the hypertensive subjects demonstrated a greater blood pressure rise than the normotensive subjects during phase 4. If analysis was limited to the overshoot part of phase 4 only (BRS_{V2}), the results were broadly similar (Table 2.5). Baroreflex sensitivity was again lower in the two hypertensive groups but not different between them, with no differences between the groups in the lag between pulse interval and systolic blood pressure or in the correlation obtained. However, the lag which yielded the best correlation was shorter than for BRS_{V1} , and the blood pressure change was naturally smaller.

BRS_{V1} was significantly correlated with clinic systolic blood pressure ($r = -0.66$, $p < 0.001$) and this relation persisted even after correction for the diastolic blood pressure and age (regression equation: $BRS_{V1} = 15.0 - 0.044 \text{ age} - 0.045 \text{ SBP} - 0.02 \text{ DBP}$; $R^2 = 45.2\%$, $p = 0.001$). Similarly BRS_{V2} was related to clinic systolic blood pressure ($r = -0.57$, $p < 0.001$), again independent of age and diastolic blood pressure (equation: $BRS_{V2} = 27.3 - 0.048 \text{ age} - 0.074 \text{ SBP} - 0.062 \text{ DBP}$; $R^2 = 32.5\%$, $p = 0.02$). Neither parameter for baroreflex sensitivity was independently related to age. The Valsalva ratio was significantly correlated with both BRS_{V1} ($r = 0.56$, $p < 0.001$) and BRS_{V2} ($r = 0.54$, $p < 0.001$).

2.4.4 Baroreceptor-cardiac reflex sensitivity testing

Phenylephrine drug injection was not associated with any side-effects. However, a mild flushing sensation, usually confined to the face, was sometimes reported at the lowest point of the blood pressure response to sodium nitroprusside infusion.

A typical example of the response to a phenylephrine bolus injection is shown in Figure 2.5. Steady-state BRS_{PE} was significantly lower in the two hypertensive groups 1 and 2 than in the normotensive group 3, but not different between them (Table 2.6). Similar findings were made with the analysis of the blood pressure ramps, although in 7 subjects ramp BRS_{PE} could not be obtained because of non-significant correlations between pulse interval and blood pressure. The mean blood pressure rise during the ramp responses was 31 ± 1.4 mmHg, and there was a tendency towards a slightly lower blood pressure response in the normotensive subjects to an equivalent dose of phenylephrine ($p = 0.10$). BRS_{PE} was not correlated with age, but was significantly correlated with clinic systolic blood pressure independent of age and diastolic blood pressure (regression equations: steady-state $BRS_{PE} = 23.0 - 0.104 \text{ age} - 0.086 \text{ SBP} - 0.035 \text{ DBP}$; $R^2 = 27.5\%$, $p = 0.005$; ramp $BRS_{PE} = 14.2 + 0.072 \text{ age} - 0.11 \text{ SBP} + 0.05 \text{ DBP}$; $R^2 = 32.7\%$, $p < 0.001$).

The baroreflex sensitivity findings with sodium nitroprusside infusion were broadly similar in pattern (Table 2.6). Both steady-state and ramp BRS_{SNP} were significantly lower with hypertension, but there were no differences between the two hypertensive groups 1 and 2. There was a smaller blood pressure reduction in the normotensive group with an equivalent maximum dose of sodium nitroprusside (maximum doses {in $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ }: Group 1: 0.63 ± 0.05 , Group 2: 0.75 ± 0.07 , Group 3: 0.77 ± 0.04 , $p > 0.10$). There was no significant correlation between BRS_{SNP} and age, but there was a significant correlation between BRS_{SNP} and clinic systolic blood pressure

Cardiovascular neural control

	Group 1 (CH)	Group 2 (ISH)	Group 3 (NT)
Phenylephrine			
Steady-state BRS _{PE}	3.4 ± 0.6*	4.0 ± 1.0	6.4 ± 0.9
BP change	32 ± 3	35 ± 2	28 ± 2
Ramp BRS _{PE}	3.1 ± 0.6**	3.5 ± 0.7††	7.7 ± 1.0
Sodium nitroprusside			
Steady-state BRS _{SNP}	2.3 ± 0.3**	4.0 ± 0.9	5.6 ± 0.3
BP change	40 ± 4	47 ± 6†	31 ± 2
Ramp BRS _{SNP}	2.1 ± 0.3**	3.6 ± 0.8†	5.4 ± 0.3

BRS: baroreflex sensitivity (msec/mmHg); BP: blood pressure (mmHg); for definitions of 'steady-state' and 'ramp' see text

* $p < 0.05$, ** $p < 0.01$ CH v NT; † $p < 0.05$, †† $p < 0.01$ ISH v NT; ‡ $p < 0.05$, ‡‡ $p < 0.01$ ISH v CH (ANOVA with Tukey's correction for multiple comparisons).

Table 2.6 Baroreflex sensitivity by the phenylephrine pressor and sodium nitroprusside depressor methods for the three groups

independent of both age and diastolic blood pressure (regression equations: steady-state $BRS_{SNP} = 8.93 + 0.06 \text{ age} - 0.06 \text{ SBP} + 0.003 \text{ DBP}$, $R^2 = 29.1\%$, $p = 0.003$; ramp $BRS_{SNP} = 9.77 + 0.05 \text{ age} - 0.06 \text{ SBP} + 0.0007 \text{ DBP}$, $R^2 = 31.1\%$, $p = 0.002$).

The baroreceptor-cardiac reflex sensitivity findings are summarised in Table 2.7 and Figure 2.6. There was a consistent reduction in baroreflex sensitivity by any method with hypertension (either ISH or CH) when compared to normotensive subjects, but no significant differences between subjects with CH (Group 1) and ISH (Group 2). The study had 90% power to detect differences between the CH and ISH groups of 1.51 msec/mmHg in BRS_{V1} , 2.29 msec/mmHg in BRS_{V2} , 1.70 msec/mmHg in BRS_{SNP} , and 2.17 msec/mmHg in BRS_{PE} at the 5% significance level. There were no differences in the BRS responses to the pharmacological pressor or depressor stimuli in the two hypertensive groups, but BRS_{PE} was significantly greater than BRS_{SNP} in the normotensive group ($p = 0.03$). The results obtained by the various methods were statistically related although the correlations were sometimes weak, notably those between the Valsalva methods (BRS_{V1} and BRS_{V2}) and BRS_{PE} (Table 2.8).

Cardiovascular neural control

		Group 1 (CH)	Group 2 (ISH)	Group 3 (NT)
Valsalva	V1	1.9 ± 0.3**	2.8 ± 0.5†	4.4 ± 0.4
	V2	3.9 ± 0.5**	5.6 ± 1.0†	8.8 ± 1.0
Phenylephrine	Steady-state	3.4 ± 0.6*	4.0 ± 1.0	6.4 ± 0.9
	Ramp	3.1 ± 0.6**	3.5 ± 0.7††	7.7 ± 1.0
Nitroprusside	Steady-state	2.3 ± 0.3**	4.0 ± 0.9	5.6 ± 0.3
	Ramp	2.1 ± 0.3**	3.6 ± 0.8†	5.4 ± 0.3

For definitions of V1, V2, 'steady-state' and 'ramp' see text; all BRS in msec/mmHg
 * $p < 0.05$, ** $p < 0.01$ CH v NT; † $p < 0.05$, †† $p < 0.01$ ISH v NT; ‡ $p < 0.05$, ‡‡ $p < 0.01$ ISH v CH
 (ANOVA with Tukey's correction for multiple comparisons).

Table 2.7 Baroreflex sensitivity by all methods for the three groups

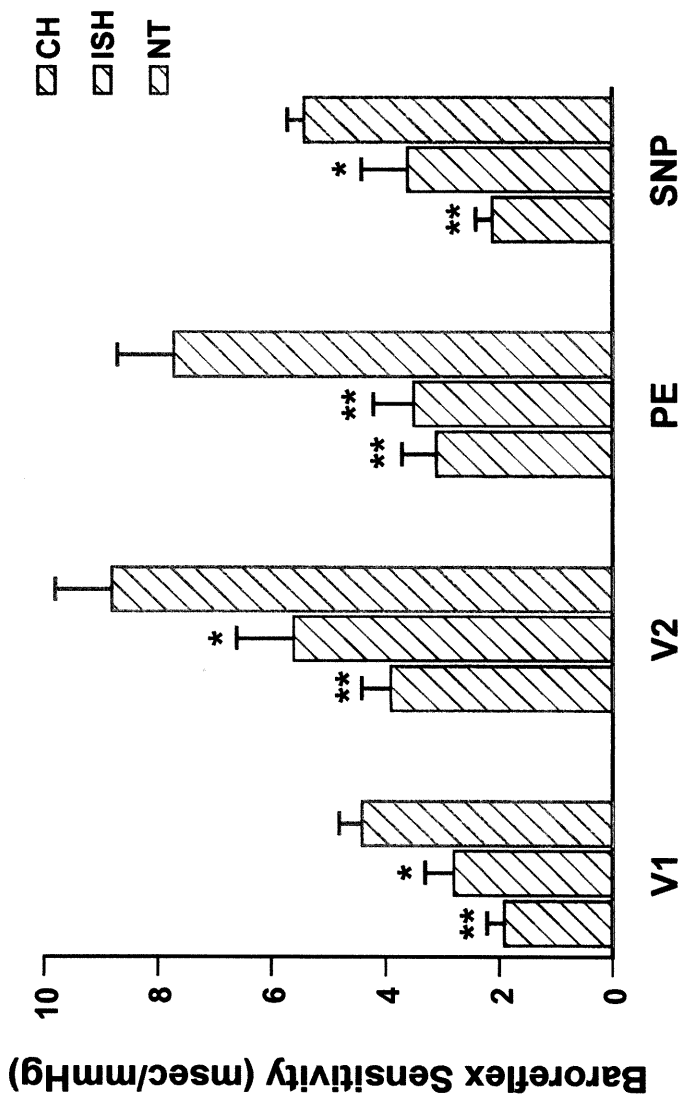


Figure 2.6 Baroreceptor-cardiac reflex sensitivity by the different methods for the combined hypertensive (CH), isolated systolic hypertensive (ISH) and normotensive (NT) groups
 V1/V2: Valsalva manoeuvre; PE: phenylephrine pressor; SNP: sodium nitroprusside depressor methods. * p < 0.05; ** p < 0.01 compared to normotensives (ANOVA)

	BRS _{V1}	BRS _{V2}	steady- state BRS _{SNP}	ramp BRS _{SNP}	steady- state BRS _{PE}
BRS _{V2}	0.89				
steady-state BRS _{SNP}	0.56	0.49			
ramp BRS _{SNP}	0.57	0.46	0.97		
steady-state BRS _{PE}	0.36	0.26	0.55	0.51	
ramp BRS _{PE}	0.42	0.33	0.58	0.53	0.79

Table 2.8 Univariate correlations (Pearson's r) between the various indices of baroreflex sensitivity

2.4.5 Baroreflex sensitivity and clinic and ambulatory blood pressure

Baroreflex sensitivity by all methods was strongly inversely correlated with the level of clinic blood pressure; the univariate correlation coefficients are shown in Table 2.9. BRS was also negatively correlated with ambulatory blood pressure, but generally with a slightly lower coefficient. Without exception BRS was more closely related to systolic than to diastolic blood pressure. Table 2.10 displays the correlations between BRS and daytime blood pressure variability. There was a tendency for daytime systolic blood pressure variability to be inversely related to BRS_{SNP} independent of age and the SBP level (regression equation: daytime SBP CV = $4.32 + 0.08 \text{ Age} - 0.17 BRS_{SNP}$; $R^2 = 35\%$, $p = 0.06$). This was not seen with the Valsalva and phenylephrine-derived BRS. For diastolic blood pressure, the relation between increasing BRS and decreasing daytime variability was more marked, again independent of age and the DBP level (regression equations: daytime DBP CV = $-4.66 + 0.26 \text{ Age} - 0.45 BRS_{V1}$, $R^2 = 31.3\%$, $p = 0.026$; daytime DBP CV = $-6.43 + 0.28 \text{ Age} - 0.17 BRS_{V2}$, $R^2 = 28.8\%$, $p = 0.07$; daytime DBP CV = $-7.08 + 0.30 \text{ Age} - 0.37 \text{ steady-state } BRS_{SNP}$, $R^2 = 31.9\%$, $p = 0.012$; daytime DBP CV = $-6.64 + 0.29 \text{ Age} - 0.42 \text{ ramp } BRS_{SNP}$, $R^2 = 33.3\%$, $p = 0.007$; daytime DBP CV = $-7.37 + 0.29 \text{ Age} - 0.17 \text{ ramp } BRS_{PE}$, $R^2 = 28.0\%$, $p = 0.08$). Furthermore, daytime heart rate variability increased significantly with increasing BRS (from the Valsalva and SNP methods) independent of age and the baseline heart rate level (regression equations: daytime HR CV = $-9.26 + 0.28 \text{ Age} + 1.27 BRS_{V1}$, $R^2 = 23.0\%$, $p = 0.001$; daytime HR CV = $-4.32 + 0.22 \text{ Age} + 0.47 BRS_{V2}$, $R^2 = 17.2\%$, $p = 0.005$; daytime HR CV = $-1.73 + 0.17 \text{ Age} + 1.03 \text{ steady-state } BRS_{SNP}$, $R^2 = 27.1\%$, $p < 0.001$;

Pearson's <i>r</i>		Valsalva		BRS _{SNP}		BRS _{PE}	
		BRS _{V1}	BRS _{V2}	Steady- state	Ramp	Steady- state	Ramp
Clinic	SBP	-0.66‡	-0.57‡	-0.53‡	-0.55‡	-0.49‡	-0.60‡
	DBP	-0.46‡	-0.46‡	-0.39†	-0.40†	-0.24	-0.33*
24-hour	SBP	-0.47‡	-0.37†	-0.32*	-0.34*	-0.36†	-0.45‡
	DBP	-0.25	-0.24	-0.12	-0.15	0.00	0.12
Daytime	SBP	-0.44‡	-0.35†	-0.27*	-0.30*	-0.29*	-0.39†
	DBP	-0.23	-0.22	-0.08	-0.12	0.08	-0.05
Night-time	SBP	-0.54‡	-0.43†	-0.38†	-0.40†	-0.47‡	-0.50‡
	DBP	-0.35†	-0.33*	-0.21	-0.23	-0.18	-0.23

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$

SBP: systolic blood pressure; DBP: diastolic blood pressure

Table 2.9 Univariate correlations between indices of baroreflex sensitivity and clinic and ambulatory blood pressure

Cardiovascular neural control

Pearson's <i>r</i>		Vaisaiva		BRS _{SNP}		BRS _{PE}	
		BRS _{V1}	BRS _{V2}	Steady- state	Ramp	Steady- state	Ramp
Daytime variability	SBP	-0.45‡	-0.37†	-0.34*	-0.35†	-0.19	-0.19
	DBP	-0.47‡	-0.38†	-0.31*	-0.36†	-0.20	-0.26
	HR	0.30*	0.26	0.37†	0.36†	0.06	0.10
Daytime CV	SBP	-0.26	-0.23	-0.24	-0.25	-0.05	0.01
	DBP	-0.36†	-0.27*	-0.29*	-0.32*	-0.25	-0.24
	HR	0.40‡	0.36†	0.50‡	0.50‡	0.11	0.14

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; CV: coefficient of variation

Table 2.10 Univariate correlations between indices of baroreflex sensitivity and daytime ambulatory blood pressure and heart rate variability

daytime HR CV = $-2.72 + 0.18 \text{ Age} + 1.11 \text{ ramp BRS}_{\text{SNP}}$, $R^2 = 28.4\%$, $p < 0.001$).

Broadly, therefore, a significant relation was seen between increasing BRS, decreasing daytime blood pressure variability and increasing heart rate variability independent of age and the blood pressure level.

2.4.6 Head-up tilting

Head-up tilt was not associated with any symptoms of dizziness or faintness in any subject. Baseline forearm blood flow (Group 1: $2.7 \pm 0.3 \text{ ml}\cdot\text{min}^{-1}\cdot 100\text{ml}^{-1}$, Group 2: 2.4 ± 0.2 and Group 3: 2.1 ± 0.2 , $p = 0.21$) and forearm vascular resistance (Group 1: 50 ± 5 arbitrary units, Group 2: 55 ± 6 and Group 3: 50 ± 4 , $p = 0.71$) were not different between the three groups. The percentage changes in FBF with head-up tilt are shown in Figure 2.7. The reductions in the ISH group were the greatest, with a statistically significant difference between the groups in the entire FBF response curves ($p = 0.008$). Subjects with ISH manifested greater increases in FVR with tilt by comparison with the other two groups (effect of group: $F=8.33$, $p < 0.001$; effect of time: $F=4.00$, $p = 0.001$; Figure 2.8). The maximum fall in blood pressure with tilt was significantly greater in the subjects with ISH compared to the normotensive group (Table 2.11), and the maximum increase in heart rate with orthostatic stress was significantly greater in the normotensives than in the ISH group.

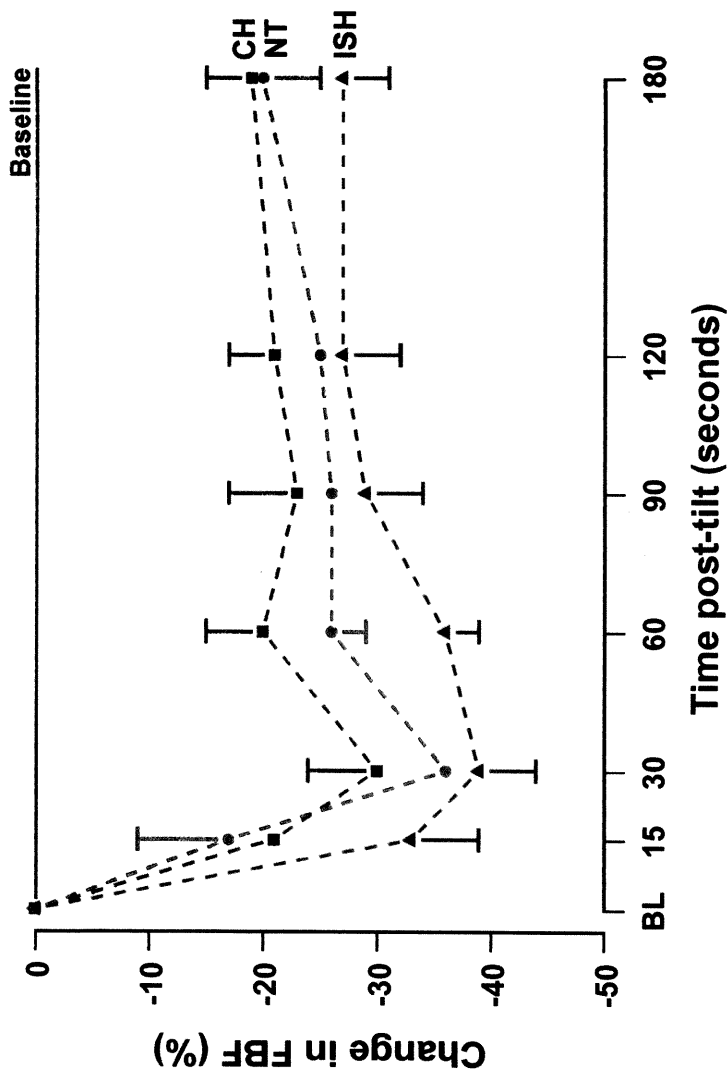


Figure 2.7 Percentage changes in forearm blood flow (FBF) with passive tilt for the combined hypertensive (CH), isolated systolic hypertensive (ISH) and normotensive (NT) groups
p<0.01 for difference between groups in the entire response
BL: baseline; some error bars omitted for clarity

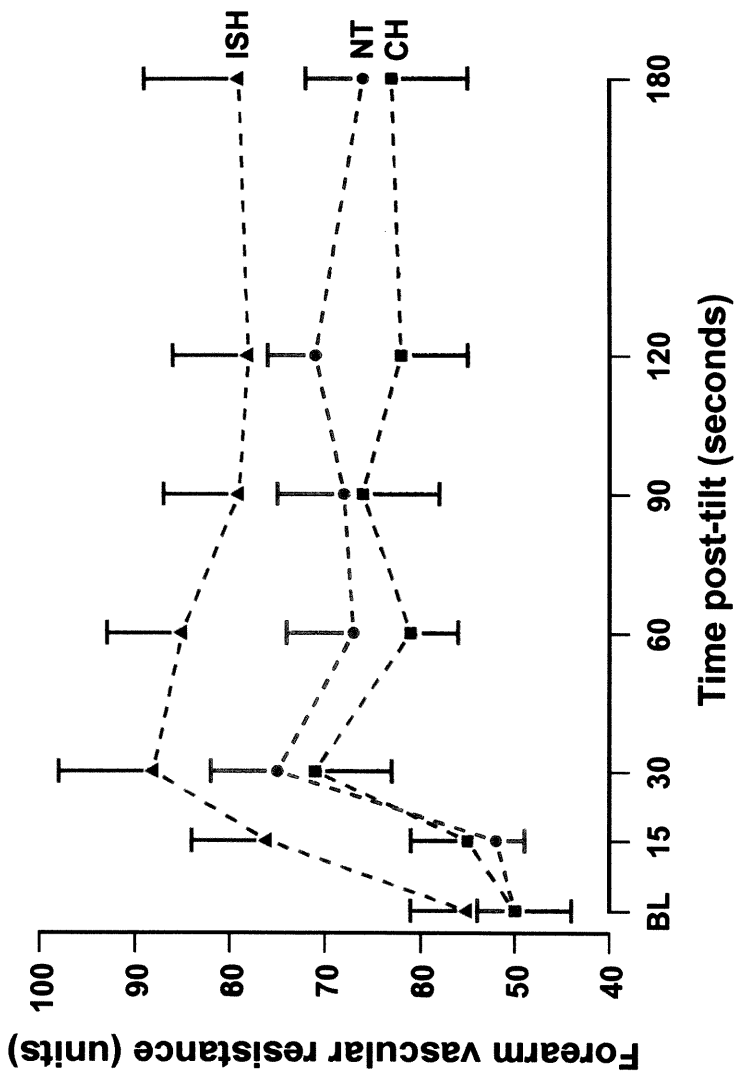


Figure 2.8 Changes in forearm vascular resistance with passive tilt for the combined hypertensive (CH), isolated systolic hypertensive (ISH) and normotensive (NT) groups
p<0.001 for difference between groups in the entire response; BL: baseline

Cardiovascular neural control

	Group 1 (CH)	Group 2 (ISH)	Group 3 (NT)
Max SBP fall (mmHg)	44 ± 5	49 ± 5*	35 ± 3
Time to trough SBP (secs)	53 ± 14	37 ± 12	36 ± 9
Max DBP fall (mmHg)	15 ± 2	17 ± 2	13 ± 2
Time to trough DBP (secs)	32 ± 10	36 ± 13	27 ± 9
Max HR increase (bpm)	18 ± 2	15 ± 2*	24 ± 4
Time to peak HR (secs)	93 ± 16	107 ± 11	89 ± 11

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate
* $p < 0.05$, ISH v NT (ANOVA with Tukey's correction for multiple comparisons).

Table 2.11 Blood pressure and heart rate responses to orthostatic stress for the three groups

2.4.7 Cold face stimulus

Again, forearm blood flow (Group 1: $2.6 \pm 0.3 \text{ ml}\cdot\text{min}^{-1}\cdot 100\text{ml}^{-1}$, Group 2: 2.3 ± 0.3 and Group 3: 2.1 ± 0.2 , $p = 0.36$) and forearm vascular resistance (Group 1: 53 ± 6 arbitrary units, Group 2: 60 ± 7 and Group 3: 52 ± 4 , $p = 0.57$) did not differ between the three groups at baseline. FBF fell on average by 20% during the first 15 seconds of the CFS, but there was no further fall and this response was not different between the three groups. Blood pressure rose modestly in all three groups, with the largest rises being seen in Group 1 (combined hypertensives) (Table 2.12). Heart rate was little changed by the CFS, but there was a significantly larger rise in Group 1 than in the other two groups. The result of these changes was a significant rise in FVR of approximately 30% in all three groups (Figure 2.9). Changes in FVR were significantly related to time ($F=3.88$, $p = 0.01$) but the differences between groups were not significant ($F=2.37$, $p = 0.10$).

Cardiovascular neural control

	Group 1 (CH)	Group 2 (ISH)	Group 3 (NT)
Max SBP increase (mmHg)	26 ± 5*	18 ± 3	15 ± 2
Time to peak SBP (secs)	29 ± 4	23 ± 4	23 ± 4
Max DBP increase (mmHg)	12 ± 3*	7 ± 1	5 ± 1
Time to peak DBP (secs)	30 ± 4	33 ± 3	27 ± 4
Max HR increase (bpm)	8 ± 3*‡	3 ± 1	3 ± 1
Time to peak HR (secs)	31 ± 4	34 ± 3†	22 ± 3

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate
 * $p < 0.05$ CH v NT; † $p < 0.05$ ISH v NT; ‡ $p < 0.05$ ISH v CH (ANOVA with Tukey's correction for multiple comparisons).

Table 2.12 Blood pressure and heart rate responses to the cold face stimulus for the three groups

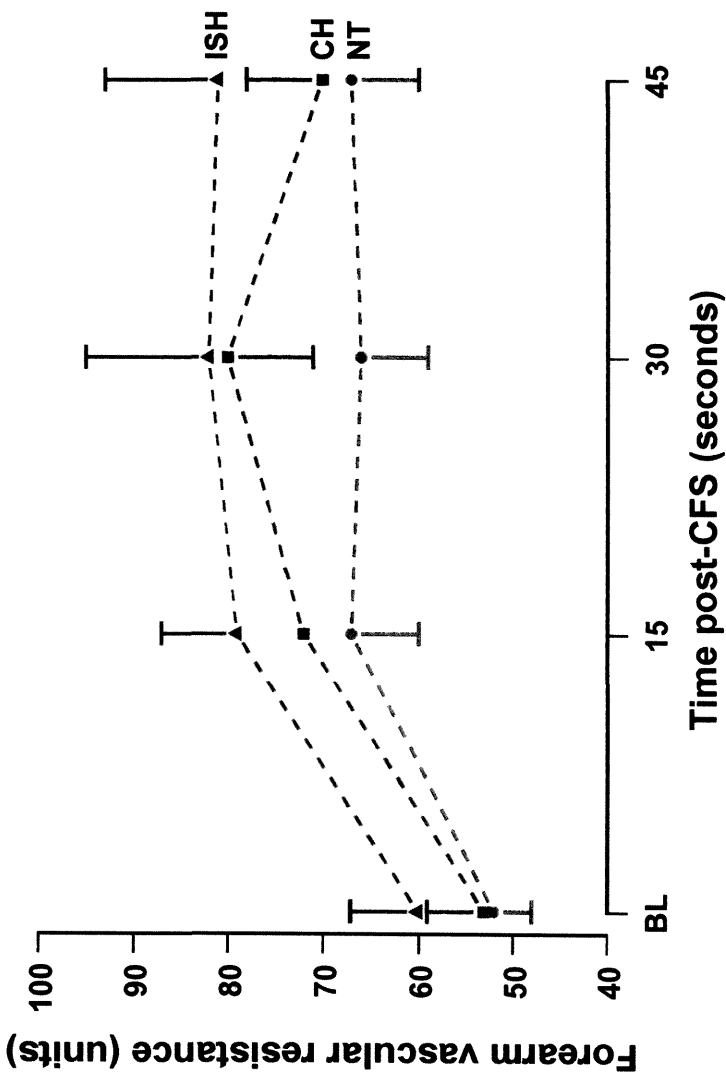


Figure 2.9 Changes in forearm vascular resistance with the cold face stimulus (CFS) for the combined hypertensive (CH), isolated systolic hypertensive (ISH) and normotensive (NT) groups
Some error bars omitted for clarity; BL: baseline

2.5 DISCUSSION

2.5.1 Blood pressure, heart rate and daytime blood pressure variability

This study provides a comprehensive evaluation of cardiovascular neural control in elderly subjects and the relation to blood pressure. The study design permits analysis of the differences in both the baroreceptor-cardiac reflex (including responses to both a pressor and a depressor stimulus) and the baroreceptor-vascular responses between elderly subjects with combined hypertension, isolated systolic hypertension and 'normal' blood pressure. In this regard, the study achieved this division in terms of clinic blood pressure, with the two hypertensive groups being matched for similar levels of clinic systolic blood pressure, with a highly significant (both statistically and clinically) 17 mmHg average difference in clinic diastolic blood pressure. As far as is possible, subjects with ISH but with a history of previous elevation of diastolic blood pressure were excluded from the study to establish separation of the groups, but it cannot be certain that contamination between the groups did not occur. The present study did not exclude from the ISH group subjects who, during the initial period of clinic observation of their blood pressure, had the occasional diastolic value between 90 and 95 mmHg provided the final average for their 'sustained' diastolic blood pressure was less than 90 mmHg. What is more, clinic diastolic blood pressure was still significantly higher in the ISH group than in the normotensives, indicating that these subjects did not strictly have 'isolated' elevation of systolic pressure, but rather 'predominant' systolic hypertension [Koch-

Weser, 1973]. Interestingly, on 24-hour ABPM, levels of diastolic blood pressure were much closer for the Group 1 (CH) and Group 2 (ISH) subjects, although values for the ISH group remained consistently lower (Figure 2.2). This occurred because, although clinic and 24 hour diastolic blood pressure were similar in the ISH group, diastolic blood pressure fell significantly with 24 hour monitoring in the CH group (by an average 13 mmHg). Both groups showed a substantial fall in systolic blood pressure on 24 hour monitoring (34 mmHg for CH, 27 mmHg for ISH). These clinic-ambulatory differences accord with those observed in other studies of elderly hypertensives using intermittent recorders [Ruddy et al, 1988; Khoury et al, 1992; Thijs et al, 1992b]. Such studies do not contain similar data on heart rate, but in the present study both clinic and 24 hour heart rate were consistently higher in the CH group, even at night, and this may suggest an underlying greater activation of the sympathetic nervous system in this group. This possible association is supported by the findings of a recent study of a correlation between resting heart rate and arterialed plasma adrenaline levels in elderly subjects, with significantly higher adrenaline levels in CH compared to normotensives [Potter et al, 1993].

Daytime blood pressure variability, when corrected for the mean level of blood pressure by calculation of the CV, was not different between the three groups. The analysis of variability was confined to the daytime BP readings (07:00 to 22:00) to avoid error in the measurement due to a low frequency of readings at night. Di Rienzo and colleagues (1983) demonstrated that the standard deviation of readings (as a measure of variability) taken at fifteen minute intervals was similar to that obtained from continuous, intra-arterial recordings. This may account for the

difference between the findings of the present study and those of Muneta et al (1991), who found a significant increase in the CV for both systolic and diastolic blood pressure in subjects with ISH compared to subjects with diastolic hypertension and normotensives. Ambulatory readings in their study were made at 30 minute intervals during the daytime, with further deletions of data if 'inconsistent increases or decreases' in blood pressure were observed. The findings from the present study agree with those of Rowlands et al (1984) who found no difference in BP variability between elderly hypertensives and normotensives on continuous 24 hour intra-arterial monitoring (despite problems with the selection of subjects for each group), and with the findings of Ruddy et al (1988), who found no difference in BP variability between elderly subjects with ISH and normal blood pressure.

2.5.2 Baroreceptor-cardiac reflex sensitivity

In the study of cardiovascular reflexes, the vagal loop was demonstrated to be unimpaired with hypertension, with no difference in RR interval variation with respiration between the three groups. Further, the Valsalva ratio was also similar between the three groups. Study of the baroreceptor-cardiac reflex, however, identified a consistent reduction in BRS with hypertension, irrespective of the method used for study (phase 4 of the Valsalva manoeuvre, phenylephrine pressor or nitroprusside depressor method; Table 2.7). BRS in the CH and ISH groups was significantly lower than for the normotensive group, but there were no differences between the two hypertensive groups. This observation is also borne out by the

multiple regression analysis, which identified systolic blood pressure as the single predictor of BRS, independent of age and diastolic blood pressure.

The present study is in agreement with that of Sumimoto et al (1990) who found reduced BRS in elderly subjects categorised as either diastolic hypertensives (continuing from middle age) or recently-diagnosed ISH, although after seven days of hospitalisation most of the subjects in their study were in fact normotensive. However, the current study contrasts with that of Jansen and Hoefnagels (1989), who found no difference in BRS from the phenylephrine method between elderly hypertensive and normotensive subjects. Where they do agree, however, is that the baroreflex when assessed by the depressor method is impaired with hypertension in elderly subjects. This discrepancy may have been because their study had insufficient power to test the observed difference between the groups in BRS_{PE} , whereas it was powerful enough to test the difference seen in depressor BRS (nitroglycerin in their study). Kawamoto et al (1989) found no difference in R-R variability and no impairment of the baroreflex measured from phase 4 of the Valsalva manoeuvre in elderly hypertensive subjects, and concluded that hypertension is not associated with impairment of BRS in the elderly. The data from the present study and that of Kawamoto and colleagues cannot be directly compared because of methodological differences, but otherwise it is difficult to account for the disparity in the results from the two studies. However, the present study has identified a distinct reduction in BRS from phase 4 of the Valsalva manoeuvre with hypertension which is entirely consistent with the results observed with other pharmacological tests of BRS.

Atherosclerosis of large arteries, which causes loss of arterial compliance and contributes to a preferential elevation of the systolic pressure and thus to ISH [O'Rourke, 1990], has previously been proposed as a cause of reduced baroreceptor sensitivity in such patients. Studies of large artery compliance in elderly subjects have indeed identified significant differences between elderly subjects with CH and ISH [Sumimoto et al, 1990]. The present study indicates no difference in BRS between elderly subjects with CH and ISH and this suggests that changes in arterial wall compliance at sites such as the aortic arch and the carotid sinus may make less of a contribution to reduced baroreceptor function than has been regarded heretofore. The observed association between systolic hypertension and orthostatic hypotension has often been attributed to afferent baroreflex dysfunction related to loss of arterial compliance [Lipsitz et al, 1985; Tonkin et al, 1991]. If subjects with ISH are more prone to orthostatic hypotension than their counterparts with CH, this study presents no evidence that this is due to differences between these two groups in baroreflex function. Of relevance is the demonstration in the present study of similar responses of systolic and diastolic blood pressure to 60° head-up tilt between the two hypertensive groups, and an increased heart rate response in the normotensives compared to the other two groups. The relationship between orthostatic responses and arterial BRS is examined in more detail in Chapter 5.

The two most often cited studies of the relation between BRS and age have included a total of three subjects aged over 60 years [Gribbin et al, 1971; Duke et al, 1976], while other studies have not confirmed an independent effect of age [Parmer

et al, 1992; Lage et al, 1993]. The present study exclusively of subjects over the age of 60 years (range 60-81) indicates that beyond this point any relation with age is diminished or lost, although with the relatively narrow age range studied a slight effect of age cannot be entirely ruled out. Thus systolic blood pressure remains the main predictor of changes in BRS independent of age and diastolic blood pressure. Nevertheless, changes in systolic blood pressure accounted for only between 27% and 45% of the variance in BRS.

Short term modulation of blood pressure and heart rate by baroreflex mechanisms leads to a reduction in blood pressure variability with a corresponding increase in heart rate variability. A significant relation was seen in the present study between increasing BRS, decreasing daytime blood pressure variability (mainly diastolic) and increasing heart rate variability independent of age and the blood pressure level. This indicates a clinically important link between BRS and blood pressure variability irrespective of blood pressure itself. This has previously been demonstrated in the young [Watson et al, 1980; Conway et al, 1984; Mancia et al, 1986; Parati et al, 1992; Siché et al, 1993, 1995] but an independent association between BRS and blood pressure variability has not been previously reported in a group of elderly subjects. This may have been due in part to negative publication bias because it is known that at least one study including the relevant data has been performed [Rowlands et al, 1984].

2.5.3 The baroreceptor-vascular reflex

Head-up tilting with measurement of the changes in forearm vascular resistance enables the study of another limb of the baroreflex: the baroreceptor-vascular reflex. Heart rate is almost immediately increased in response to tilt via the arterial baroreceptor-cardiac reflex, with withdrawal of vagal activity and sympathetic stimulation, which is also responsible for the slower-acting increases seen in vascular resistance. Cardiopulmonary reflexes act in synergy to cause sympathetic activation. In the present study baseline forearm blood flow and vascular resistance were not different between subjects with CH, ISH and normal blood pressure, but reductions in FBF were greatest in the ISH group, with greater increases in FVR than those seen in the CH or NT groups (Figure 2.8). Relative preservation of the baroreceptor-cardiac reflex in the normotensive subjects was reflected in significantly greater increases in heart rate in this group which also tended to occur sooner following tilt, and this moderated the blood pressure changes to some extent, with less of a blood pressure fall in the normotensives than in the other two groups. The absolute blood pressure changes in all three groups were greater than usually described [Jansen et al, 1989] and this is probably due to the use of the continuous Finapres method for recording blood pressure, rather than an intermittent method. The continuous method ensured the capture of the nadir of blood pressure whenever it occurred. Vardan et al (1993), using intra-arterial blood pressure monitoring, similarly found much larger falls in systolic blood pressure in the elderly with passive tilting than are usually found with intermittent recordings.

London et al (1987) observed no difference in the heart rate response but greater increments in total peripheral resistance with tilting in young hypertensive subjects (of mean age 33 years, all below 50 years) from an already higher baseline level when compared to age-matched normotensives, and suggested that this may be due to hypertension-related structural alterations in the resistance vasculature amplifying the vasoconstrictive response [Folkow, 1973]. In their study, London and colleagues measured total peripheral resistance by the indocyanine green dilution method and thus included the vasoconstrictive response of all vascular beds. The splanchnic bed is regarded as of particular importance in the response to orthostasis [Bannister & Mathias, 1988] and may differ from the forearm in being predominantly regulated by arterial baroreceptors, while the forearm vessels are principally regulated by cardiopulmonary receptors [Abboud et al, 1979]. In elderly subjects Tonkin & Wing (1994) found no difference in the forearm vascular responses to head-up tilt between normotensives and subjects with ISH. They based their analysis on blood pressure and FBF data obtained after ten minutes of tilt (5 minutes at 30° followed by 5 minutes at 60°) when a large part of the adaptative response would have already occurred. The methods used in the present study permitted a more detailed analysis of the entire response over time, and it was this analysis that exposed the differences between the groups. Indeed if the analysis were confined solely to data obtained at the end of tilt (i.e. at three minutes in the current study) there would have been no significant differences between the three groups. Thus the data obtained by Tonkin & Wing at ten minutes (in about half the number of subjects as in the present study) risked missing the greater part of the differences in the baroreceptor-vascular response. An explanation such as that offered by London et al (1987) may apply in

part to the data seen in the present study, in that despite substantial impairment of the baroreceptor-cardiac reflex by comparison with normotensives, the blood pressure fall with head-up tilting in elderly subjects with ISH may be partly compensated by an enhanced baroreceptor-vascular response, possibly mediated through hypertension-related structural alterations. However, this does not explain the observations in the CH group who, despite a reduction in arterial BRS similar to that seen in the ISH group but with no similar increase in their FVR with tilt, had no greater fall in blood pressure with tilt. A structural factor, however, is more likely to account for the observed changes than any increased level of sympathetic activation in response to orthostatic stress, which tends to be attenuated in elderly hypertensive subjects and is no greater than in normotensives [Rowlands et al, 1984; Jansen et al, 1989; Lipsitz et al, 1990; Lucini et al, 1993].

2.5.4 Non-baroreceptor-mediated reflexes

The cold face stimulus tests non-baroreceptor mediated vasoconstrictive responses and leads to a rise in blood pressure and a sympathetically-mediated fall in musculoskeletal blood flow [Khurana et al, 1980; Anderson et al, 1988; Heath & Downey, 1990]. Young hypertensives have previously been shown by Bolli and colleagues (1981) to exhibit greater vasoconstriction with a cold stress than normotensives, although their study found baseline FBF to be greater in the hypertensives, in contrast to the findings of London et al (1987). In the elderly Tonkin & Wing (1994) showed greater increases in FVR in subjects with ISH,

although blood pressure and heart rate changes were similar to those seen in normotensives. The present study has demonstrated similar changes in FVR between CH, ISH and NT subjects (an increase of approximately 30%) but greater increments in blood pressure and heart rate in the combined hypertensives. These findings could be considered together with that of a higher heart rate in the combined hypertensives in the clinic and on 24-hour ABPM (both during the day and at night), to suggest a greater degree of baseline sympathetic activity in those subjects. In response to an sympathetic-mediated stimulus such as the CFS, an augmented response from an already higher baseline level may be observed. However, baseline FBF and FVR were not different between the hypertensive and normotensive subjects, although there was a non-significant trend towards an augmented vasoconstrictive response in the CH group. Although the differences seen between the CH subjects and those with ISH with the CFS are small, if taken together with the differences seen in the baroreceptor-vascular response to tilt may indicate that while baroreceptor-cardiac function is not different between them, there may be distinctions between the groups related to the sympathetic nervous system and vascular structural adaptation.

2.5.5 Study limitations

The interpretation of these results needs to take account of the limitations of the study. The methods used for the assessment of the baroreceptor-cardiac reflex are at their most accurate when used to study subjects with higher levels of baroreflex

sensitivity; the 'ramp' techniques cannot be used in subjects in whom pulse interval changes very little in response to substantial blood pressure changes as a significant regression will not be found. The alternative method involving a modification of Korner's 'steady-state' technique [Korner et al, 1974] can avoid this obstacle and a figure for BRS could thus be obtained for all subjects. It must be remembered however, that observations concerning the ramp methods have excluded seven subjects from amongst those with lower values for BRS. Notably these considerations have not featured in other studies of subjects who have exhibited similarly low values for BRS [Goldstein et al, 1982; Palmero et al, 1981; Rowlands et al, 1984].

The assessment of the sympathetic response to stimuli such as the cold face stimulus and tilting has been confined to measurement of changes in forearm blood flow and vascular resistance in the present study. Ideally the assessment of changes in peripheral vascular resistance should include the whole-body measurement of changes thus including the splanchnic bed, so alterations in total peripheral resistance cannot necessarily be deduced from changes measured only in the forearm. The best technique for assessing sympathetic responses would be microneurography [Wallin, 1988; Ebert et al, 1992], but this method was not available for the present study. Thus caution is required in regarding the data from the current study as descriptive of the entire sympathetic response. Similarly, care is required in interpreting the responses to tilt as the cardiovascular changes are complex and do not discriminate between high- and low-pressure baroreflexes, which may be differentially affected by hypertension. Time constraints prevented the study of all

the constituent components involved in the response using lower body negative pressure or passive leg raising (to offload and stimulate cardiopulmonary receptors) and the data should be regarded as descriptive only of the integrated response to tilt.

2.6 CONCLUSIONS

1. Reduced arterial baroreceptor-cardiac reflex sensitivity in elderly hypertensive subjects is principally related to the level of systolic blood pressure.
2. This observation is consistent irrespective of the method of baroreceptor-cardiac reflex testing used.
3. There is no difference in arterial baroreflex sensitivity between elderly hypertensive subjects with combined systolic-diastolic hypertension and isolated systolic hypertension, matched for similar levels of systolic blood pressure.
4. In contrast to what has been described in younger hypertensive subjects, in the elderly, ranging in age from 60 to 81 years, there is no independent relation between increasing age and declining baroreflex sensitivity.

5. There is an independent relation between blood pressure variability and heart rate variability assessed from intermittent ambulatory recordings and baroreflex sensitivity in elderly subjects.
6. Ambulatory blood pressure monitoring demonstrates similar twenty-hour blood pressure between subjects with combined hypertension or isolated systolic hypertension. Twenty-four hour pulse rate, however, is higher in the combined hypertensive subjects than in those with either isolated systolic hypertension or normotension.
7. Baseline forearm blood flow and vascular resistance is not different with hypertension. However, an enhanced increment in forearm vascular resistance with passive tilt is seen in elderly subjects with isolated systolic hypertension.
8. Despite this, systolic blood pressure falls to a greater extent in subjects with isolated systolic hypertension than those with normal blood pressure.
9. Blood pressure and heart rate responses to the non-baroreflex-mediated cold face stimulus are increased in the combined hypertensives compared to those with isolated systolic hypertension or normal blood pressure.

Chapter 3

Arterial blood pressure and resistance artery
structure in elderly subjects

3.1 SUMMARY

This study was designed to investigate the relation between blood pressure and resistance artery structure in elderly subjects from an age group in which a widening of the pulse pressure is a typical finding, with the blood pressure parameters being characterised from 24 hour ambulatory blood pressure monitoring. Resistance vessels were retrieved from biopsies of skin and subcutaneous fat taken from the gluteal region under local anaesthesia in 32 subjects of mean age 70 years, 21 of whom were untreated hypertensives and 11 were normotensive. Morphology studies were made under light microscopy after mounting of the vessels in an isometric myograph.

Media:lumen ratio was higher in the hypertensive subjects than in the normotensives ($18.6 \pm 1.6\%$ vs. $12.8 \pm 1.2\%$, $p < 0.01$). Media:lumen ratio was also positively correlated with age, clinic systolic blood pressure, 24-hour systolic blood pressure and 24-hour pulse pressure. Stepwise multivariate regression analysis identified clinic and 24-hour pulse pressure as the only significant predictors of media:lumen ratio independent of age, other parameters of clinic blood pressure and blood pressure variability ($R^2 = 41\%$, $p < 0.05$). These findings are consistent with the results from younger subjects showing an increased media:lumen ratio with hypertension, and confirm and extend the findings from animal models of hypertension of the importance of the pulse pressure in relation to cardiovascular structural adaptation.

3.2 BACKGROUND and AIMS

Several studies have examined the relation between blood pressure and vascular structural adaptation in younger hypertensive subjects. However, recent studies in experimental hypertension have suggested that pulse pressure may be a previously unrecognised determinant of hypertensive cardiovascular adaptation. The elderly manifest both a widening of the pulse pressure and an increase in the peripheral resistance, so the study of small arteries in this age group presents a particular opportunity to examine the significance of the different parameters of blood pressure, including the pulse pressure, in determining structural alteration.

The aims of this study were therefore twofold:

1. To describe the structure of resistance arteries in the elderly;
2. To examine the relation between resistance artery structure and blood pressure in this age group. This would be achieved by the careful characterisation of blood pressure with 24 hour ambulatory blood pressure monitoring.

3.3 MATERIALS and METHODS

3.3.1 Study subjects and blood pressure measurement

This study involved 32 elderly (older than 60 years) subjects drawn from hospital referrals, their friends and relatives and from respondents to a local newspaper

Resistance artery structure

advertisement. The study received the approval of the local ethics committee and all subjects gave written informed consent. All subjects attended the research clinic where blood pressure was measured supine after five minutes rest, with a large cuff when necessary, using a standard mercury sphygmomanometer. A minimum of three clinic blood pressure readings were taken on three separate occasions at least two weeks apart and the reported clinic blood pressure is the average of the resultant nine or more readings. Those subjects with a clinic blood pressure ≥ 160 mmHg systolic and/or ≥ 90 mmHg diastolic (Korotkoff phase V) were classified as hypertensive. By these criteria, 21 subjects were hypertensive and 11 normotensive. No subject had previously received any anti-hypertensive medication. All subjects had a normal physical examination and electrocardiogram, and no subject had evidence of secondary hypertension or coronary artery disease by history and examination, and standard laboratory and radiological tests. Subjects with a previous history of angina, myocardial infarction, diabetes mellitus or stroke were excluded. On their third visit to the clinic, a fasting blood sample was taken for the measurement of cholesterol and triglyceride levels, and subjects were fitted with a SpaceLabs 90207 blood pressure monitor (SpaceLabs Inc., Redmond, Washington, USA). Monitor validation and recording technique was as described in Chapter 2. Mean systolic blood pressure, mean arterial pressure and diastolic blood pressure values were obtained for the full 24 hours and for the respective daytime and nighttime periods. Pulse pressure was taken as the systolic minus the diastolic pressure. Daytime blood pressure variability was taken as the standard deviation of all the daytime readings taken at 15 minute intervals.

3.3.2 Myograph protocol

Within 2 weeks of ambulatory blood pressure recording, subjects attended the research clinic at 8 am for the donation of a biopsy of skin and subcutaneous fat from the gluteal region (approximately 2 cm × 0.5 cm × 1 cm), taken under local anaesthesia (3-5 ml of 2% lignocaine hydrochloride). Between two and four silk stitches were used to suture the biopsy site, and these were removed 7-10 days later. The biopsy was placed in a sterile receptacle containing cold (4°C) physiological salt solution (PSS) of composition NaCl 118 mmol/l, KCl 4.5 mmol/l, CaCl₂ 2.5 mmol/l, MgSO₄·7H₂O 1.0 mmol/l, KH₂PO₄ 1.0 mmol/l, NaHCO₃ 25 mmol/l, glucose 6 mmol/l for transfer to the laboratory without delay. Small arteries were then dissected from the biopsy and mounted as ring preparations on two parallel 40 µm stainless steel wires in a Mulvany-Halpern myograph (JP trading, Aarhus, Denmark; Figure 3.1) [Mulvany & Halpern, 1977; Aalkjær, 1993]. Both dissection and mounting were carried out in cold PSS. The central recess of the myograph (of volume 10 mL) contained two pairs of horizontally opposed 'jaws' on which the ring preparation was mounted, with one wire connected to a micrometer and the other to a force transducer to measure isometric tension (Figure 3.2). The output from the force transducer was routed to a flatbed 2-channel chart recorder (Graphic 20002, Lloyds Instruments, Loughborough, UK) where changes in tension were plotted against time. The vessels were held without tension in 10 mL PSS at 37°C and bubbled with a gaseous mixture of 5% CO₂/95% O₂ to achieve a pH of 7.4. Following mounting in the myograph, the vessels were allowed to equilibrate for 30 minutes before the myograph was transferred to the stage of a microscope for

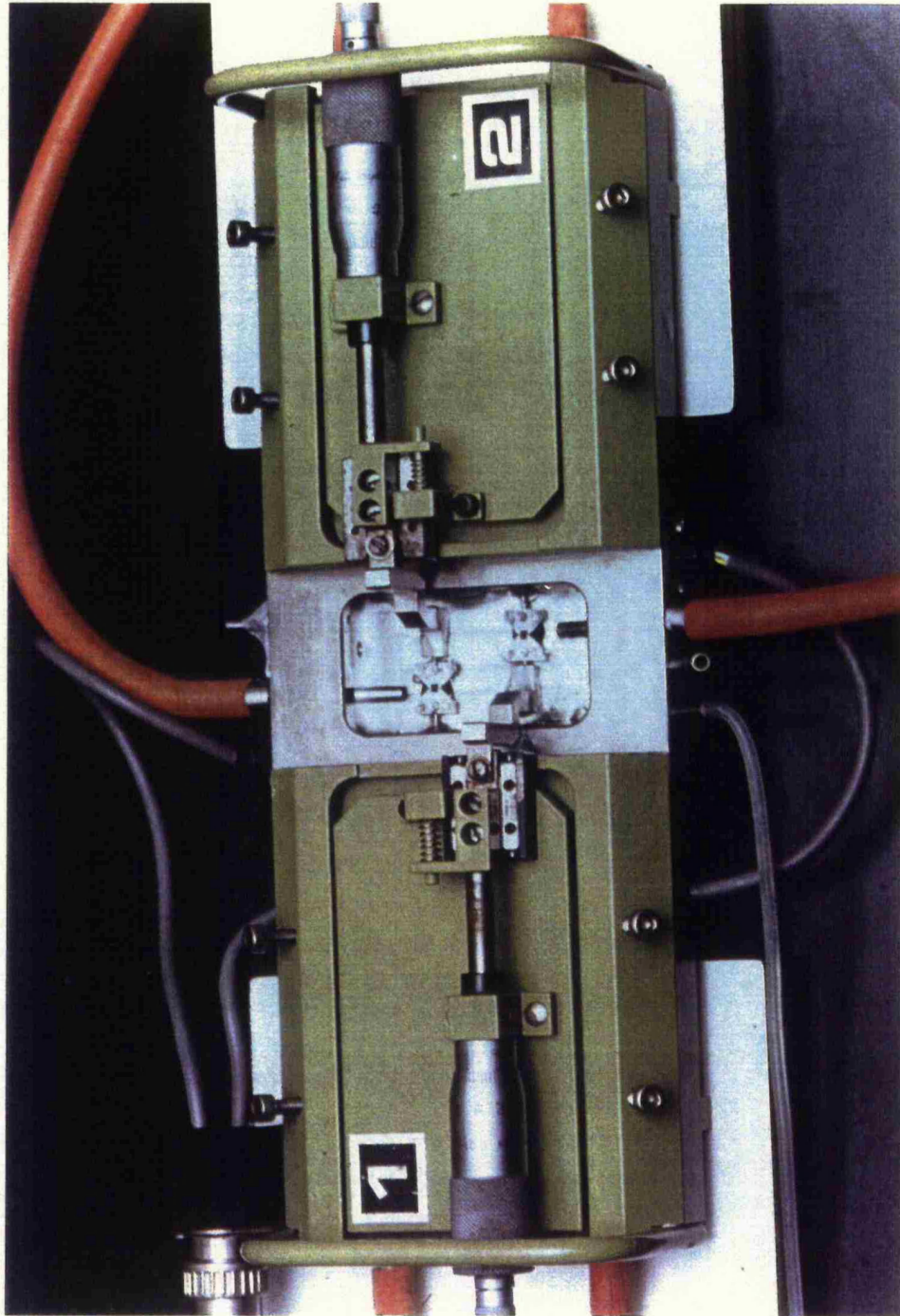


Figure 3.1

The Mulvany-Halpern myograph

Resistance arteries were mounted between each set of 'jaws' within the central recess, each positioned over a small glass aperture to enable light microscopic measurements

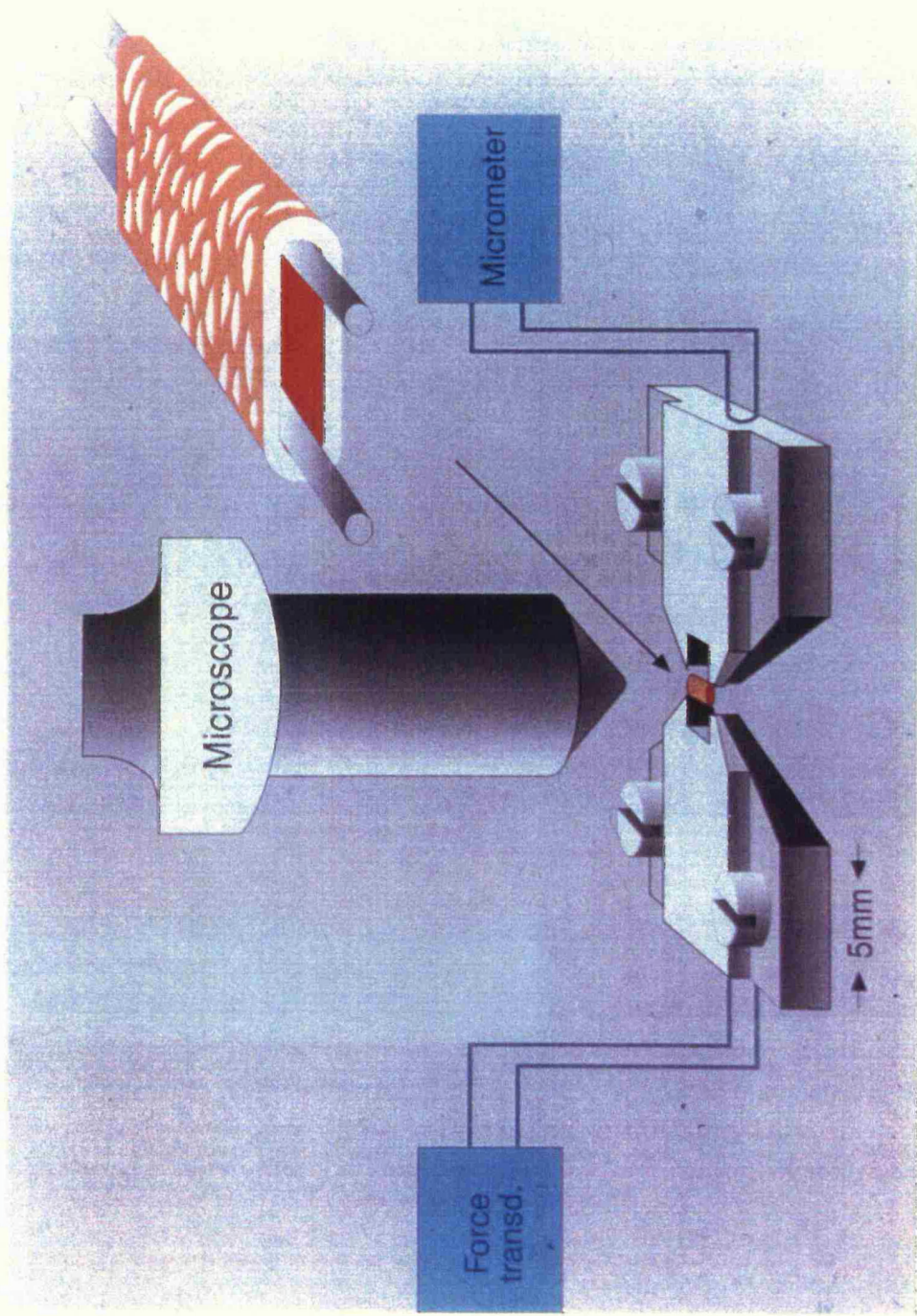


Figure 3.2 Schematic diagram of a resistance artery mounted on two parallel $40\ \mu\text{m}$ wires between the 'jaws' of the myograph

The micrometer is used to set the vessel to $L_{0.9}$ (see text), with the generated tension recorded from the output of the force transducer

Resistance artery structure

measurement of vessel length and media thickness under minimum tension. The myograph recess contained a glass aperture beneath each set of jaws to enable such measurements to be made by light microscopy, using an immersion objective lens and a graduated eyepiece.

After a further 30 minutes of equilibration, the vessels were exposed to predetermined stepwise increases in tension to determine the length-tension characteristic from the Laplace equation ($P = T/r$, where P is the transmural pressure, T is the wall tension and r the internal radius of the vessel). Then the internal diameter was set to $0.9 \times L_{100}$, where L_{100} is the internal diameter that the vessel would have *in vivo* when relaxed at $P = 100$ mmHg. This is described as the normalized lumen diameter, $L_{0.9}$, and is the diameter at which the generated isometric tension is greatest [Mulvany & Halpern, 1977]. Morphology measurements obtained for each vessel included length (mm), normalized lumen diameter ($L_{0.9}$, μm), media cross-sectional area (μm^2), normalized media thickness (μm) and media:lumen ratio (%).

3.3.3 Statistical methods

Results are expressed as mean \pm standard error of the mean (SEM). Data were obtained from two vessels in 24 subjects and these data were averaged before analysis but in 8 subjects only one vessel could be retrieved from the biopsy. The results were analyzed in two ways. Firstly, a comparison between hypertensive and normotensive groups was made using Student's two-tailed unpaired t test (after

testing for normality with the Shapiro-Wilk W test) and the χ^2 test. Over the entire study group blood pressure parameters were normally distributed, and blood pressure within each hypertensive or normotensive subgroup also satisfied the Shapiro-Wilk test, enabling parametric comparisons. Secondly, Pearson's correlation and least squares regression analysis were used to test for linear relationships between structural parameters and continuous variables such as blood pressure and age. Simultaneous independent effects of these variables on structure were assessed by stepwise multivariate linear regression. A p value of < 0.05 was regarded as statistically significant.

3.4 RESULTS

3.4.1 Subject characteristics and blood pressure

Of the 32 subjects recruited, of mean age 70.1 ± 0.9 years, 17 (53%) were male. There were no differences in age, gender, smoking history, family history of hypertension, weight, alcohol intake, fasting cholesterol or triglycerides between the hypertensive and normotensive groups. Eight of the hypertensive subjects had isolated systolic hypertension on the basis of their clinic blood pressure. These subjects were of similar age, with 24 hour systolic blood pressure similar to those with combined systolic-diastolic hypertension and 24 hour diastolic blood pressure similar to the normotensive subjects, but 24 hour pulse pressure was not significantly different in subjects with isolated systolic hypertension compared with the combined

Resistance artery structure

hypertension subgroup (analysis of variance with Tukey's post hoc correction). Data comparison was therefore confined to that between the 21 hypertensive and 11 normotensive subjects. Clinic and 24 hour ambulatory blood pressure measurements for the study group, together with resistance vessel structure, are shown in Table 3.1. Clinic blood pressure and heart rate, and 24 hour blood pressure, were significantly lower in the normotensive group when compared to the hypertensive group. 24 hour ambulatory blood pressure was significantly lower than clinic blood pressure (all $p < 0.001$), but there was no difference in heart rate. There were no blood pressure differences with gender, with the exception of the 24 hour pulse pressure which was higher in females than males (68 ± 3 mmHg v. 59 ± 3 mmHg respectively, $p < 0.05$). Pulse pressure was not related to height.

3.4.2 Resistance vessel structure

The media:lumen ratio was significantly greater in the hypertensive subjects compared to the normotensives. This was probably the result of the combined effects of non-significant trends to decreased lumen diameter and increased media thickness in the hypertensives (Table 3.2). There was a tendency for the media:lumen ratio to be higher in females than males ($18.9 \pm 1.8\%$ v. $14.6 \pm 1.6\%$, $p = 0.08$) but sex was not an independent predictor of media:lumen ratio after correction for the difference in pulse pressure.

Univariate correlation was performed between resistance vessel structure and age, the various measures of clinic and ambulatory blood pressure, daytime blood

Resistance artery structure

Variable		All	Hypertensive	Normotensive
Number		32	21	11
Age (years)		70 ± 1	71 ± 1	68 ± 2
Male:female		17:15	10:11	7:4
Clinic				
Blood pressure	Systolic	171 ± 5	189 ± 5	135 ± 3‡
	Mean arterial	117 ± 3	128 ± 3	97 ± 2‡
	Diastolic	91 ± 3	97 ± 3	78 ± 2‡
Pulse pressure		80 ± 4	92 ± 4	57 ± 4‡
Heart rate (bpm)		72 ± 3	75 ± 4	66 ± 2*
24 hour				
Blood pressure	Systolic	147 ± 3	158 ± 2	127 ± 3‡
	Mean arterial	106 ± 2	112 ± 2	93 ± 3‡
	Diastolic	84 ± 2	88 ± 2	76 ± 3†
Pulse pressure		63 ± 2	70 ± 2	51 ± 2‡
Heart rate (bpm)		73 ± 2	74 ± 3	71 ± 2
Daytime BP variability				
	Systolic	14.3 ± 0.7	16.1 ± 0.8	10.6 ± 0.6‡
	Diastolic	10.7 ± 0.6	11.8 ± 0.6	8.5 ± 0.9†

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ for hypertensive v. normotensive groups
 All blood pressures are in mmHg. BP: blood pressure; bpm: beats per minute

Table 3.1 Blood pressure and heart rate for the whole study group and the hypertensive and normotensive subgroups

Variable	All	Hypertensive	Normotensive
Lumen diameter (μm)	266 \pm 16	255 \pm 18	286 \pm 30
Media CSA (μm^2)	35774 \pm 2362	36611 \pm 2968	34174 \pm 4034
Media thickness (μm)	40.6 \pm 2.0	42.7 \pm 2.5	36.5 \pm 3.5
Media:lumen ratio (%)	16.6 \pm 1.2	18.6 \pm 1.6	12.8 \pm 1.2†

† $P < 0.01$ for hypertensive v. normotensive groups

Table 3.2 Resistance artery structure for the whole study group and the hypertensive and normotensive subgroups

Resistance artery structure

pressure variability, and heart rate. The correlation matrix is shown in Table 3.3.

Increasing age was associated with a significant decrease in lumen diameter and an increase in media:lumen ratio. Media:lumen ratio was significantly correlated with clinic and 24 hour systolic blood pressure, but there was a stronger correlation between media:lumen ratio and 24 hour pulse pressure ($r = 0.56$, $p < 0.001$; Figure 3.3A). Dividing the 24 hour blood pressure record into daytime and nighttime periods resulted in similar correlations, again with only systolic blood pressure (day: $r = 0.39$, $p < 0.05$; night: $r = 0.42$, $p < 0.05$) and pulse pressure (day: $r = 0.53$, $p < 0.01$; night: $r = 0.41$, $p < 0.05$) being significantly related to media:lumen ratio. Normalised media thickness and media:lumen ratio correlated significantly with daytime blood pressure variability, but not with either clinic or 24 hour heart rate.

There were significant positive associations between age and clinic systolic blood pressure ($r = 0.48$, $p < 0.01$), clinic mean arterial pressure ($r = 0.37$, $p < 0.05$), clinic pulse pressure ($r = 0.54$, $p < 0.001$) and 24-hour pulse pressure ($r = 0.53$, $p < 0.01$). There was no association between age and diastolic blood pressure or heart rate. There were also significant associations between blood pressure variability and 24-hour systolic blood pressure (SBP variability: $r = 0.74$, $p < 0.001$, DBP variability: $r = 0.57$, $p < 0.001$) and between BP variability and 24-hour pulse pressure (SBP variability: $r = 0.77$, $p < 0.001$, DBP variability: $r = 0.62$, $p < 0.001$). In a stepwise multiple linear regression model with media:lumen ratio as the dependent variable, 24-hour pulse pressure was significantly associated with media:lumen ratio independent of age, 24-hour systolic and mean arterial pressure, and BP variability ($R^2 = 31\%$, $p < 0.05$). Enlarging the model by the addition of the clinic blood

Resistance artery structure

Variable	Media: lumen ratio	Media thickness	Media CSA	Lumen diameter	
Age	0.44*	0.12	-0.11	-0.46†	
Clinic					
BP	Systolic	0.35*	0.32	0.22	-0.18
	Mean arterial	0.34	0.29	0.22	-0.17
	Diastolic	0.27	0.22	0.18	-0.15
Pulse pressure	0.32	0.30	0.19	-0.15	
Heart rate	-0.07	0.04	0.02	0.02	
24 hour					
BP	Systolic	0.40*	0.32	0.23	-0.12
	Mean arterial	0.28	0.25	0.19	-0.04
	Diastolic	0.04	0.19	0.17	0.08
Pulse pressure	0.56‡	0.32	0.21	-0.24	
Heart rate	-0.13	-0.05	-0.09	0.00	
Daytime BP variability					
	Systolic	0.53†	0.38*	0.15	-0.28
	Diastolic	0.38*	0.35*	0.06	-0.22

* $P < 0.05$; † $P < 0.01$; ‡ $P = 0.001$

BP: blood pressure; CSA: cross-sectional area

Table 3.3 Correlation matrix for the relation between resistance artery structure and age, clinic and 24 hour blood pressure, heart rate, and daytime blood pressure variability

Resistance artery structure

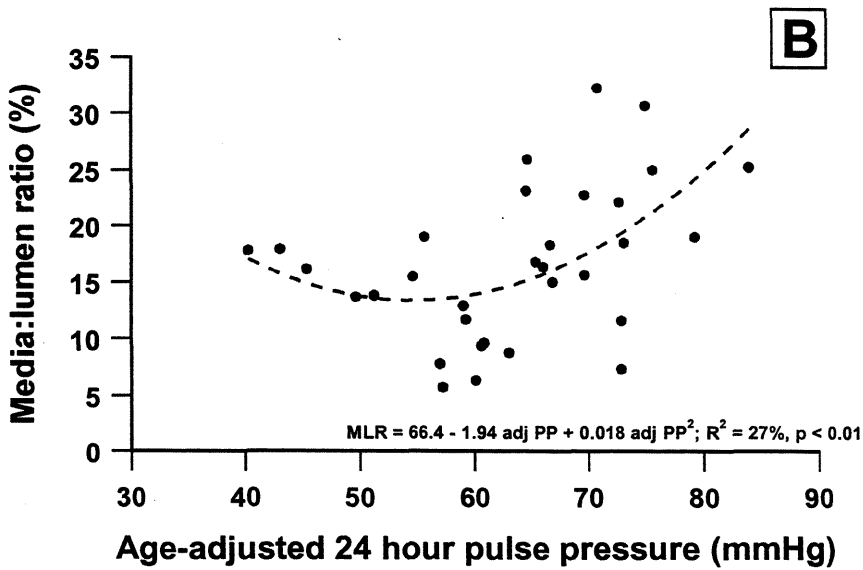
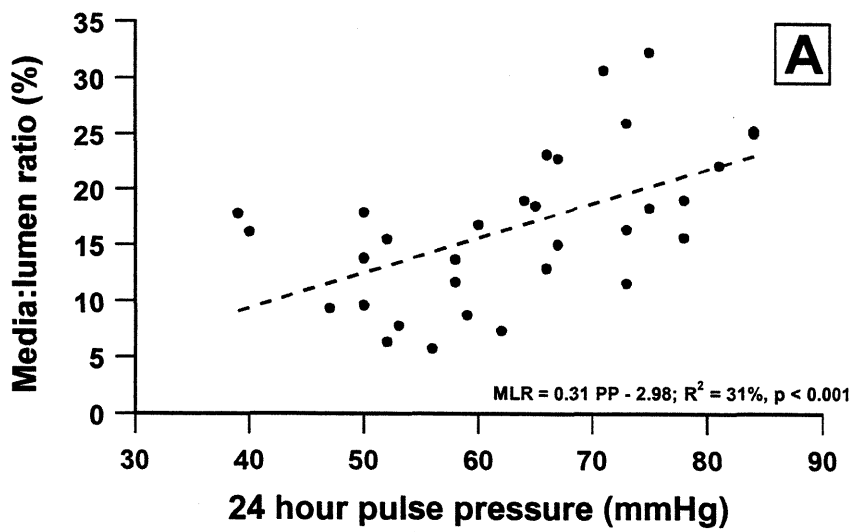


Figure 3.3 Relation between media:lumen ratio and 24-hour pulse pressure (A) without and (B) with adjustment for age

MLR: media:lumen ratio; adj PP: age-adjusted pulse pressure

Resistance artery structure

pressure parameters as predictor variables indicated that only 24-hour pulse pressure and clinic pulse pressure were significant independent predictors of media:lumen ratio ($R^2 = 41\%$, $p < 0.05$). This would suggest that the relation seen between increasing age and increasing media:lumen ratio was mediated predominantly through increasing pulse pressure. The relation between media:lumen ratio and pulse pressure is shown in Figure 3.3, with and without adjustment for age. The relation between media:lumen ratio and unadjusted pulse pressure was linear (Media:lumen ratio = $0.31PP - 2.98$, $R^2 = 31\%$, $p < 0.001$; Figure 3.3A), but after adjustment for age the relationship was curvilinear (Media:lumen ratio = $66.4 - 1.94 \text{ adjPP} + 0.018 \text{ adjPP}^2$, $R^2 = 27\%$, $p < 0.01$; Figure 3.3B). Thus there appeared to be a threshold effect at a pulse pressure of approximately 55 mmHg.

3.5 DISCUSSION

3.5.1 Vascular structure and blood pressure

This study has demonstrated a significant relationship between media:lumen ratio and pulse pressure independent of age, systolic and mean arterial pressure, and blood pressure variability, in a group of untreated elderly subjects most of whom were hypertensive. Media:lumen ratio is the principal parameter of interest when examining structural adaptation in the resistance vasculature, and the finding of a significantly greater media:lumen ratio in these elderly hypertensive subjects is consistent with the results from other studies in younger patients using either the isometric myograph or the newer pressure-perfusion myograph [Aalkjaer et al, 1987; Schiffrin, 1992; Heagerty et al, 1993; Falloon & Heagerty, 1994]. Furthermore, the present findings lend further evidence to the hypothesis that it is cyclical alteration in blood pressure which acts as the main stimulus to vascular adaptation in human hypertension [Hajdu et al, 1990; Baumbach et al, 1991; Baumbach, 1991; Christensen, 1991]. After adjustment for age, the results suggest that pulse pressure has an important influence on media:lumen ratio above a threshold of approximately 55 mmHg. Ideally the study would have included distinct hypertensive subgroups with either isolated systolic hypertension or combined hypertension, to explore the hypothesis that if systolic blood pressure were identical between the two groups, any differences in resistance artery structure may be attributable to differences in pulse pressure. However, the homogeneity of sustained hypertension in the elderly group studied prevented a clear division, with the combined hypertensives having a

Resistance artery structure

tendency to higher systolic blood pressure values which equalized pulse pressure between the two subgroups. An analysis of the data by arbitrary groups has therefore been largely avoided, and instead the analysis has been mainly confined to one treating blood pressure as a continuous variable.

The present study has demonstrated no significant increase in media cross-sectional area with blood pressure, which, combined with trends to increasing media thickness and, to a lesser extent decreasing lumen diameter, suggests that rearrangement of the cellular and interstitial components of the vessel wall (remodeling) is the main adaptation which leads to a higher media:lumen ratio, as opposed to the observation of predominant smooth muscle cellular hypertrophy in animal models of hypertension [Mulvany, 1992; Heagerty et al, 1993]. This is consistent with other studies of subcutaneous vessels in human essential hypertension in which remodeling predominates over growth in the adaptive response of resistance vessels [Korsgaard et al, 1991].

The results from the present study are largely in agreement with studies on the relation between blood pressure and resistance vessel structure in animal models of hypertension. In such experiments pulse pressure was reduced by treatment with hydralazine or the angiotensin converting enzyme inhibitor cilazapril [Hajdu et al, 1990], or with carotid clipping [Baumbach et al, 1991], which reduces pulse pressure but raises systolic and mean blood pressure. Such treatments prevented hypertrophy of cerebral arterioles in the stroke-prone spontaneously hypertensive rat (SHR). In another study, Christensen (1991) found that resistance vessel structural

Resistance artery structure

regression was greatest in SHRs with the largest reductions in pulse pressure with treatment. In Christensen's study, an independent effect of heart rate on media:lumen ratio was also identified, but this observation has not been confirmed in the current study. Christensen's analysis has been criticized for failing to take account of treatment effects [Harrap & Forbes, 1993] and his study was interesting in that 24 hour heart rate in the SHR was not reduced by β -blocker treatment.

In the one other study which has examined the relationship between pulse pressure and vascular structure in human resistance arteries, Cooper et al (1993) did not find a significant relation between pulse pressure measured casually and media:lumen ratio in a larger but younger group of hypertensives. In the older subjects from the present study, consistently better correlations were seen between media:lumen ratio and 24 hour rather than casual blood pressure measurements, presumably arising from the greater reproducibility of 24 hour blood pressure. Also it is likely that in a younger group of hypertensives levels of pulse pressure are lower and this may diminish any effect. Cooper et al do not give the pulse pressure of the subjects in their study, which has only been published in abstract form, so the possible effect of the threshold observed in the present study cannot be assessed, but it may be that in the absence of subjects with higher levels of pulse pressure more typical of the elderly a significant relation with media:lumen ratio would not be seen.

There are no data currently available in the literature for the age group of 60 years and above, and yet this age group offers a more powerful opportunity to study the

Resistance artery structure

differential effects of pulse pressure (pulsatile) and mean arterial pressure (steady-state) on arterial structure. In this context the use of 24 hour ambulatory blood pressure monitoring could be regarded as obligatory, since it has been shown to reveal large discrepancies between systolic and pulse pressure measured in the clinic and by ambulatory methods [Silagy et al, 1990; Cox et al, 1991; Thijs et al, 1992]. By the same token, the use of 24 hour ABPM in this study considerably strengthened the correlations between systolic blood pressure, pulse pressure, and vascular structure.

3.5.2 Blood pressure and ageing

Increasing age is accompanied by a number of important haemodynamic changes. Increasing large arterial rigidity with ageing tends to preferentially increase systolic blood pressure and, at a given level of peripheral resistance, to decrease diastolic pressure [O'Rourke, 1990; Safar, 1993; Safar & Laurent, 1993; Palatini & Graniero, 1993]. Furthermore, although in young subjects pulse pressure in peripheral arteries tends to be higher than in central arteries, this difference declines with age so that above the age of 50 years pulse pressure tends to be similar in both central and peripheral arteries in both normotensive and hypertensive subjects [O'Rourke, 1990; Safar & Laurent, 1993]. One effect of this in the young is to reduce the apparent contribution of pulse pressure to changes at the level of the resistance artery when blood pressure is measured at the brachial artery. In the elderly, pulse pressure measured at the brachial artery may more closely reflect the level of pulse pressure in the resistance vasculature and this has implications when considering

Resistance artery structure

the relationship between the two. However, it remains that brachial blood pressure is only a substitute measure for the level of blood pressure prevailing in the resistance vessels of the gluteal region from which the biopsies were taken, and in humans the brachial-gluteal blood pressure difference is unquantified. Moreover, the small arteries may be an important site for the generation of reflected waves, which amplify the systolic peak and contribute to a widened pulse pressure and disproportionate systolic hypertension [Safar, 1993; Palatini & Graniero, 1993]. Therefore it remains plausible that an increased systolic and pulse pressure are a consequence of greater structural alteration at the level of the small arteries rather than its cause.

Comparison of the data from the present study with other studies which have included normotensive controls from younger age groups shows the media:lumen ratio in normotensive elderly subjects to be considerably increased, attaining to the levels observed in younger hypertensives [Aalkjær et al, 1986, 1987; Heagerty et al, 1988; Schiffrin, 1992; Schiffrin et al, 1994]. No subject in the normotensive group of the present study had any past history of high blood pressure however remote, thus excluding as far as possible any subject with 'burned out' hypertension [Amery et al, 1989]. 'Normotensive' blood pressure levels in the control group were higher than in equivalent control groups of younger subjects, but not greatly so and the difference in blood pressure would seem to be insufficient to solely explain the difference in media:lumen ratio. Thus, despite the haemodynamic considerations described above, this would tend to suggest an independent effect of age. A 'thickening' of arteries and arterioles with age in many different sites has been known

for more than twenty-five years [Auerbach et al, 1968]. However, this issue could only be resolved by a study of young and older subjects matched for blood pressure.

3.5.3 Study limitations

Some limitations of this work need to be considered, the most significant of which arises from the cross-sectional nature of the study. Thus the interpretation needs to be tempered by the two possibilities that the observed increase in pulse pressure could be the cause or the consequence of the increased resistance artery structural alteration. This issue could be addressed by a longitudinal study, but naturally techniques involving gluteal biopsies are not suitable for such studies, although some workers have performed two biopsies on subjects before and after drug treatment [Heagerty et al, 1988; Schiffrin et al, 1994; Thybo et al, 1995]. Recently Agabiti-Rosei and colleagues (1995) have demonstrated a good correlation between the media:lumen ratio of resistance arteries and post-ischaemic minimum forearm vascular resistance, which would enable the repeated study of surrogate measures of structure over time, and perhaps address the cause-effect issue.

The study was not able to measure the level of blood pressure prevailing in the gluteal arteries, substituting instead the brachial artery pressure from intermittent recording. The brachial-gluteal blood pressure difference is about 30 mmHg [Aalkjaer et al, 1987] but this difference is probably constant between the two groups of hypertensive and normotensive subjects so the effect of this on the relations seen between blood pressure and structure is uncertain.

3.6 CONCLUSIONS

1. Media:lumen ratio in subcutaneous resistance arteries from elderly subjects is markedly increased with hypertension.
2. Media:lumen ratio is most closely related to the prevailing pulse pressure, particularly when this is measured by 24-hour monitoring.
3. The increased media:lumen ratio with similar media cross-sectional area suggests that the rearrangement of the cellular and interstitial elements of the vessel wall (remodeling) is the main adaptation in human vessels.
4. In elderly normotensive subjects, media:lumen ratio would appear to be increased to a greater extent than can be explained by the small difference in blood pressure compared to younger normotensives. This suggests other effects on resistance vessel structure that may be associated with increasing age.

Chapter 4

Endothelial and vascular smooth muscle cell
function in resistance arteries from elderly subjects

4.1 SUMMARY

This study was designed to examine the functional behaviour of the endothelium and vascular smooth muscle of small arteries in elderly subjects. Resistance arteries were obtained from gluteal biopsies taken under local anaesthesia in 28 subjects of mean age 70 years (range 60-76) and studied in an isometric myograph. Eighteen subjects had untreated essential hypertension, and 10 were normotensive. After measurement of contractile responses to noradrenaline (both in the absence and presence of cocaine) and angiotensin II, relaxation responses to a variety of endothelium-dependent (acetylcholine and bradykinin) and endothelium-independent (iloprost and sodium nitroprusside) mechanisms were assessed in vessels precontracted with noradrenaline. Endothelium-dependent responses were also studied after incubation with N^G-nitro-L-arginine to inhibit nitric oxide synthase.

There were no significant differences in the contraction or relaxation responses between elderly subjects with or without high blood pressure. Inhibition of nitric oxide synthase prevented any relaxation with acetylcholine and significantly attenuated the relaxation with bradykinin. Near-complete relaxation was however achieved with the endothelium-independent vasodilator sodium nitroprusside. Results were consistent with other studies which have suggested an age-related decline in endothelium-dependent relaxation with relative preservation of endothelium-independent responses. However, the results did not suggest a further impairment of resistance artery function with hypertension in the elderly beyond that already conferred by ageing.

4.2 BACKGROUND and AIMS

The function of the endothelium in hypertension has risen to prominence in recent years, and in particular the role of the potent vasodilator moiety EDRF/NO, the active product of the L-arginine-nitric oxide synthase pathway. This has led to much interest in possible dysfunction of this and other endothelium-dependent mechanisms in the pathophysiology of hypertension and other disease states, including the prospect of a role in hypertension in the elderly. Also the possibility that a vascular smooth muscle defect may lead to enhanced excitation-contraction coupling in hypertension has been suggested as contributing to the raised vascular resistance seen in the condition.

Recent studies have also indicated a decline in endothelium-dependent vasodilation with ageing, but the further effect of hypertension on resistance vessel function in an elderly age group is unknown but of particular interest. The aims of this study were therefore:

1. To examine the possible differences in the contractile function of resistance arteries between elderly hypertensive and normotensive subjects.
2. To examine differences in endothelium-dependent and endothelium-independent relaxations between the same groups.

4.3 MATERIALS and METHODS

4.3.1 Study subjects

The study involved 28 elderly subjects (age {mean \pm SEM} 69.5 \pm 0.9 years, range 60–76) of whom 15 were male. Subjects were recruited from those attending the outpatient department of a large teaching hospital for management of their hypertension, and from a collaboration with several large local general practices. 'Normal' subjects were recruited from among the spouses, relatives and friends of the hypertensive subjects, and from the respondents to a local newspaper advertisement. All subjects were active and ambulant, and living independently in the community. The study received local ethical committee approval, and all participants gave written informed consent.

Clinic blood pressure was measured according to the protocol described in Chapter 3. Blood pressure was recorded three times on each of three visits using a standard mercury sphygmomanometer with the subject supine after five minutes rest. Clinic blood pressure was defined as the average of the resultant nine readings, and subjects with a clinic blood pressure of systolic \geq 160 mmHg and/or diastolic \geq 90 mmHg (Korotkoff phase V) were classified as hypertensive. By these criteria 18 subjects were hypertensive and 10 were normotensive. No subjects had previously received any antihypertensive medication. No subject had any history of angina, myocardial infarction, stroke or diabetes mellitus, and none of the

Resistance artery function

normotensive subjects had any past history of hypertension or pre-eclampsia. No subject had evidence of secondary hypertension or coronary artery disease from history and examination, electrocardiogram or standard laboratory and radiological tests.

4.3.2 Preparation of arteries

Preparation and mounting of resistance arteries was conducted in the manner described in Chapter 3. Briefly, subjects donated a biopsy of skin and subcutaneous fat from the gluteal region from which resistance arteries were dissected and mounted as ring preparations on two parallel 40 μm stainless steel wires between a micrometer attachment and a force transducer in a Mulvany-Halpern myograph (JP trading, Aarhus, Denmark). The output from the force transducer was routed to a 2-channel chart recorder (Graphic 20002, Lloyds Instruments, Loughborough, UK) where changes in tension were plotted against time. The vessels were held without tension in physiological salt solution (PSS) of composition NaCl 118 mmol/L, KCl 4.5 mmol/L, CaCl_2 2.5 mmol/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.0 mmol/L, KH_2PO_4 1.0 mmol/L, NaHCO_3 25 mmol/L, glucose 6 mmol/L, at 37°C and bubbled with 5% CO_2 /95% O_2 to achieve a pH of 7.4. Following mounting in the myograph, the vessels were equilibrated for 30 minutes before the measurement of vessel length and media thickness by light microscopy. After a further 30 minutes of equilibration, the vessels were exposed to predetermined stepwise increases in tension to determine the length-tension characteristic calculated from the Laplace equation ($P = T/r$, where P is the transmural pressure, T is the wall tension and r the internal radius of the vessel).

Resistance artery function

Then the internal diameter was set to $0.9 \times L_{100}$, where L_{100} is the internal diameter that the vessel would have *in vivo* when relaxed at $P = 100$ mmHg. This is described as the normalised lumen diameter, $L_{0.9}$, and is the diameter at which the generated isometric tension is greatest [Mulvany & Halpern, 1977].

4.3.3 Myograph protocol

After setting to their normalised internal diameter, the vessels were maintained in PSS at 37°C for a further 60 minutes before being stimulated three times with a high-potassium PSS in the myograph (high-potassium PSS [KPSS] is standard PSS with KCl substituted for NaCl on an equimolar basis giving $[K^+] = 123$ mmol/L). After each contraction the bath was rinsed out several times with PSS to allow the vessel tension to return to baseline. A maximum contraction was then obtained to KPSS with noradrenaline 10^{-5} mol/L. Cumulative dose-response curves were then performed to noradrenaline (10^{-8} to 3×10^{-5} mol/L, approximately 2 minutes between successive doses) before and after the incubation of the vessels with cocaine 10^{-6} mol/L for 20 minutes to inhibit neuronal reuptake of noradrenaline. Vessels were washed out with PSS following each dose-response curve and rested for 15 minutes. A further contraction study was then performed in the same manner to Angiotensin II (10^{-10} to 10^{-7} mol/L).

Relaxation studies were performed following maximal precontraction with 10^{-5} mol/L noradrenaline when a stable plateau had been reached (usually after two minutes). Relaxation responses were measured to the following substances with 15

Resistance artery function

minutes between each contraction/relaxation curve: the EDRF/NO-dependent vasodilators acetylcholine (ACh; 10^{-9} to 10^{-5} mol/L) and bradykinin (BK; 10^{-9} to 10^{-5} mol/L), the synthetic prostacyclin analogue iloprost (10^{-10} to 10^{-6} mol/L), and the endothelium-independent nitrovasodilator sodium nitroprusside (SNP; 10^{-9} to 10^{-4} mol/L).

Following this, the L-arginine analogue N^G-nitro-L-arginine (NOARG; 10^{-6} mol/L) was incubated in the bath for 60 minutes to inhibit nitric oxide synthase. Under these conditions ACh curves were repeated in five subjects and BK curves repeated in 22 subjects.

Previous studies under identical conditions in our laboratory have shown no significant change in human artery contraction or relaxation over the time period of the protocol. All drugs were obtained from Sigma Chemicals Co.(Poole, Dorset, UK), apart from the iloprost which was kindly donated by Schering AG (Berlin, Germany). All drugs were dissolved in distilled water to achieve the molarities given as the final molarity in the organ bath (i.e. after addition to the 10 mL PSS in the bath).

4.3.4 Statistical methods

Two vessel segments were studied simultaneously in the myograph and the results averaged to give one value for each individual, except in four subjects in whom only one vessel could be retrieved from the gluteal biopsy. Group results are given as mean \pm SEM. Contractions are expressed as active wall tension T (force

Resistance artery function

per unit length of the vessel; mN/mm) and active media stress σ (active wall tension corrected for media thickness; mN/mm²). Relaxations are expressed as the percentage of the maximal noradrenaline precontraction. Sensitivity to agonists is expressed as the ED₅₀, the dose required to produce 50% of the maximum response. Between-group differences in these summary statistics, and comparison of responses before and after cocaine and NOARG, were examined using Student's two-tailed *t*-test (paired or unpaired, as appropriate), with a value of $p < 0.05$ being regarded as statistically significant.

4.4 RESULTS

The characteristics of the study group, together with the physical data for the vessels studied, are shown in Table 4.1. There were no significant differences in lumen diameter, media thickness or cross-sectional area between hypertensive and normotensive subjects, but media:lumen ratio was significantly increased in hypertensive subjects (see Chapter 3). Nevertheless, the contractile responses (expressed either as active tension T or active media stress σ) to KPSS, KPSS + noradrenaline and 3×10^{-5} mol/L noradrenaline were not different between the hypertensive and normotensive groups (Table 4.2), and similarly sensitivity to noradrenaline (as expressed by the ED₅₀; Table 4.2 and Figure 4.1) was not different. There was no evidence of any alteration of presynaptic noradrenaline reuptake in hypertension, with both the maximal contractile response and ED₅₀

	Hypertensive	Normotensive	<i>p</i>
<i>n</i>	18	10	
Age	70.3 ± 1.0	68.2 ± 1.8	0.33
Blood pressure (mmHg)			
Systolic	187 ± 6	136 ± 3	< 0.0001
Diastolic	95 ± 2	78 ± 3	0.0001
Pulse Pressure	92 ± 5	58 ± 4	< 0.0001
Lumen Diameter (µm)	258 ± 21	288 ± 33	0.46
Media Thickness (µm)	43.0 ± 2.4	36.4 ± 3.8	0.17
Media Cross-sectional Area (µm ²)	37007 ± 2894	33781 ± 4439	0.55
Media Lumen Ratio (%)	18.6 ± 1.8	12.7 ± 1.3	0.013

Table 4.1 Subject characteristics and morphology data for the vessels studied from hypertensive and normotensive subjects

Resistance artery function

	Hypertensive	Normotensive	<i>p</i>
123 mmol/L K ⁺ max <i>T</i>	1.10 ± 0.13	1.13 ± 0.20	0.92
KPSS + NA max <i>T</i>	1.32 ± 0.13	1.31 ± 0.20	0.98
NA max <i>T</i>	1.44 ± 0.15	1.45 ± 0.20	0.95
NA max σ	34.5 ± 4.1	45.1 ± 8.5	0.28
NA ED ₅₀	0.48 ± 0.12	0.39 ± 0.13	0.64
NA + cocaine max <i>T</i>	1.46 ± 0.15	1.42 ± 0.20	0.87
NA + cocaine max σ	34.8 ± 4.1	44.1 ± 8.5	0.35
NA + cocaine ED ₅₀	0.36 ± 0.08	0.32 ± 0.11	0.75
Angiotensin II max <i>T</i>	0.80 ± 0.09	0.91 ± 0.18	0.59
Angiotensin II max σ	19.6 ± 2.5	27.8 ± 6.3	0.25
Angiotensin II ED ₅₀	9.63 ± 1.65	5.95 ± 1.55	0.09

KPSS: high-potassium salt solution (for composition see text); NA: noradrenaline
 Max *T*: maximum tension (mN/mm); max σ : maximum active media stress (mN/mm²); ED₅₀: sensitivity to agonists (μ mol/L for NA, nmol/L for angiotensin II).

Table 4.2 Maximum contractile responses and sensitivities for the resistance vessels studied from hypertensive and normotensive subjects

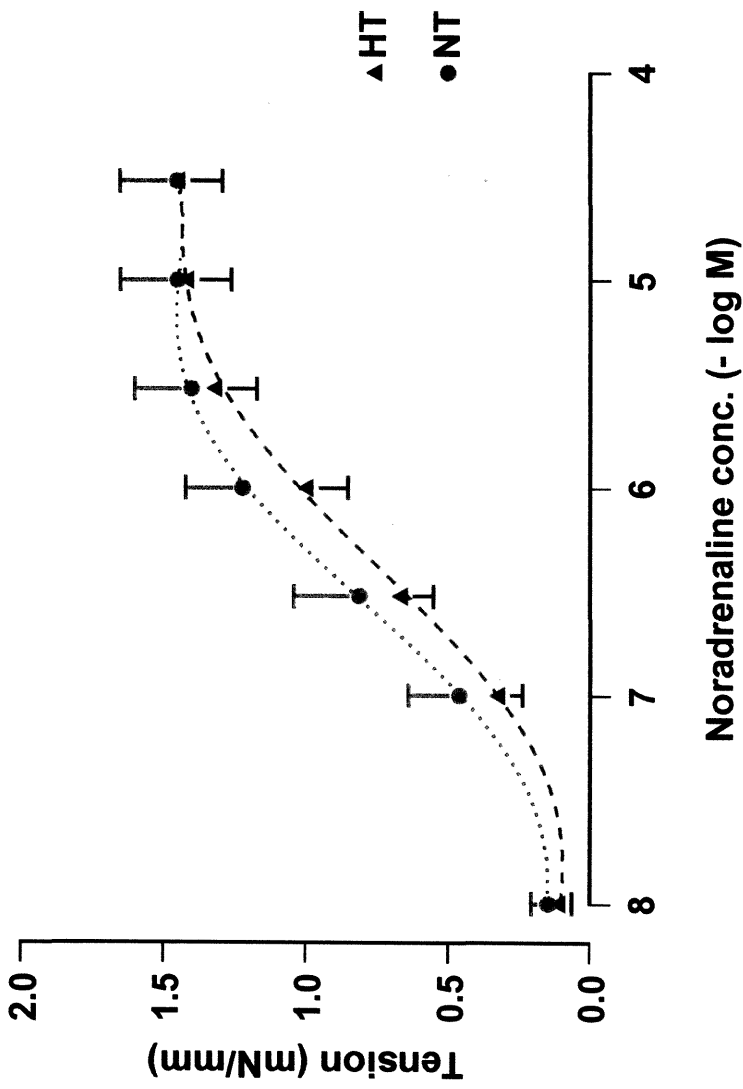


Figure 4.1 Dose-response curves for resistance arteries to noradrenaline

Resistance artery function

unaffected by the presence of cocaine (Table 4.2 and Figure 4.2). Maximum response to Angiotensin II was no different between the hypertensive and normotensive groups, but there was a tendency towards reduced sensitivity to Angiotensin II in the hypertensive group with a higher value for ED₅₀ (Table 4.2 and Figure 4.3).

Maximum relaxation responses and ED₅₀ results for the various vasodilators studied are shown in Table 4.3. There were no significant differences between the hypertensive and normotensive groups for the acetylcholine (Figure 4.4), bradykinin (Figure 4.5), iloprost (Figure 4.6) or sodium nitroprusside (Figure 4.7) relaxation responses.

Incubation with NOARG abolished acetylcholine relaxation altogether in five subjects (4 hypertensive, one normotensive). Following NOARG, maximum bradykinin relaxation was significantly attenuated but not abolished in the same way ($p = 0.005$ for hypertensives, $p = 0.002$ for normotensives for comparison with bradykinin relaxation alone; Table 4.3, Figure 4.8). The bradykinin sensitivities following NOARG were much more widely spread but not significantly different from the sensitivities to bradykinin in the absence of NOARG ($p = 0.29$ for hypertensives and $p = 0.35$ for normotensives).

The results for the various vasodilator substances are summarised in Figure 4.9. The maximum response was seen with sodium nitroprusside, with progressively less relaxation achieved with iloprost, bradykinin and acetylcholine. NOARG attenuated

Resistance artery function

the bradykinin relaxation, but even in the presence of NOARG the bradykinin response was significantly greater than the relaxation achieved with acetylcholine.

Resistance artery function

	Hypertensive	Normotensive	<i>p</i>
Acetylcholine maximum	31.8 ± 8.4	28.3 ± 11.0	0.81
Acetylcholine ED ₅₀	0.14 ± 0.03	0.16 ± 0.05	0.74
Bradykinin maximum	56.7 ± 7.0	58.1 ± 8.5	0.90
Bradykinin ED ₅₀	46.8 ± 16.0	51.4 ± 24.0	0.88
Iloprost maximum	71.5 ± 6.6	72.0 ± 7.6	0.96
Iloprost ED ₅₀	22.8 ± 6.0	16.2 ± 6.8	0.47
SNP maximum	84.9 ± 4.7	87.8 ± 4.1	0.65
SNP ED ₅₀	1.16 ± 0.49	1.07 ± 0.34	0.88
BK-NOARG maximum	45.6 ± 6.9	42.6 ± 9.2	0.80
BK-NOARG ED ₅₀	109.4 ± 64.5	90.3 ± 56.8	0.83

Maximum (%): maximum relaxation expressed as a percentage of the noradrenaline precontraction. ED₅₀: sensitivity to agent (μmol/L for acetylcholine and sodium nitroprusside, nmol/L for bradykinin and iloprost). SNP: sodium nitroprusside; BK-NOARG: bradykinin relaxation following incubation with N^G-nitro-L-arginine

Table 4.3 Maximum relaxation responses and sensitivities for the resistance vessels studied from hypertensive and normotensive subjects

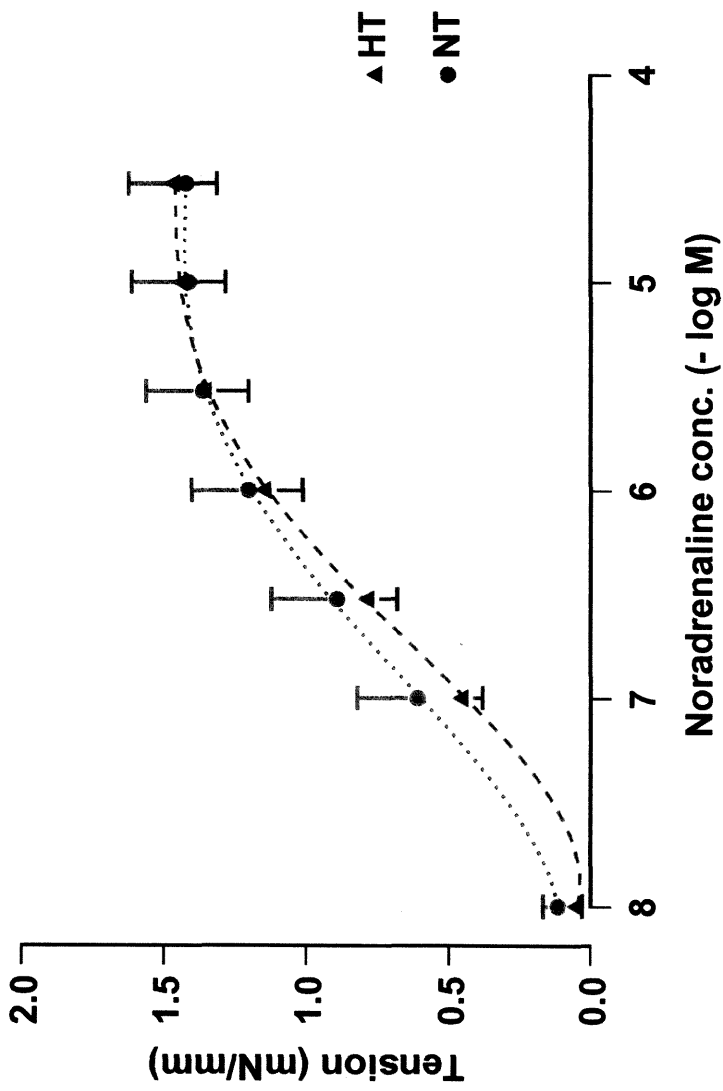


Figure 4.2 Dose-response curves for resistance arteries to noradrenaline in the presence of cocaine

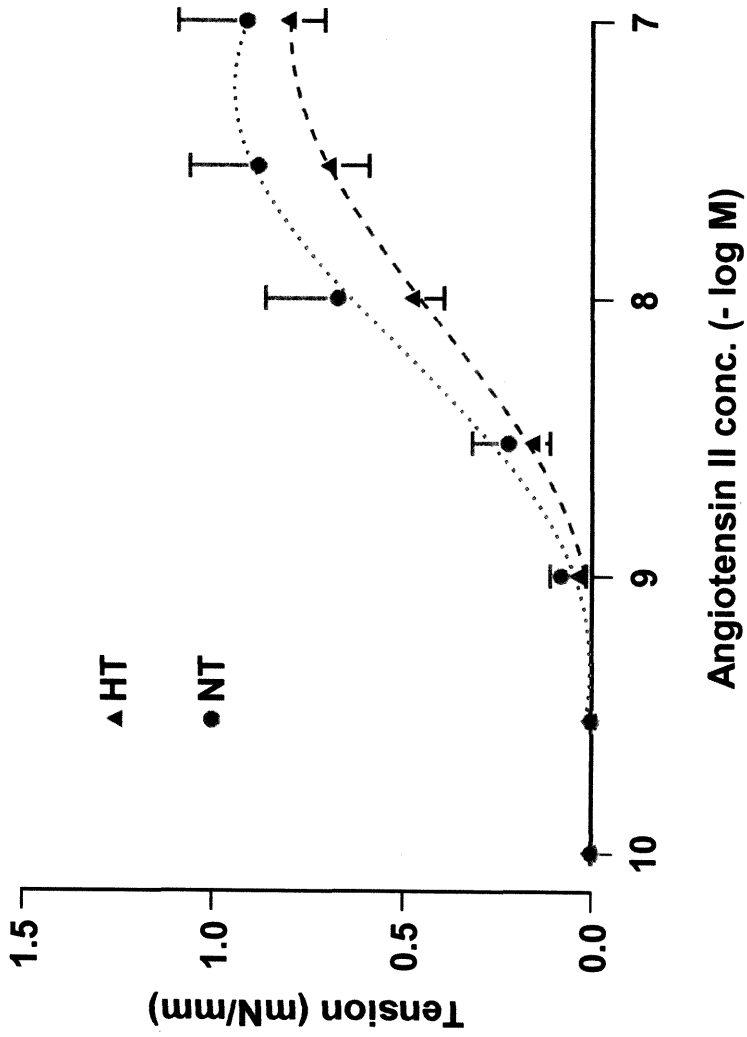


Figure 4.3 Dose-response curves for resistance arteries to Angiotensin II

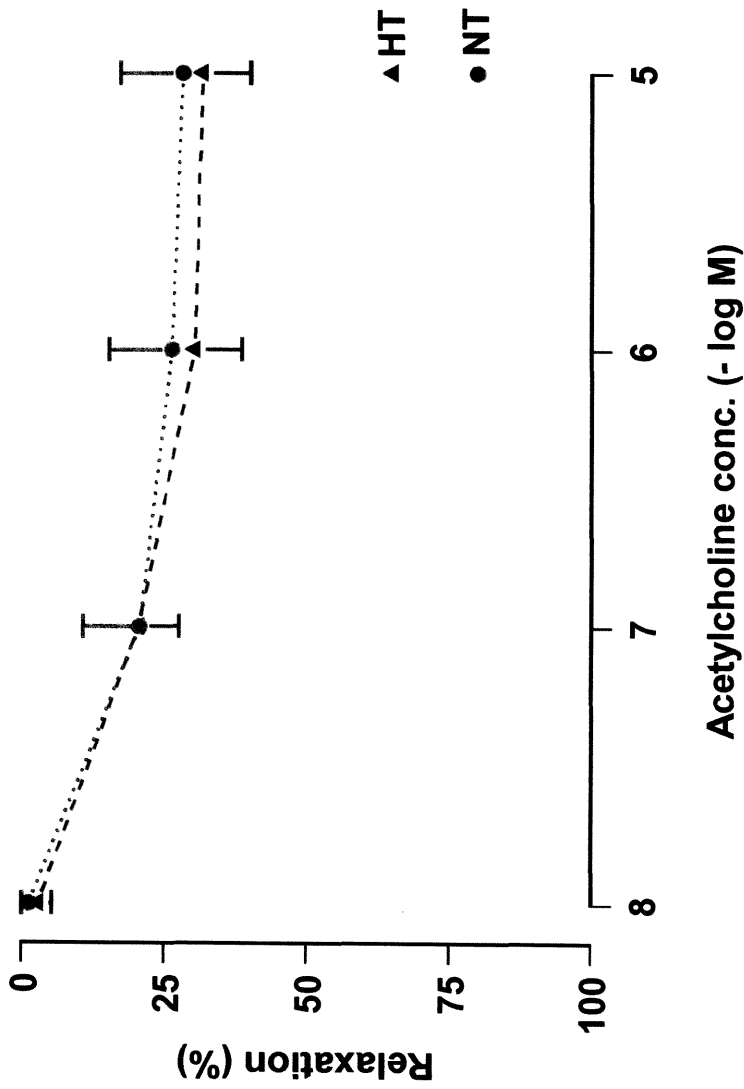


Figure 4.4 Dose-response curves for resistance arteries to acetylcholine

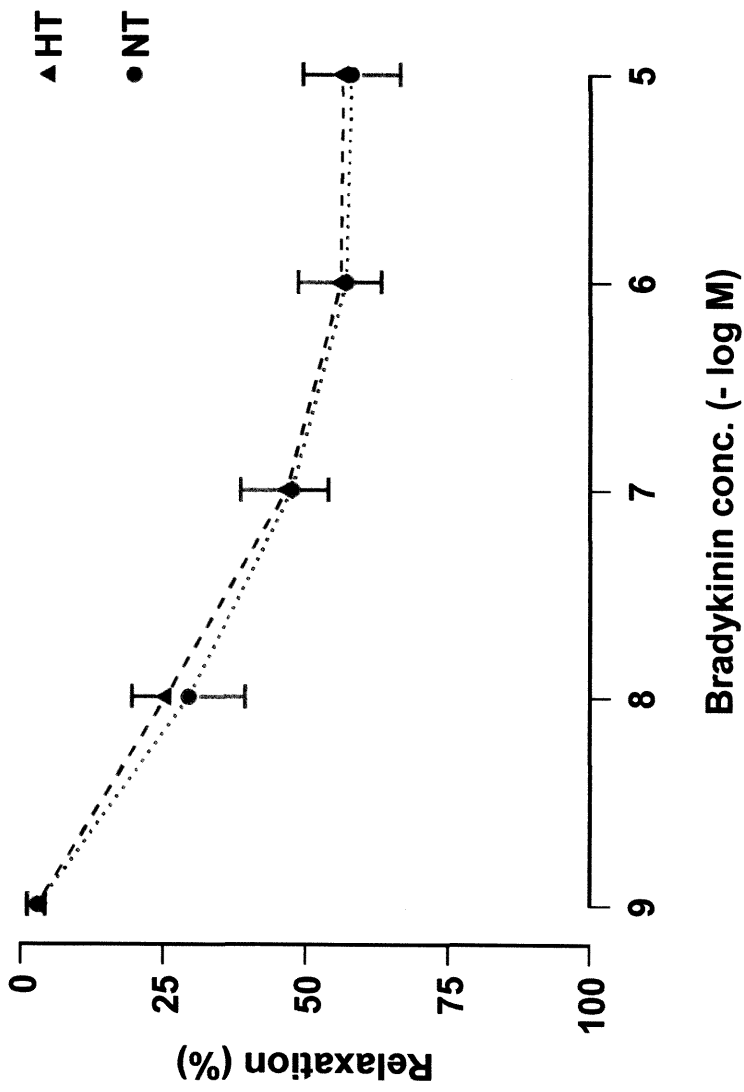


Figure 4.5 Dose-response curves for resistance arteries to bradykinin

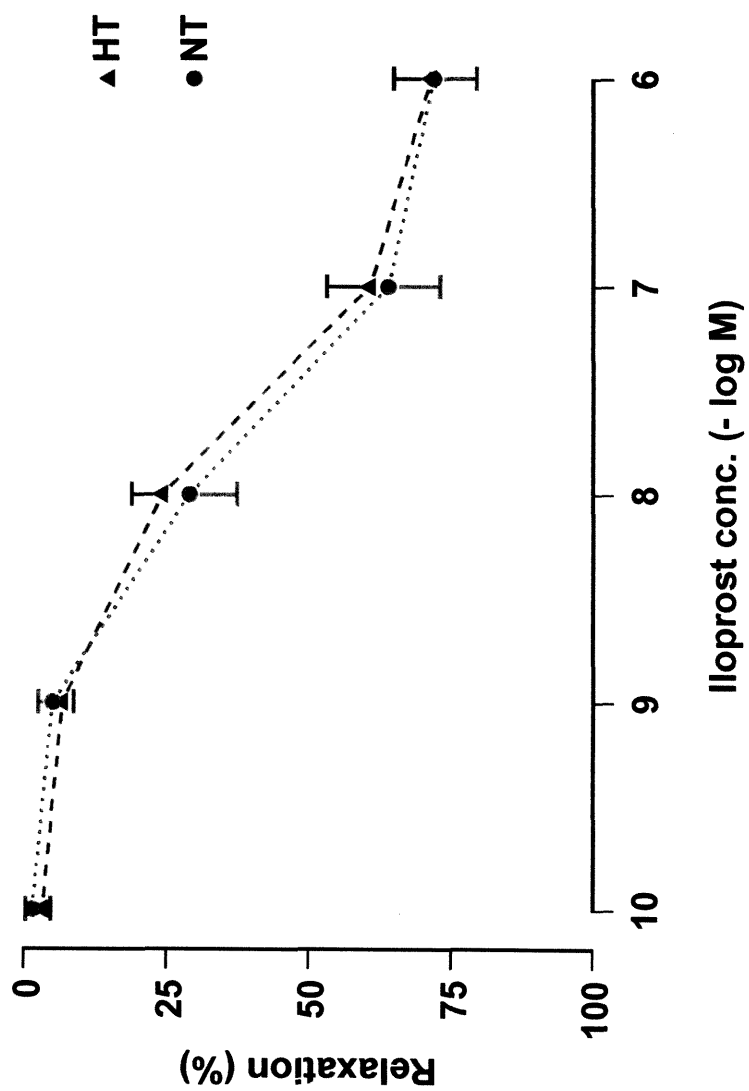


Figure 4.6 Dose-response curves for resistance arteries to iloprost

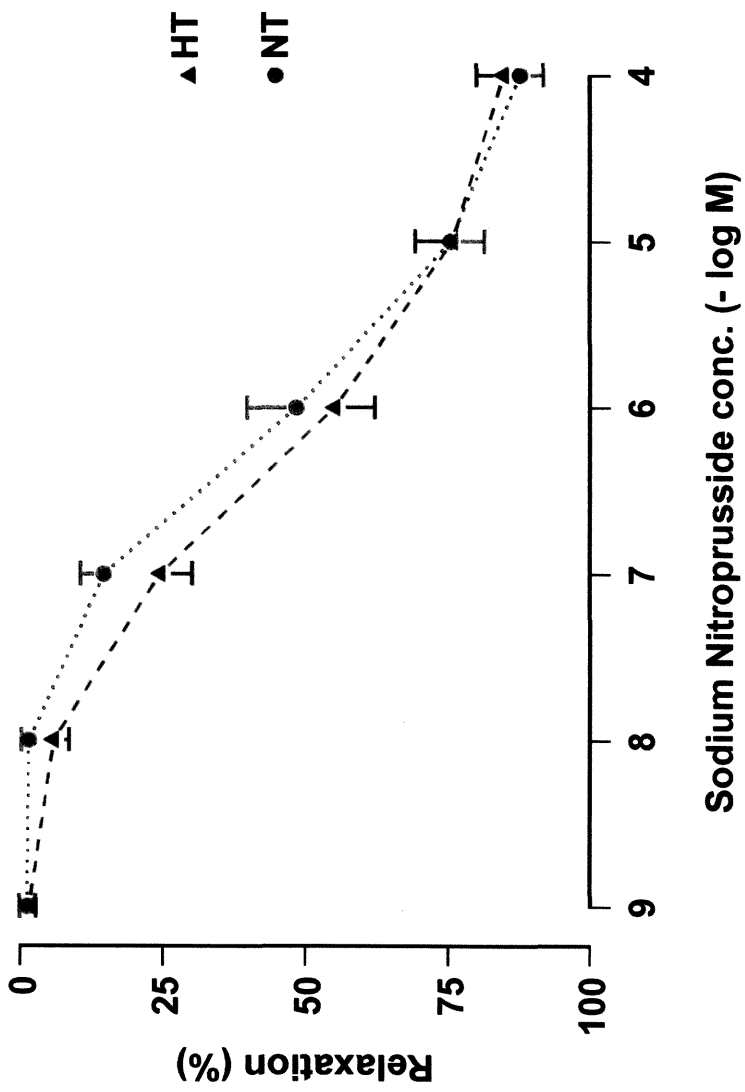


Figure 4.7 Dose-response curves for resistance arteries to sodium nitroprusside

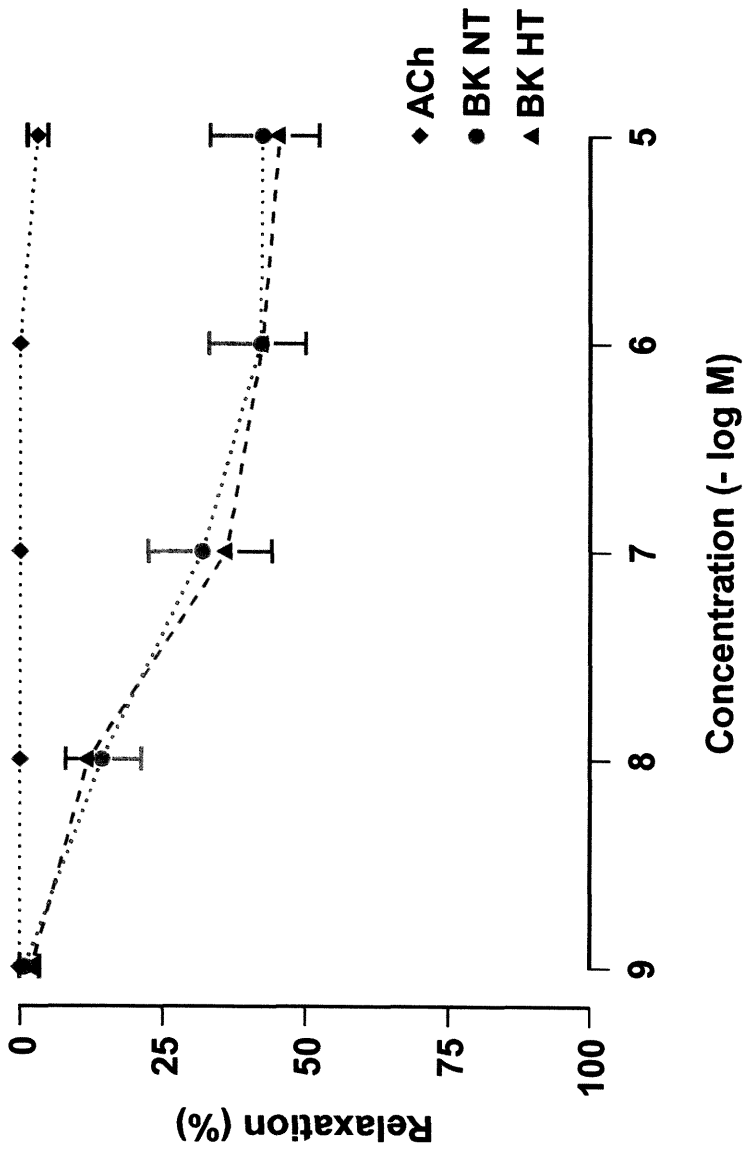


Figure 4.8 Dose-response curves for resistance arteries to acetylcholine (ACh) and bradykinin (BK) following incubation with N⁶-nitro-L-arginine

NT: normotensive; HT: hypertensive

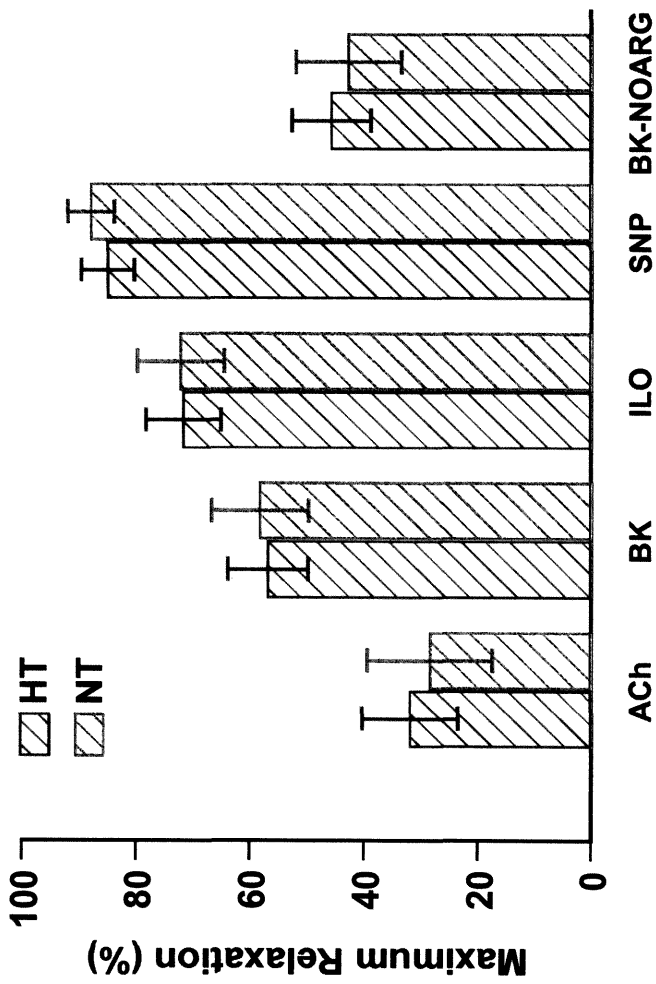


Figure 4.9 Maximum relaxation responses to the various agents studied

ACh: acetylcholine; BK: bradykinin; Ilo: iloprost; SNP: sodium nitroprusside;
NOARG: Nitro-L-arginine
HT: hypertensive; NT: normotensive

4.5 DISCUSSION

4.5.1 Differences between hypertensive and normotensive subjects

The principal results from this study show that there is no difference between hypertensive and normotensive elderly subjects in the reactivity of isolated subcutaneous resistance vessels to a variety of contractile and relaxant agents. This is despite some differences in structure between the two groups, most notably an increased media:lumen ratio in the hypertensive subjects, as discussed in Chapter 3. However, media thickness and media cross-sectional area were similar in the two groups, and the active media stress generated by noradrenaline or angiotensin II was not different with hypertension (Table 4.2). The isometric myograph, by the nature of its ring preparation under standardised conditions for both hypertensive and normotensive vessels, tends to reduce the effects of structure on reactivity in resistance arteries [Schiffirin, 1992; 1995]. Nonetheless, this study offers no support for an increased reactivity of resistance arteries to pressor agonists in hypertension. The present study has shown no significant effect of incubation with cocaine, suggesting no effect of hypertension on the neuronal reuptake of noradrenaline. In younger subjects, subcutaneous vessels from hypertensives have shown an increased effect of cocaine compared to normotensives, suggesting increased neuronal reuptake of noradrenaline with hypertension in a younger age group [Aalkjaer et al, 1987].

Resistance artery function

Endothelial dysfunction has also been postulated as contributing to the aetiology of hypertension, including hypertension in the elderly, but the results from this study do not support that contention [Applegate & Rutan, 1992; Byyny, 1995]. The pattern of altered vascular smooth muscle cell relaxation observed in this study does however suggest some significant changes to the mechanisms involved in resistance artery vasodilation in the elderly. The observation of intact sodium nitroprusside responses indicated normal function of the smooth muscle cell guanylate cyclase-cGMP pathway in producing relaxation. However, markedly lower responses to acetylcholine indicated impairment of the endothelial M_2 -receptor-L-arginine-NO pathway if the final activation in smooth muscle cells was intact. Abolition of acetylcholine relaxation with the NO-synthase inhibitor NOARG would indicate that what relaxation was seen occurred by this pathway, with little or no stimulation of other vasodilating agents such as EDHF by acetylcholine. This is contrary to the observation from one recent study of younger hypertensive patients [Deng et al, 1995], in whom up to 70% of the relaxation response to acetylcholine was mediated via mechanisms other than EDRF/NO. Relaxation responses to bradykinin were also impaired by comparison to sodium nitroprusside but less so, suggesting that at least part of the response to bradykinin may be achieved by alternative pathway(s). This is supported by the results of bradykinin relaxation following incubation with NOARG. Relaxation was significantly attenuated (by about 23%) but not abolished, and the relaxation obtained was still significantly greater than with acetylcholine alone. Over 75% of the relaxation with bradykinin may thus be achieved via alternative pathways possibly including production of EDHF and stimulation of prostacyclin synthetase, and dysfunction of the L-arginine-NO pathway may divert the effects of bradykinin

towards these alternatives. Similar results have been seen in another study in younger hypertensives from Panza et al (1995), who demonstrated by the brachial artery drug infusion method that although impaired by hypertension, the vasodilating response to bradykinin was not any further reduced by inhibition of NO synthesis with L-NMMA, whereas in normotensive subjects administration of L-NMMA reduced the response to the levels seen in hypertensives. In the current study, the possibility of the replacement of the L-arginine-NO pathway as the main route for vasodilation was also supported by the findings with the prostacyclin analogue iloprost, which achieved significantly greater relaxation than that seen with either acetylcholine or bradykinin. This suggests that with the failure of the L-arginine-NO pathway in the elderly, alternative mechanisms may come to predominate, although the role for these other pathways *in vivo* remains unconfirmed.

The findings of the present study are consistent with and supported by the evidence from other studies using other methods, although there are no comparable data from isolated subcutaneous resistance arteries in elderly humans. By comparison with reactivity data obtained in our laboratory using the same myograph techniques in younger normotensive subjects [McNally et al, 1994], there would appear to be consistent age-related decline in endothelium-dependent relaxations. In the control group from that study (normotensive subjects of mean age 28 ± 2 years), mean acetylcholine relaxation was $73 \pm 8\%$, mean bradykinin relaxation $83 \pm 6\%$, while mean sodium nitroprusside relaxation was $91 \pm 2\%$ (as opposed to $28 \pm 11\%$, $58 \pm 8\%$ and $88 \pm 4\%$ respectively in the normotensive subjects from the present study). Thus from this data there is the strong suggestion of an age-related

Resistance artery function

decline in endothelium-dependent relaxation with relative preservation of endothelium-independent responses. Comparison of the present study with the data from the study of McNally et al also suggests an age-related decline in maximum contractile responses to 123 mmol/L K^+ , noradrenaline and angiotensin II. Other studies performed in experimental hypertension have indicated a decline in maximum contractile responses to potassium and catecholamines with increasing blood pressure and age [Tuttle, 1966; Schiffrin, 1992], suggesting depressed excitation-contraction coupling with ageing. Such differences are clearly best explored by the future study of a group of subjects of all ages matched for blood pressure and other possible confounding factors such as cholesterol, but these observations raise some interesting possibilities regarding the effect of age on vascular smooth muscle and endothelial function. The causes of this decline with age are not known, but could include the effects of cholesterol or other lipid abnormalities over a number of years [Flavahan, 1992], thereby possibly accounting for the gender differences found by Celermajer et al and others [Lieberman et al, 1994; Kauser & Rubanyi, 1995], and/or the accumulation of glycosylation end-products or the effects of oxidative stress and free-radical damage (the free radical theory of ageing) [Bucala et al, 1991; Vlassara et al, 1992; Martin, 1992].

4.5.2 Study limitations

Some consideration is required of the limitations of this study. With regard to the study size, it is unlikely that the conclusions drawn would be affected by too few

subjects being studied, although the trend towards reduced sensitivity to angiotensin II in the hypertensive group may have achieved statistical significance with more subjects in each group. In view of the identical nature of the other responses between the two groups, differences would be most unlikely to become apparent even with greatly increased group sizes. The pharmacological actions of the agents used are very well characterised even in the short history of the study of the endothelium and its function [Lüscher & Vanhoutte, 1990], although the suggestion of a greater rôle for the cyclooxygenase pathway could be further explored by the study of responses, particularly to bradykinin, after blockade with indomethacin. The results of this could not be predicted in that, in the SHR, indomethacin restores endothelium-dependent relaxation by blocking production of an endothelial, cyclooxygenase-dependent contracting factor, possibly thromboxane A₂, prostaglandin H₂ or superoxide ions [Bennett et al, 1992; Lüscher & Dubey, 1995], although production of this agent in humans has not been demonstrated [Deng et al, 1995]. A thromboxane analogue (U 46619) and receptor antagonist (SQ 30741) are available which may help further discriminate the actions of the various prostanoids [Mayhan et al, 1990; Tschudi & Lüscher, 1995]. The endothelium-dependence of such responses could be confirmed by mechanical removal of the endothelium [Osol et al, 1989]. In other studies, the response to acetylcholine has been used as an indicator that endothelial structure and function have not been damaged by the process of vessel preparation [Sunman et al, 1993] and such reassurance has not been available in this study, but the techniques used have been identical to those employed in other studies of younger subjects conducted simultaneously in this laboratory achieving quite predictable acetylcholine responses. The normal sodium

Resistance artery function

nitroprusside responses indicate normal function of vascular smooth muscle under the experimental conditions described.

The study describes only stimulated EDRF/NO responses *in vitro*, and the *in vivo* significance of the respective pathways cannot be necessarily inferred. In particular, the observation of large relaxation responses to the synthetic prostacyclin analogue iloprost in both groups is of interest but gives no indication the role of this pathway in achieving relaxation in intact man. In order to examine basal EDRF/NO activity it would be valuable to study the effects of infusion of a nitric oxide synthase inhibitor such as N^G-monomethyl-L-arginine (L-NMMA), either in the forearm circulation by intrabrachial artery administration, or the whole body effects from intravenous infusion [Haynes et al, 1993; Hansen et al, 1994; Castellano et al, 1995]. In younger subjects this led to a significant rise in mean arterial pressure and systemic vascular resistance indicating basal generation of EDRF/NO, and it could be speculated that if a persistent defect of the L-arginine-NO pathway existed in elderly subjects, these effects of L-NMMA infusion would not be seen.

4.6 CONCLUSIONS

1. Contractile responses to noradrenaline and angiotensin II in resistance arteries from elderly hypertensive subjects are not different from those seen in vessels from normotensives of similar age.

Resistance artery function

2. No difference is seen in the neuronal reuptake of noradrenaline (judged from the effects of incubation with cocaine) between hypertensive and normotensive subjects.
3. Similarly, there is no difference in endothelium-dependent and endothelium-independent relaxations in vessels from elderly hypertensive and normotensive subjects.
4. Blockade of the L-arginine-NO pathway with NOARG attenuates but does not prevent the relaxation effects of bradykinin, suggesting that other pathways may have a role in achieving endothelium-dependent relaxation in elderly subjects.
5. These observations militate against a significant role for defects of endothelium-dependent relaxation, or a 'functional' role of resistance arteries, in the pathogenesis of hypertension in the elderly.

Chapter 5

The tilt test, vascular structure and baroreflexes
in elderly subjects

5.1 SUMMARY

This study was designed to examine the relation between the cardiovascular responses to orthostasis and both resistance artery structure and baroreflex sensitivity in elderly subjects. Twenty-five subjects of mean age 70 years (range 60-76) were studied, sixteen of whom were hypertensive and nine were normotensive. Arterial baroreflex sensitivity (from the Valsalva manoeuvre, and the phenylephrine and sodium nitroprusside drug methods) and the blood pressure and forearm vascular responses to tilt and the cold face stimulus were assessed, and the morphology of resistance arteries measured in vessels retrieved from biopsies of gluteal skin and subcutaneous fat taken under local anaesthesia.

The fall in blood pressure with either active standing or passive tilt was greater in subjects with higher levels of systolic blood pressure. Increased media:lumen ratio in the resistance vessels was correlated with greater rises in blood pressure with non-baroreflex-mediated, but not baroreflex-mediated stimuli, and the hypertensive group showed greater increases in forearm vascular resistance with the cold face stimulus. However, the increased structural change seen in the hypertensive subjects was insufficient to compensate for their observed reduction in baroreflex sensitivity and thus hypertensives had greater falls in blood pressure with orthostasis. Diminished arterial baroreflex sensitivity was associated with a greater orthostatic blood pressure fall independent of the level of blood pressure.

5.2 BACKGROUND and AIMS

A combination of the techniques described in the foregoing chapters enables the study of the relationship between the baroreceptor-vascular reflex and vascular structure at the level of the effector mechanism of the reflex - the resistance artery. It also enables an analysis of the Folkow hypothesis in as much as it relates to sympathetically-mediated vasoconstriction; it could be postulated that the remodeling of resistance arteries that accompanies hypertension (as described in Chapter 3) might mitigate the blood pressure changes induced by a sympathetic stress such as orthostasis by amplifying the vasoconstrictor response, counteracting to some extent the effect of the impaired baroreceptor-cardiac reflexes described in Chapter 2. It remains controversial whether any disorder of cardiovascular neural control contributes to orthostatic hypotension in elderly subjects.

The aims of this study were therefore:

1. To examine the baroreflex mediated responses to head-up tilt and non-baroreflex mediated reflexes such as the cold face stimulus in combination with information on vascular structure from *in vitro* studies.
2. To examine the relation between baroreflex sensitivity and the blood pressure and heart rate responses to orthostasis.

5.3 MATERIALS and METHODS

5.3.1 Study subjects

The study involved 25 elderly subjects all aged over 60 years, of whom 12 were female. Sixteen were hypertensive subjects who had not previously received treatment (sustained clinic systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 90 mmHg) and nine were normotensive. Sources for recruitment, inclusion and exclusion criteria were identical to those described in Chapters 2 and 3. All subjects gave written informed consent to participate in the study, which received prior approval from the local research ethics committee. Subjects attended the research clinic in the morning where clinic blood pressure was measured on three separate occasions at least one week apart, using a standard mercury sphygmomanometer and a cuff of the appropriate size. Supine blood pressure was recorded on each visit as the mean of three readings taken supine after at least five minutes rest. Standing blood pressure readings were taken after one, two and three minutes of standing, and the results averaged for each visit. A final value for supine and standing blood pressure for each subject was calculated from the mean of the values taken at each clinic visit.

5.3.2 Study protocol

The techniques used in this study have been described in detail in Chapters 2 and 3. Subjects attended the cardiovascular laboratory for testing of baroreceptor-

Baroreflexes and orthostasis

cardiac reflexes (from the Valsalva manoeuvre, phenylephrine injection and sodium nitroprusside infusion methods) and baroreceptor-vascular responses (changes in forearm blood flow [FBF] and forearm vascular resistance [FVR] with 60° head-up tilt) as described in detail in section 2.3.2. The blood pressure and heart rate responses to isometric exercise were recorded in the same manner, together with the responses to the cold face stimulus. Subjects then donated a biopsy of gluteal skin and subcutaneous tissue as described in section 3.3.2, and vessels were mounted in the myograph for the measurement of vessel morphology by light microscopy. Morphology measurements obtained for each vessel included length (mm), normalized lumen diameter ($L_{0.9}$, μm), media cross-sectional area (μm^2), normalized media thickness (μm) and media:lumen ratio (%). Where two vessels were obtained from the biopsy and mounted in the myograph, the average value for each parameter was taken.

5.3.3 Statistical methods

Results are expressed as mean \pm standard error of the mean (SEM). The linear relation between variables was tested using Pearson's correlation coefficient and least squares linear regression analysis. Simultaneous independent effects of continuous variables were assessed by multivariate linear regression and calculation of the the partial correlation coefficient [Altman, 1991]. For the tilt and CFS data, statistical analysis was performed using the General Linear Models procedure (to allow for unbalanced data i.e. differing group sizes) for repeated measures ANOVA,

with group and time point as factors. Summary statistics (maximum changes in SBP, DBP, heart rate, FBF and FVR, and time to maximum change) were also studied, and differences between groups in these parameters were tested using Student's unpaired two-tailed *t* test. A *p* value of < 0.05 was regarded as statistically significant.

5.4 RESULTS

5.4.1 Clinic blood pressure and vascular structure

Mean age for the 25 subjects was 70 ± 1 years (range 60–76 years). Mean clinic supine systolic blood pressure was 172 ± 7 mmHg, and mean diastolic blood pressure 91 ± 3 mmHg. The equivalent standing values were $168 \pm 6 / 98 \pm 3$ mmHg respectively. The mean change in clinic systolic blood pressure on standing was 5 ± 2 mmHg, range -14 (the negative sign representing a rise in blood pressure on standing) to 37 mmHg. Postural blood pressure change was greater in those subjects with higher initial systolic blood pressure ($r = 0.67$, $p < 0.001$). As a result, the blood pressure fall with active standing was greater in the hypertensive group compared to the normotensive group; hypertensives 8 ± 3 mmHg, normotensives -2 ± 2 mmHg ($p = 0.012$).

Baroreflexes and orthostasis

The vascular structural parameters are shown in Table 5.1. Broadly, the findings were similar to those described in Chapter 3 i.e. no significant difference between hypertensive and normotensive subjects in media cross-sectional area but a significant increase in media:lumen ratio in the hypertensives.

5.4.2 Laboratory cardiovascular tests

The response to isometric exercise is shown in Table 5.2. Although baseline and peak blood pressures were higher in the hypertensive subjects, there were no differences in the absolute change in blood pressure between hypertensives and normotensives, averaging at $51 \pm 3 / 27 \pm 2$ mmHg.

The blood pressure, heart rate, FBF and FVR responses to 60° head-up tilt are shown in Table 5.3. Baseline FBF and FVR were not different between the two groups, neither were the changes in both parameters provoked by tilting. Systolic and diastolic blood pressure fell to a greater extent with tilt in the hypertensives, and the nadir tended to occur at a later point after tilting. Furthermore, the hypertensive group also exhibited a reduced heart rate increment with tilt compared to the normotensives.

The blood pressure, heart rate, FBF and FVR responses to the cold face stimulus (CFS) are shown in Table 5.4 and Figure 5.1. The rises in systolic and diastolic blood pressures with the CFS were significantly greater in the hypertensive subjects,

	All	Hypertensive	Normotensive
Lumen diameter (μm)	283 \pm 19	273 \pm 22	301 \pm 35
Media CSA (μm^2)	36751 \pm 2841	37468 \pm 3652	35477 \pm 4742
Media thickness (μm)	40.3 \pm 2.5	42.2 \pm 3.1	36.9 \pm 4.1
Media:lumen ratio (%)	15.4 \pm 1.3	17.2 \pm 1.8*	12.2 \pm 1.3

CSA: cross-sectional area

* $P < 0.05$ for hypertensive v. normotensive groups**Table 5.1** Vascular morphology parameters for the study group as a whole, and for the hypertensive and normotensive groups

Baroreflexes and orthostasis

		Hypertensive	Normotensive	<i>p</i>
Baseline	SBP	196 ± 7	144 ± 4	< 0.0001
	DBP	94 ± 4	83 ± 4	< 0.05
	HR	74 ± 3	66 ± 3	< 0.05
Peak	SBP	247 ± 7	191 ± 6	< 0.001
	DBP	121 ± 4	109 ± 6	0.11
	HR	91 ± 4	82 ± 3	0.11
Change	SBP	51 ± 5	50 ± 4	0.78
	DBP	27 ± 3	26 ± 4	0.96
	HR	17 ± 3	16 ± 3	0.83
Time to peak (secs)	SBP	220 ± 18	250 ± 22	0.30
	DBP	210 ± 22	253 ± 12	0.10
	HR	246 ± 19	203 ± 37	0.33

SBP: systolic blood pressure(mmHg); DBP: diastolic blood pressure (mmHg); HR: heart rate (beats per minute)

Table 5.2 Blood pressure and heart rate responses to isometric handgrip for the hypertensive and normotensive groups

Baroreflexes and orthostasis

	Hypertensive	Normotensive	<i>p</i>
Max SBP fall (mmHg)	45 ± 4	29 ± 6	0.04
Time to trough SBP (secs)	70 ± 16	27 ± 7	0.023
Max DBP fall (mmHg)	17 ± 2	10 ± 1	0.011
Time to trough DBP (secs)	53 ± 16	31 ± 17	0.38
Max HR increase (bpm)	16 ± 2	24 ± 4	0.02
Time to peak HR (secs)	120 ± 11	106 ± 14	0.42
Baseline FBF (ml.min ⁻¹ .100ml ⁻¹)	3.0 ± 0.4	2.3 ± 0.3	0.16
Baseline FVR (units)	50 ± 7	49 ± 6	0.89
Max reduction in FBF (%)	47 ± 5	50 ± 6	0.74
Max increase in FVR (units)	47 ± 11	38 ± 7	0.52

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; FBF: forearm blood flow; FVR: forearm vascular resistance

Table 5.3 Blood pressure, heart rate, forearm blood flow and forearm vascular resistance responses to orthostatic stress for the hypertensive and normotensive groups

Baroreflexes and orthostasis

	Hypertensive	Normotensive	<i>p</i>
Max SBP increase (mmHg)	27 ± 4	14 ± 2	0.01
Time to peak SBP (secs)	26 ± 4	14 ± 5	0.06
Max DBP increase (mmHg)	11 ± 3	5 ± 1	0.05
Time to peak DBP (secs)	32 ± 3	18 ± 5	0.03
Max HR increase (bpm)	5 ± 2	1 ± 1	0.04
Time to peak HR (secs)	36 ± 4	23 ± 6	0.08
Baseline FBF (ml.min ⁻¹ .100ml ⁻¹)	2.7 ± 0.4	2.1 ± 0.3	0.29
Baseline FVR (units)	58 ± 8	52 ± 6	0.56
Max reduction in FBF (%)	25 ± 5	20 ± 3	0.28
Max increase in FVR (units)	29 ± 7	13 ± 3	0.05

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; FBF: forearm blood flow; FVR: forearm vascular resistance

Table 5.4 Blood pressure, heart rate, forearm blood flow and forearm vascular resistance responses to the cold face stimulus for the hypertensive and normotensive groups

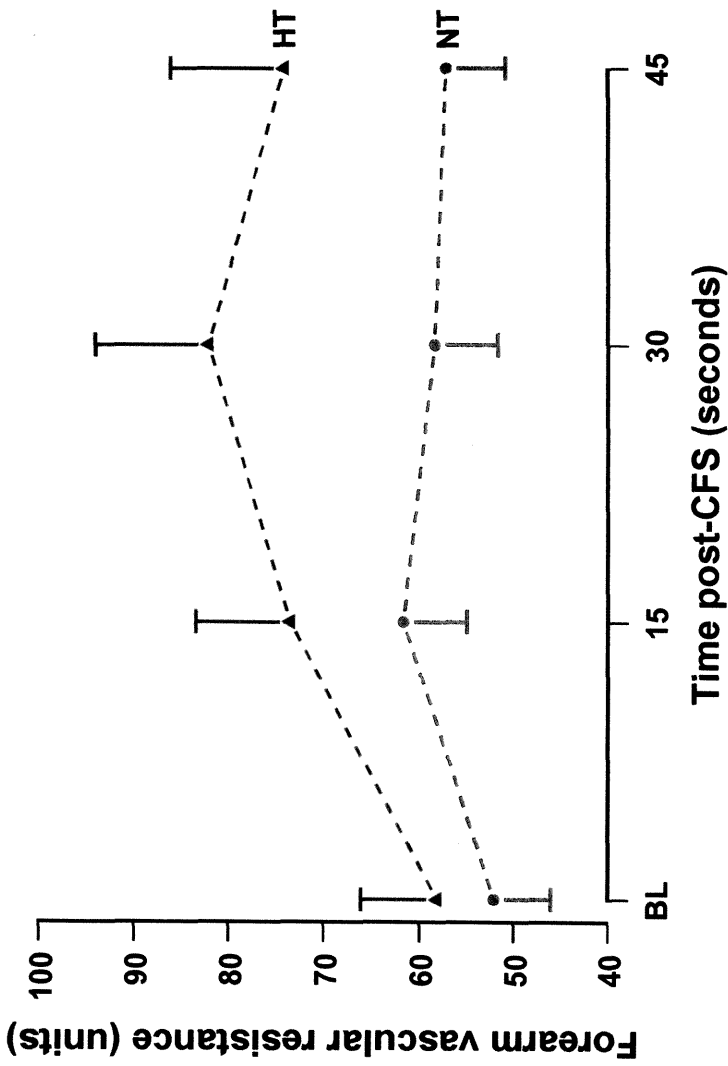


Figure 5.1 Changes in forearm vascular resistance with the cold face stimulus (CFS) for the hypertensive (HT) and normotensive (NT) groups
 $p < 0.05$ for difference between groups in the entire response; BL: baseline

and occurred significantly later following the onset of the stimulus. Once again baseline FBF and FVR, and the change in FBF with the CFS, were not different between the two subgroups, but the maximum increase in FVR with the CFS was larger in the hypertensive subjects, and there was a significant difference between the two groups when the entire response was examined (effect of time $F=1.11$, $p = 0.35$; effect of group $F=3.80$, $p < 0.05$; Figure 5.1).

Baroreceptor-cardiac reflex sensitivities from the Valsalva manoeuvre and from the steady-state and ramp analyses of the phenylephrine and sodium nitroprusside responses are shown in Table 5.5. Broadly the finding of reduced baroreflex sensitivity with hypertension was consistent with the results described in Chapter 2, with a reduction in statistical significance due to the smaller group sizes.

5.4.3 Relation between vascular responses and vascular structure

Media:lumen ratio was correlated with baseline blood pressure in the same manner as was observed in Chapter 3 (systolic blood pressure: $r = 0.44$, $p < 0.05$; diastolic blood pressure: $r = 0.25$, $p > 0.2$; pulse pressure: $r = 0.48$, $p < 0.02$). The univariate correlations between media:lumen ratio and the induced changes in blood pressure, heart rate, FBF and FVR are shown in Table 5.6 for the baroreflex-mediated tilt responses and the non-baroreflex-mediated isometric exercise and cold stress. In view of the association between media:lumen ratio and baseline blood pressure, the correlations between media:lumen ratio and blood pressure change in

Baroreflexes and orthostasis

		Hypertensive	Normotensive	<i>p</i>
Valsalva manoeuvre	V1	2.2 ± 0.5	4.8 ± 0.8	0.02
	V2	4.5 ± 0.8	9.4 ± 2.3	0.07
Sodium nitroprusside	Steady-state	3.1 ± 0.8	5.7 ± 0.5	0.01
	Ramp	3.0 ± 0.8	5.5 ± 0.5	0.02
Phenylephrine	Steady-state	3.3 ± 0.7	5.9 ± 0.9	0.03
	Ramp	3.1 ± 0.6	7.2 ± 0.7	0.0006

All values in msec/mmHg; for definitions of V1, V2, steady-state and ramp refer to section 2.3.4

Table 5.5 Arterial baroreceptor-cardiac reflex sensitivity by the different methods for the hypertensive and normotensive groups

Baroreflexes and orthostasis

	Tilt		Cold Face Stimulus		Isometric Exercise	
	simple	partial	simple	partial	simple	partial
Δ SBP	0.38	0.10	0.50†	0.44*	0.43*	0.44*
Δ DBP	0.45*	0.41*	0.39	0.28	0.07	0.10
Δ HR	-0.37	-0.34	0.43*	0.43*	-0.15	-0.17
Δ FBF	-0.05	-0.05	0.12	0.15	-	-
Δ FVR	0.12	0.03	0.14	0.06	-	-

Δ : change in; SBP: systolic blood pressure (mmHg); DBP: diastolic blood pressure (mmHg); HR: heart rate (bpm); FBF: forearm blood flow (%); FVR: forearm vascular resistance (units)
* $p < 0.05$; † $p < 0.01$

Table 5.6 Simple and partial correlation coefficients (correcting for baseline values) between media:lumen ratio and changes in blood pressure, heart rate, forearm blood flow and forearm vascular resistance responses to head-up tilt, the cold face stimulus and isometric exercise

response to each stimulus were corrected for the level of blood pressure by calculation of the partial correlation coefficients. These are shown in the adjacent columns in Table 5.6.

5.4.4 Orthostatic responses and baroreceptor-cardiac reflex sensitivity

Blood pressure changes in response to active standing in the clinic and in response to 60° head-up tilt in the laboratory were significantly correlated ($r = 0.73$, $p < 0.001$), although the absolute values seen with passive tilting were much greater ($p < 0.0001$ for difference between active standing and passive tilt). The relation between postural blood pressure change (both in the clinic and with passive head-up tilt) and baroreceptor-cardiac reflex sensitivity is shown in Table 5.7 and Figure 5.2. It can be seen that with all methods of baroreflex assessment, and in particular irrespective of the pressor or depressor nature of the baroreflex stimulus, there is a consistent association between diminished baroreceptor-cardiac reflex sensitivity and postural hypotension. A greater blood pressure fall with orthostasis was also correlated with increasing age ($r = 0.58$) but this was not independent of simultaneous changes in baroreflex sensitivity and systolic blood pressure.

As mentioned above, the fall in blood pressure with both active standing and passive tilt was strongly correlated with the prevailing level of systolic blood pressure (Δ SBP standing: $r = 0.67$, $p < 0.001$; Δ SBP tilt: $r = 0.60$, $p = 0.001$). The fall in blood pressure with tilt was independently related to baroreflex sensitivity at any given level

	Valsalva manoeuvre		Sodium nitroprusside		Phenylephrine	
	V1	V2	Steady-state	Ramp	Steady-state	Ramp
Δ SBP active standing	-0.61†	-0.53†	-0.58†	-0.56†	-0.60†	-0.58†
Δ SBP passive tilt	-0.72‡	-0.62‡	-0.46*	-0.43*	-0.61‡	-0.58†

Δ SBP: change in systolic blood pressure (mmHg); * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$

Table 5.7 Univariate Pearson's correlation coefficients between the various measures of baroreflex sensitivity and orthostatic blood pressure changes with active standing and passive 60° head-up tilt

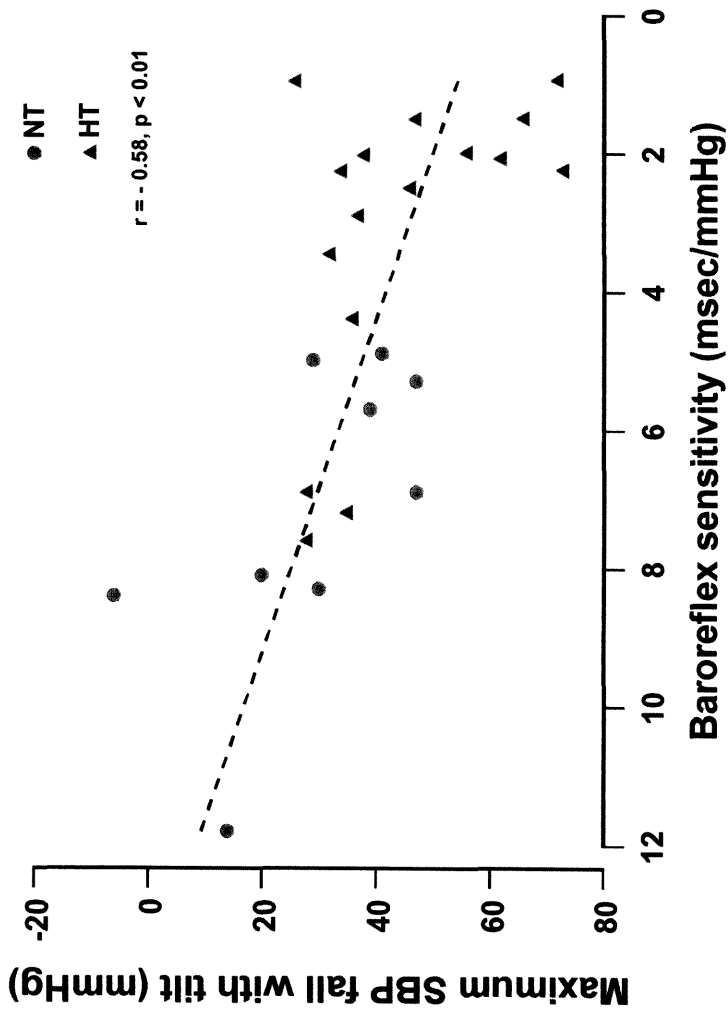


Figure 5.2 Relation between orthostatic systolic blood pressure (SBP) fall and arterial baroreflex sensitivity
NT: normotensive; HT: hypertensive subjects

Baroreflexes and orthostasis

of systolic blood pressure (BRS_{V1} : $R^2 = 54.8\%$, $p = 0.007$; BRS_{V2} : $R^2 = 48.4\%$, $p = 0.055$; BRS_{PE} steady-state: $R^2 = 45.0\%$, $p = 0.08$; BRS_{PE} ramp: $R^2 = 49.2\%$, $p = 0.028$), except for the sodium nitroprusside-based indices of baroreflex sensitivity, which were not related to orthostatic blood pressure change independent of systolic blood pressure.

5.5 DISCUSSION

5.5.1 The baroreceptor-vascular reflex and vascular structure

The main findings of this study of the baroreceptor-vascular reflex and resistance artery structure suggest that the changes seen in vascular structure are insufficient to compensate for the simultaneous deficits of the baroreflex seen in hypertension. The Folkow hypothesis would suggest that the structural changes seen in hypertension, and in particular an increased media:lumen ratio, can amplify vasoconstrictor responses leading to larger increases in peripheral vascular resistance to a given sympathetic stimulus. This phenomenon has been observed in the present study in that the hypertensive subjects showed a larger response of systolic and diastolic blood pressure, and a greater increment in FVR, with the non-baroreflex, sympathetically mediated cold face stimulus. Furthermore, the increase seen in systolic, and to a lesser extent diastolic, blood pressure was correlated with media:lumen ratio independent of the baseline blood pressure level (Table 5.6). A similar relation between media:lumen ratio and systolic blood pressure increment was also seen with isometric exercise. However, whereas little change in heart rate (and cardiac output) is seen with cold stress, isometric exercise provokes a potent sympathetically-mediated increase in heart rate and cardiac output [Fisher et al, 1973] which results in much larger rises in blood pressure than those seen with the cold face stimulus. Ewing et al (1973) observed reduced responses of blood pressure and heart rate to isometric handgrip in subjects with hypertensive left

Baroreflexes and orthostasis

ventricular hypertrophy, which raises the possibility that augmented changes in the peripheral vasculature with hypertension (as suggested by the significant relation between media:lumen ratio and Δ SBP in the present study) are opposed by diminished cardiac sympathetic responses due to hypertensive heart disease, resulting in no difference between hypertensive and normotensive subjects as seen in the present study.

No relation was seen in the present study between changes in FBF or FVR with tilt or the cold face stimulus and vascular structure. Folkow's original hypothesis was based on the observation of reduced maximal post-ischaemic vasodilatation in hypertension, indicating a residual structural alteration separate from any functional changes [Folkow et al, 1958]. Such techniques have not been employed in the present study, but it would be consistent with the hypothesis to observe greater reductions in FBF (and/or increases in FVR) with a standard stimulus such as the cold stress. There is some indication of this with a significantly greater increment in FVR to the cold face stimulus in the hypertensive subjects (Table 5.4), but no correlation was seen between maximal changes in FBF or FVR and media:lumen ratio with either the cold face stimulus or head-up tilt (Table 5.6). This is an important observation since despite a significantly increased media:lumen ratio in the hypertensive subjects, there were no significant differences in the FVR response to tilt, and this combined with an impairment of the baroreceptor-cardiac reflex (with the attendant reduction in the heart rate responses to tilt compared to the normotensives) may have led to the observed greater fall in blood pressure with tilt in the hypertensives. Although the FVR response to tilt was the same as that seen in

Baroreflexes and orthostasis

the normotensive subjects, it was not in proportion to the greater decrement in blood pressure and was itself insufficient to prevent it. It has previously been held that sympathetic activation is the most important adaptive response to postural change, particularly as the accompanying fall in cardiac filling pressure limits the extent to which cardiac factors can act to compensate. This study has suggested that, although it is apparent both that sympathetic efferent function is not impaired with hypertension and that the structural factor is present to theoretically augment any vasoconstrictive responses, postural hypotension is still greater in subjects with higher blood pressure and strongly correlated with impaired baroreceptor-cardiac reflexes.

It is in this regard that the results of the present study are substantially different from those of Tonkin and colleagues (1991, 1994). In their study of two groups of elderly subjects with either normal blood pressure or isolated systolic hypertension [Tonkin & Wing, 1994], they found no differences in blood pressure or heart rate responses to 60° tilt, the cold pressor test or isometric exercise between the groups. However, there are two important problems with their study. Not only did they fail to detect potentially important blood pressure changes early during tilt by measuring blood pressure only at the end of ten minutes of tilt (in the present study the nadir of systolic blood pressure occurred within 1-2 minutes of tilting), but ironically they chose to minimise one of the more interesting findings of their study, stating in the text that there were no differences in FVR responses between elderly normotensive and hypertensive subjects while the data in their table indicate that the hypertensives had an larger increment in FVR than the normotensives to cold stress, but not to tilt.

Baroreflexes and orthostasis

This observation is in agreement with the results of the present study, but it did not deflect Tonkin & Wing from concluding that there is no difference in cardiovascular reflex behaviour with systolic hypertension beyond that already due to age, and that there is no evidence for a causative association between high blood pressure and orthostatic hypotension. In their study of the responses to tilt examined by spectral analysis of heart rate variability, Yo et al (1994) found no difference in the changes in parasympathetic activity or sympathovagal balance between older subjects with or without hypertension. However, this was despite a significantly greater fall in blood pressure in the hypertensives, again raising the issue of the adequacy of these sympathetic responses in the face of larger orthostatic blood pressure changes.

London and colleagues (1987) observed in a young group of hypertensive subjects not only increased whole-body peripheral vascular resistance compared to normotensives, but also greater increments in response to tilt, and hypothesized that this was due to augmentation of vasoconstriction by hypertension-related vascular hypertrophy. The present study offers a unique opportunity to examine this hypothesis, albeit in an elderly group with many other intervening physiological changes, and found no evidence of a relation between vascular structure (media:lumen ratio) and the alterations in vascular resistance that follow a baroreflex-mediated sympathetic stress. Furthermore, baseline vascular resistance (at least in the forearm vascular bed) was not different with hypertension in the present study.

5.5.2 Hypertension and orthostatic hypotension

The observation of a relation between hypertension and orthostatic hypotension in the elderly has been reiterated by many studies [MacLennan et al, 1980; Lipsitz et al, 1985; Robinson et al, 1994]. This has been explained previously by the hypothesis that progressive impairment of cardiovascular reflexes with ageing produces inadequate homeostatic responses to environmental influences, be they due to food [Lipsitz & Fullerton, 1986] or changing posture. However, it has not been demonstrated previously that any further impairment of these reflexes as may occur with hypertension in already aged subjects is responsible for any further impairment of cardiovascular responses. The data from the present study offer evidence that this is indeed the case. Greater blood pressure falls with both active standing and passive tilt were strongly associated with impaired arterial baroreflex sensitivity, and intact sympathetic efferent function (as judged by similar blood pressure responses to a non-baroreflex mediated sympathetic stress) and blood pressure-related resistance artery structural changes (increased media:lumen ratio) were insufficient to compensate. This synthesis suggests two conclusions:

1. It mitigates against one of the popular theories for the genesis of hypertension based on the observation of impaired afferent baroreflex function, that is that reduced inhibitory influences permit the 'sympatho-excitation' that is thought to lead to peripheral vasoconstriction - the present data suggest no evidence of increased baseline peripheral vascular resistance (at least in the musculocutaneous vascular bed of the forearm) in the intact subject with hypertension;
2. It proposes the opposite conclusion to that drawn by Tonkin & Wing (1994) in

Baroreflexes and orthostasis

suggesting that the impairments of cardiovascular homeostasis seen with hypertension are likely to also be responsible for the associated orthostatic hypotension. The results from multiple regression analysis in the present study support this, with baroreflex sensitivity being related to orthostatic blood pressure change independent of the prevailing level of systolic blood pressure.

This is the first study to compare orthostatic blood pressure changes and direct measurement of arterial baroreceptor-cardiac reflexes in otherwise healthy and asymptomatic elderly persons with or without high blood pressure. Thirty years ago, Johnson and colleagues (1965) described an association between orthostatic hypotension and impaired blood pressure responses to the Valsalva manoeuvre in institutionalised elderly patients. However, Johnson's group (1983) later went on to report no differences in the heart rate response to tilting between hospitalised elderly subjects with or without orthostatic hypotension but with similar levels of systolic blood pressure. White (1980) demonstrated a smaller and slower rise in heart rate in elderly subjects with orthostatic hypotension. Rowlands et al (1984) did study the responses both to tilt and phenylephrine injection in elderly hypertensive and normotensive subjects, and found greater falls in systolic blood pressure after 15 minutes of tilt in the hypertensives, but no attempt was made in their study to relate responses to orthostatic stress to baroreceptor-cardiac reflex sensitivity. In young patients with symptomatic orthostatic hypotension, El-Sayed & Hainsworth (1995) found that a reduced tolerance to orthostatic stress was related to increased (not decreased) carotid baroreceptor-cardiac reflex sensitivity (using the neck suction method). They suggested that a baroreflex sensitivity of less than 10 msec/mmHg

Baroreflexes and orthostasis

was effective protection against orthostatic intolerance. There was an unusual group to study, consisting of otherwise healthy young subjects with unexplained syncopal episodes. Consequently it is difficult to translate the possible implications of their study to a group of asymptomatic elderly subjects, particularly as virtually all subjects in the present study had baroreflex sensitivities of less than 10 msec/mmHg. Indeed the present study would suggest the opposite conclusion, that the reduced baroreceptor-cardiac reflex sensitivity seen with hypertension is independently associated with inadequate baroreceptor-vascular responses to postural change and thereby with orthostatic hypotension.

5.5.3 Study limitations

This study has a number of limitations that need to be acknowledged. First, it has not included any attempt to measure sympathetic responses to tilt from either venous catecholamines or from microneurographic studies [Wallin, 1988]. The problems of inferring alterations in sympathetic function from venous plasma catecholamines are widely recognised [Floras et al, 1986], but Rowlands et al (1984) did not find any greater increment in plasma noradrenaline with tilt in elderly hypertensive subjects compared to normotensives, and Lye et al (1990) found that the increment in plasma noradrenaline with tilt was not related to the postural blood pressure change. Second, the vascular structural changes described relate particularly to the subcutaneous vasculature of the gluteal region, yet comparisons were drawn with the vascular responses measured from forearm plethysmography. Agabiti-Rosei and

Baroreflexes and orthostasis

colleagues (1995) have recently found reasonable agreement between minimum forearm vascular resistance and media:lumen ratio from gluteal subcutaneous vessels, so these regions may not be so dissimilar despite some differences in the prevailing level of blood pressure in the two vascular beds. While it may be a reasonable assumption to consider gluteal vessels typical of the vessels of the skin and musculature, it cannot be so readily assumed that they describe the structure of the splanchnic bed, which probably plays a more important role in the vasoconstrictive response to orthostasis [Bannister & Mathias, 1988]. Also this study has included no attempt to distinguish the separate responses to orthostasis of the low-pressure cardiopulmonary reflexes from those of the high-pressure arterial baroreflexes. The response to tilt is a complex one invoking stimulation of both systems but the test does distinguish between them. Non-hypotensive lower body negative pressure and passive leg raising can be used to stimulate and inhibit cardiopulmonary receptors exclusively, and these responses would be of interest in view of the known impairment of such reflexes with both ageing and hypertension, particularly if the latter is accompanied by left ventricular hypertrophy [Trimarco et al, 1986; Grassi et al, 1988b; Ferrari et al, 1989]. The two latter points raised above may be related in view of the previous observation that high pressure baroreflexes appear to predominantly increase splanchnic vasoconstriction, while cardiopulmonary responses act principally on the skeletal muscle and renal vasculatures [Abboud et al, 1979], although this differentiation is not confirmed by other workers [Escourrou et al, 1993]. Baroreflex-mediated venoconstriction, which also contributes to the adaptive response to postural change has also not been assessed, but the significant contribution of this side of the reflex is implied by the

prior observation of orthostatic hypotension related to varicose veins [Caird et al, 1973].

5.6 CONCLUSIONS

1. Orthostatic blood pressure changes assessed either by active standing or passive tilting are greater in elderly subjects with higher systolic blood pressure.
2. The increased media:lumen ratio observed in elderly hypertensive subjects is associated with greater increases in systolic blood pressure with non-baroreflex mediated, but not baroreflex-mediated sympathetic stress.
3. An increased media:lumen ratio in the subcutaneous vasculature is not related to any increase in the forearm vascular resistance response to a sympathetic stress.
4. Thus hypertensive subjects display inadequate forearm vasoconstriction and have greater falls in blood pressure with active standing and passive tilt despite their increased media:lumen ratio in resistance arteries.
5. The fall in blood pressure with orthostasis is associated with decreased arterial baroreflex sensitivity, independent of the blood pressure level.

Chapter 6
Discussion

6.1 SUMMARY of PRINCIPAL STUDY FINDINGS

Chapter 2 consisted of a study using well-established methodology to examine cardiovascular neural control, a topic that has received an enormous amount of attention in the literature, particularly since the seminal work of the Oxford group in describing a standardised method for the quantification of the baroreceptor-cardiac reflex [Smyth et al, 1969; Bristow et al, 1969]. Work based on this methodology has contributed considerably to the understanding of hypertension, yet it is intriguing that in the 40 years that have elapsed since the first descriptions of baroreflex resetting in hypertension, the issue of cause and effect still remains controversial [Conway et al, 1973; Ito & Scher, 1979; Norman, 1981; Sleight, 1979, 1986, 1991; Aksamit et al, 1987; Kuchel et al, 1987; Karemaker et al, 1989]. The work conducted in chapter 2 was motivated by the lack of clarity about the relation between reduced baroreflex sensitivity, age and hypertension in the elderly, and by the clinical importance of isolated systolic hypertension in this age group. The study demonstrated a consistent reduction in baroreflex sensitivity in both combined hypertension and isolated systolic hypertension, but no difference between them (with 90% power at the 5% level to detect a clinically important difference; see section 2.5.2). It also demonstrated that changes in baroreflex sensitivity in the elderly were independent of increasing age or diastolic blood pressure, being associated only with changes in systolic blood pressure. The study also demonstrated an independent relation between blood pressure and heart rate variability and baroreflex sensitivity in elderly subjects. Although baseline forearm blood flow and vascular resistance were not

Discussion

different between subjects with normal or high blood pressure, elderly persons with isolated systolic hypertension showed greater increments in forearm vascular resistance with passive tilt. However, this was insufficient to prevent a greater fall in blood pressure with tilt in these subjects when compared to the normotensives.

As if by contrast with the long-established methodology used in Chapter 2, Chapters 3 and 4 used relatively recent techniques to conduct the first studies of resistance artery structure and function in elderly subjects. The *in vitro* study of small arteries has been advanced by the development of the Mulvany myograph, first described in 1977 by Mulvany and Halpern, together with the retrieval of human small arteries from gluteal biopsies taken under local anaesthesia. The work described in Chapter 3 demonstrated considerable structural modification with hypertension in the elderly, manifest as a significant increase in the media:lumen ratio of resistance arteries. This parameter was mainly related to the level of pulse pressure, particularly when this was measured by 24-hour ambulatory blood pressure monitoring. The observed increase in media:lumen ratio was associated with similar media cross-sectional area in the normotensive and hypertensive subjects, suggesting that the main adaptation with high blood pressure was wall remodeling rather than hypertrophy.

In Chapter 4 the isometric myograph was used to study the contractile function of resistance arteries and the function of the vascular endothelium. No differences were found in the behaviour of resistance arteries from elderly hypertensive and normotensive subjects in the contractile responses to noradrenaline and angiotensin

Discussion

II. Neither were any differences found with high blood pressure in the relaxation responses to the stimulated release of endothelium-dependent factors. Near-maximal relaxation was seen in both groups with the endothelium-independent vasodilator sodium nitroprusside. Significant relaxation to bradykinin was preserved even after blockade of nitric oxide synthase, suggesting that the L-arginine-NO pathway may not be a major pathway for achieving vasodilation in the elderly. The studies described in these latter two chapters were inevitably exploratory, being the first such studies in this age group, and raise almost as many questions as they answer.

Chapter 5 describes a study taking advantage of the techniques used in chapters 2 and 3 to examine the relation between cardiovascular reflex responses and in particular head-up tilting, and baroreflex function and small artery structure. The study demonstrated a statistically and clinically significant relation between reduced baroreflex sensitivity and a greater fall in blood pressure with orthostasis independent of the level of blood pressure, but no relation between vascular structure and the baroreceptor-vascular reflex. Hypertensive subjects showed reduced baroreflex-mediated vasoconstriction with orthostasis leading to a greater fall in blood pressure in that group compared to the normotensives. Thus the study tended not to support other studies which have suggested that augmented vascular resistance responses in hypertensives are attributable to hypertension-related structural alteration in resistance vessels.

6.2 IMPLICATIONS OF THESE FINDINGS FOR THEORIES OF THE AETIOLOGY OF HYPERTENSION IN THE ELDERLY

To consider the implications of these findings for our understanding of the aetiology of hypertension in the elderly, it is perhaps best to begin with a consideration of the rise in systolic blood pressure that accompanies ageing in Western societies. Two results of this trend are that the majority of elderly hypertensives have isolated systolic hypertension [Wilkings et al, 1988], and in the elderly the incidence of cardiovascular complications is related principally to the systolic blood pressure, and less so to the diastolic blood pressure [Staessen et al, 1990; Potter, 1994]. The changes that occur in the large arteries of humans with ageing lead to loss of compliance and distensibility which results in preferential elevation of systolic blood pressure. This effect is augmented by increased pulse wave velocity leading to summation of the incident and reflected waves at the systolic peak. The 'splinting' effect of a more rigid arterial wall is thought to cause a loss of mechanical-electrical coupling in the baroreceptor and a reduction in baroreceptor sensitivity, which leads to a fall in the central inhibition of sympathetic outflow. This, combined with a loss of peripheral β -adrenoceptor sensitivity leads to a state of excess sympathetic activity and increased peripheral vascular resistance. An increase in peripheral resistance is reported in hypertension in the elderly, even when it is predominantly systolic [Koch-Weser, 1973; Messerli et al, 1983; Berger & Li, 1990].

Discussion

The studies presented in this thesis have shown a significant reduction in baroreceptor-cardiac reflex sensitivity with hypertension in elderly subjects. The values for baroreflex sensitivity seen in the normotensive controls were also much less than those reported in the literature for younger subjects [Smyth et al, 1969; Gribbin et al, 1971] and are consistent with a progressive reduction with ageing. However, in this age group of subjects beyond 60 years, no further independent effect of ageing is seen, although the relatively narrow 21-year age range may have precluded the detection of a small independent effect of age. The findings of the present study constitute an extrapolation of the original work demonstrating a relation between age and declining baroreflex sensitivity by Gribbin, Pickering, Sleight and Peto (1971) in 81 subjects using the phenylephrine pressor method, one of whom was aged over 60 years. Their landmark study, frequently cited as confirming the decline in baroreflex sensitivity with ageing, deserves further examination. They demonstrated a logarithmic rather than a linear relation between declining baroreflex sensitivity and age, and the data from their original 81 subjects are replotted on a linear scale in Figure 6.1A. Although an independent relation between age and baroreflex sensitivity is described, this is an over-simplification. In fact in subjects beyond the age of forty there is no significant linear relation between age and baroreflex sensitivity, rather like in the present study beyond 60 years. There is a remarkable concordance between their results from 25 years ago and those from the present study, using similar methodology, which are plotted together in Figure 6.1B. If the results from the 54 subjects from the present study are added to the graph from ages 60 to 81, there is no significant change to the polynomial regression equation

Discussion

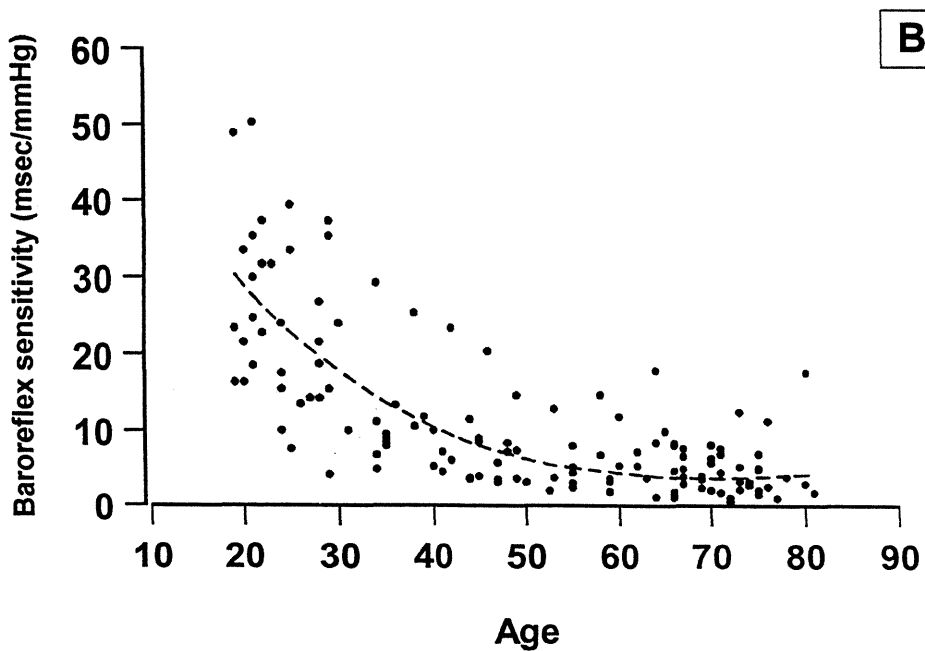
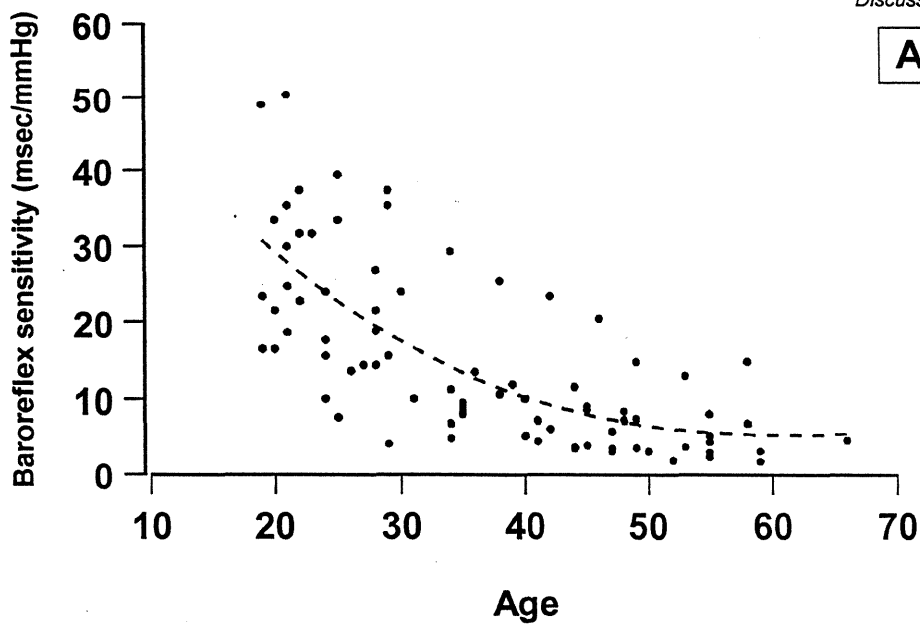


Figure 6.1 The relationship between baroreceptor-cardiac reflex sensitivity and age [A] Data replotted from Gribbin et al (1971) [B] Combined data from Gribbin et al and the present study
Equation for both regression lines $y = 70.7 - 2.76x + 0.038x^2 - 0.00017x^3$, $R^2 = 52\%$

Discussion

relating age to baroreflex sensitivity for the entire group ($y = 70.7 - 2.76x + 0.038x^2 - 0.00017x^3$; $R^2 = 0.52$, $p < 0.001$). The linear correlation coefficient between baroreflex sensitivity and age for all the subjects over 40 years is -0.24 ($p = 0.02$), and for the subjects over 50 years is -0.04 . Whilst accepting the reservations one would have about comparing or combining the results of two studies separated by twenty-five years, the degree of agreement between them is all the more remarkable. This 'meta-analysis' would suggest that the majority of the reduction that is seen in baroreflex sensitivity with age has already occurred by the fifth decade.

What implications does this have for the argument about the role of baroreflex dysfunction (in as much as this is quantified solely by measurement of baroreceptor-cardiac reflex sensitivity) in the aetiology of hypertension in the elderly? The cause-effect relationship has always been hampered by the assumption that with ageing in humans, baroreflex sensitivity fell and blood pressure rose in parallel. It would appear that the majority of the rise in blood pressure with ageing actually occurs well after the main fall in baroreflex sensitivity, such that while baroreflex sensitivity changes little between 50 and 80 years, average systolic blood pressure rises by 20 mmHg or more [Rose, 1985; Staessen et al, 1990], whereas between 20 and 40 years there is a sharp decline in baroreflex sensitivity while blood pressure changes little. The effect of a reduction in baroreflex sensitivity on blood pressure is not immediate [Sleight, 1979], but is more likely to result in the longer-term consequences of a reduction in baroreflex-mediated sympathetic inhibition or increased blood pressure variability. However, this argument does run counter to that which states that impaired baroreflexes result from the effects of a raised blood

Discussion

pressure [Abboud, 1982]. Those subjects in the present study with lower blood pressure and higher levels of baroreflex sensitivity may be those who, for whatever reason, have retarded the progressive impairment in baroreflex sensitivity that began many years previously and is thus associated with advancing age. It would be wrong, however, to assume that the two factors of ageing and hypertension are mutually exclusive and it remains quite possible that an elevated blood pressure also confers a further impairment of baroreceptors due both to a mechanical effect and local paracrine factors. Some further light can be shed on this from the comparison of subjects with combined hypertension and isolated systolic hypertension. Elderly subjects in the latter group have previously been shown to have reduced large artery compliance compared to their peers with systolic-diastolic hypertension at the same level of systolic blood pressure [Sumimoto et al, 1990]. Randall et al (1976) found an independent reduction in compliance and baroreflex sensitivity with both age and hypertension in young subjects. Lage and co-workers (1992) demonstrated that impaired baroreflexes were correlated with reduced arterial compliance in their study of younger (age 26 to 62) normotensive and hypertensive subjects. However, they did not correct their compliance parameters for the prevailing level of blood pressure, and thus were unable to advance the argument beyond the description of epiphenomena. If elderly subjects with isolated systolic hypertension do indeed have more rigid arteries than combined hypertensives with similar systolic blood pressure, this would not appear to lead to a further reduction in afferent baroreceptor sensitivity in these patients.

Discussion

Reduced large artery compliance alone produces elevation of systolic blood pressure and a fall in diastolic blood pressure, with a widening of the pulse pressure that is characteristic of the changes in the blood pressure profile that occur with advancing age. The study described in chapter 3 examined the relation between systolic blood pressure, pulse pressure and the structure of small arteries of less than 300 μm internal diameter, where a substantial proportion of the peripheral resistance to blood flow occurs. The strongest relation was that between pulse pressure, particularly when measured over 24-hours, and media:lumen ratio. Two possible explanations arise for this observation: firstly that the study describes the 'downstream' effects on the resistance vasculature of exposure to a widened pulse pressure caused by changes in large arteries, or that the increased pulse pressure observed in the study was itself the consequence of increased wave reflections from the structurally altered resistance vasculature. The former hypothesis may explain why the elevated systolic blood pressure associated with large artery rigidity in elderly subjects is not usually accompanied by a fall in diastolic blood pressure, as the structural changes that are evoked in the small arteries may to some extent maintain the diastolic blood pressure [Salvetti et al, 1995]. Similarly, it may contribute to the relative pre-eminence of systolic blood pressure as a risk factor in the elderly, if a higher systolic and wider pulse pressure were a reflection of greater vascular structural change. However the study, being cross-sectional, was unable to address this issue directly, and indeed the techniques used preclude the repeated study of subjects in a longitudinal manner.

Discussion

Differences in endothelial function have been described between young hypertensive and normotensive subjects, and the role of the endothelium in the genesis and maintenance of arterial hypertension has received considerable interest of late. The study described in chapter 4 did not confirm that this observation extends into the elderly population, and perhaps weakens the hypothesis of endothelial factors and a functional defect of resistance arteries contributing to the aetiology of hypertension as it applies to this age group. However, the results are consistent with a significant age-related decline in endothelium-dependent vasodilation that other workers have found [Celermajer et al, 1994; Taddei et al, 1995], such that by the age of the group studied, any additive effect of hypertension has been lost. In the study of Taddei and colleagues, the decline in endothelium-dependent relaxation in the normotensive subjects was steeper than in the elderly hypertensives, such that over the age of 60 years the difference between the two groups was much reduced, and no further decline in endothelial function with age was seen in their study in either group beyond 60 years. As discussed in Chapter 4, the causes of this age-related decline are not known, but could include the accumulation of glycosylation end-products or the effects free-radical damage [Bucala et al, 1991; Vlassara et al, 1992; Martin, 1992]. This latter factor may contribute to the observed epidemiological association between antioxidant use and protection from high blood pressure [Salonen et al, 1988].

There is also increasing evidence of the modulation of mechanical-electrical transduction in arterial baroreceptors by endothelial factors including prostacyclin, oxygen free radicals, platelet-derived factors and endothelin. Chapleau and

Discussion

colleagues (1995) have demonstrated increased baroreceptor sensitivity in animal models of hypertension and atherosclerosis with treatment with exogenous prostacyclin or the free-radical scavengers superoxide dismutase and catalase. Furthermore in their studies aggregating platelets caused baroreceptor inhibition, and this process may affect areas of endothelial damage (either structural or functional) at sites prone to shear stress such as the carotid sinus or aortic bifurcations [Karemaker et al, 1989]. These observations may give an alternative explanation for impairment of baroreceptor function long before it could be attributed to structural modification related to higher blood pressure levels or atheroma, as discussed above. A similar hypothesis would derive from the observation of reduced carotid baroreceptor sensitivity with sodium loading in humans without a change in blood pressure [Creager et al, 1991], and this may be attributable to alteration of the ionic environment around the baroreceptor or to attenuation of responses by renin or angiotensin. Alternatively, there may be effects of these factors on the central integration of baroreflexes. It is difficult to study central mechanisms in humans, but in animal studies it has been shown that sympathetic nerve activity is released from baroreceptor-mediated inhibition in older animals despite a similar level of afferent baroreflex activity to younger animals [Hajduczuk et al, 1991]. This observation would be consistent with the hypothesis that a degree of comparative sympatho-excitation in elderly subjects is due to loss of the inhibition of sympathetic efferents by the baroreflex, be it through afferent or central failure [Bertel et al, 1980; Goldstein 1983b; Shimada et al, 1985; Karemaker et al, 1989].

Discussion

Modulation of baroreflexes by other endothelium-related factors may also include a contribution from the L-arginine-NO pathway. Although the hypertension caused by inhibitors of NO-synthase such as L-NMMA and L-NAME is usually attributed to blockade of endothelial NO production [Rees et al, 1989b; Vallance et al, 1989], there is also the possibility of inhibition of neuronal NO in the nucleus tractus solitarius or the rostral ventrolateral medulla with these agents [Togashi et al, 1992; Harada et al, 1993]. NO-synthase is expressed both in the carotid sinus and in the nucleus tractus solitarius [Vincent et al, 1992; Hohler et al, 1994] and injection of L-NMMA into the latter area in animals leads to a marked rise in both sympathetic nerve activity and blood pressure [Harada et al, 1993]. Inhibition of the L-arginine-NO pathway may have an effect on the central inhibition of arterial baroreflexes, as suggested by animal studies in which L-NAME treatment was accompanied by progressive attenuation of such reflexes [Scrogin et al, 1994; Lantelme et al, 1994]. However, although a substantial contribution of the sympathetic nervous system to L-NAME-induced hypertension has been observed in animal studies [Jimbo et al, 1994; Sander et al, 1995], in intact humans a sympathetic component to the rise in blood pressure with NO inhibitors has not been demonstrated [Hansen et al, 1994; Castellano et al, 1995].

It could be postulated that an age-related decline in the activity of the L-arginine-NO pathway accounts for both the decline in endothelium-dependent relaxation seen with age with a contribution to the rise seen in peripheral vascular resistance, as well as an impairment of the central integration of baroreflexes leading to both reduced baroreceptor-cardiac reflex sensitivity and a loss of inhibition of sympathetic

autonomic outflow. There are many components to this synthesis that remain untested in either animal or human experiments, not the least of which is why one pathway might be affected to a greater extent by ageing than another, and this serves to indicate the large gaps that remain in our knowledge and understanding of hypertension in the elderly. However, while this thesis has concentrated on possible differences between elderly normotensive and hypertensive subjects, as is so often the case in the elderly the phenomena that have been observed in the groups studied may reflect processes affecting both the neural control of blood pressure and the peripheral vasculature the major part of which occurred many years earlier.

6.3 IMPLICATIONS OF THESE FINDINGS FOR THE TREATMENT OF HYPERTENSION IN THE ELDERLY

The results of these studies may have some relevant clinical implications. Historically, the systolic and pulse pressures have not received the same attention as other parameters of blood pressure in terms of their cardiovascular risk. However, together with the evidence of the particular importance of systolic blood pressure as a risk factor in the elderly [Kannel et al, 1971, 1981; Staessen et al, 1990; Silagy & McNeil, 1992], there is increasing evidence of the importance of pulse pressure as a risk factor for future cardiovascular events, including myocardial infarction [Darne et al, 1989; Madhavan et al, 1994; Scuteri et al, 1995]. Structural regression in the resistance vasculature is likely to become an important goal of anti-hypertensive

Discussion

therapy, particularly if it proves to be related to beneficial effects elsewhere such as an improvement in coronary reserve and reduction in myocardial infarction [Mulvany, 1991; Mancia et al, 1995]. In order to achieve structural regression, the study suggests that antihypertensive treatment must reduce pulse pressure as well as the more traditional goals of a reduction in mean arterial or diastolic blood pressure. This will be particularly relevant in those elderly subjects with disproportionate systolic hypertension, in which treatments which predominantly lower systolic blood pressure and pulse pressure irrespective of their effects on mean arterial or diastolic blood pressure may be preferable. As the SHEP study has shown [SHEP Co-operative Research Group, 1991], a reduction in systolic blood pressure and pulse pressure can be achieved in the elderly with few of the previously perceived adverse consequences on quality of life and with considerable benefit, including significant reductions in coronary heart disease not achieved in studies of younger age groups. So far in human studies, the main class of antihypertensive agent that has been shown to cause structural regression in the resistance arteries of hypertensive subjects are the angiotensin-converting enzyme inhibitors, exerting an effect not seen with β -adrenoceptor blocking agents despite similar reductions in blood pressure [Schiffrin et al 1994; Thybo et al, 1995]. Whether this class of drug possesses any further benefit in terms of outcome when compared to other agents (thiazide diuretic and calcium-channel blocker) in the treatment of elderly patients with isolated systolic hypertension is currently being tested in the SYST-EUR study [Slovick et al, 1993].

Discussion

It could be argued that the restoration of endothelial function in the elderly would also be a valuable goal for treatment. This would facilitate the recovery of a normal physiological vasodilating response and may be particularly valuable in counteracting endothelial dysfunction in the coronary circulation [Yasue et al, 1990; Egashira et al, 1993]. It may also reverse some of the increase seen in vascular resistance with an improvement in blood pressure as a result. Preservation or restoration of function in the L-arginine-NO pathway may also enhance the anti-thrombotic effects through the anti-aggregating properties of EDRF/NO [Lüscher & Vanhoutte, 1990]. This might be achieved through the action of angiotensin receptor antagonists or angiotensin converting enzyme inhibitors to prevent the breakdown of bradykinin, antioxidants, or through the enhancement of prostacyclin availability [Feletou et al, 1992]. These latter two factors raise the possibility of a role for vitamin C in the treatment or prevention of high blood pressure [Bulpitt, 1990; Hemilä, 1991]. Although a significant antihypertensive effect of vitamin C has not been seen in small trials in elderly subjects [Lovat et al, 1993; Ghosh et al, 1994], hypertensive subjects do have lower levels of ascorbic acid together with increased markers of endothelial damage (such as von Willebrand factor) [Tse et al, 1994]. Agents such as those mentioned above are also among those which may improve baroreceptor sensitivity by alteration of local paracrine activity [Chapleau, 1995]. In human studies of the response of baroreflex sensitivity to the drug treatment of high blood pressure, one study has shown a greater improvement in baroreflex sensitivity with an angiotensin-converting enzyme inhibitor than with a calcium antagonist for the same reduction in blood pressure [Egan et al, 1993]. It is possible that angiotensin-converting enzyme inhibitors exert their effect on the baroreflex by a central mode of action. Central

Discussion

administration results in increased baroreflex sensitivity in the spontaneously hypertensive rat [Berecek et al, 1983], and angiotensin II receptors in the area postrema may be partly responsible for the antagonism of baroreflexes [Mancia et al, 1982]. This might make angiotensin-converting enzyme inhibitors particularly suitable for the treatment of hypertension in the elderly, and may contribute to their observed efficacy in this age group despite the comparatively low plasma renin activity in such patients [Shapiro et al, 1987].

An increase in baroreflex sensitivity concurrent with a reduction in blood pressure with treatment offers two principal gains: reduction in blood pressure variability with increased heart rate variability, and an improved response to orthostasis. The study described in chapter 5 is the first such study to confirm an association in elderly subjects between diminished baroreceptor-cardiac reflex sensitivity and the postural fall in blood pressure, although such an association is often postulated or inferred [Ibrahim et al, 1975; MacLennan et al, 1980; Tonkin et al, 1991]. A fear of exacerbating orthostatic intolerance has often made physicians reluctant to use antihypertensive therapy in some elderly subjects, particularly in view of the association between systolic hypertension and orthostatic hypotension [Jackson et al, 1976; Rowlands et al, 1984; Giannattasio et al, 1994]. The findings from the study presented here suggest that the converse may be true and if baroreflex sensitivity is improved by treatment, this may actually improve orthostatic tolerance. This was suggested by the study of Egan et al (1993), who found that increased baroreflex sensitivity with angiotensin-converting enzyme inhibition reversed the postural fall in blood pressure seen with placebo.

Discussion

An increasing body of data also points to the independent significance of blood pressure variability in bestowing further cardiovascular risk at any given level of blood pressure [Parati et al, 1987; Palatini et al, 1992; Frattola et al, 1993]. As demonstrated in the present studies, diminished arterial baroreflex sensitivity in the elderly is correlated with both increased blood pressure variability and decreased heart rate variability. Reduced heart rate variability is related to increased mortality, and is thought to be the mechanism linking reduced baroreflex sensitivity and mortality following myocardial infarction [Kjellgren & Gomes, 1992]. Whether or not blood pressure variability can be improved by drug therapy, including with agents known to have beneficial effects on baroreflex sensitivity, is not yet known. In a large study of the effects of several weeks of angiotensin-converting enzyme inhibitor or calcium blocker therapy on blood pressure variability assessed from intermittent ambulatory recordings, Mancia et al (1995) found no reduction in the coefficient of variation for 24-hour, daytime or night-time blood pressure, despite substantial reductions in clinic and ambulatory blood pressure. However, they did find that it was the subjects with higher levels of blood pressure variability prior to treatment who had the greatest reductions with treatment. Other studies have usually contained too few subjects to detect a significant change in blood pressure variability with treatment [Egan et al, 1993]. Also the effects of treatment on short-term blood pressure variability have not been elucidated. Therefore while reduced blood pressure variability can be regarded as an important goal of treatment, a question remains about whether it can be achieved even with drugs previously shown to beneficially affect baroreflex sensitivity.

6.4 PROSPECTS FOR FURTHER STUDIES IN HYPERTENSION IN THE ELDERLY

The findings from the studies presented in this thesis suggest many possible further lines of investigation. As mentioned, the studies of resistance artery structure and function were inevitably exploratory, and raise many questions regarding the small arteries and endothelium in the elderly. A larger study of blood pressure and small artery structure across a wider spectrum of ages might cast further light on the relationship between the various parameters of blood pressure and structure, and would perhaps be able to address the cause-effect question. A search for the origins of diminished endothelial function in elderly subjects might explain some of the rise in blood pressure and vascular resistance seen with ageing, and further characterisation of the particular defects in endothelium-dependent relaxation may indicate possibilities for drug interventions. The role of basal EDRF/NO activity in the regulation of blood pressure in elderly subjects could be studied using intravenous administration of L-NMMA. It would be of particular interest to see if drug treatments were able to reverse some of the structural and functional defects associated with hypertension, as has been shown with some agents in younger subjects with lesser degrees of vascular structural alteration and more preservation of endothelial responses, or to examine the effects of dietary manipulation with antioxidants such as vitamins C, E or β -carotene on endothelial function. Comparison of elderly subjects on normal and vegetarian diets would provide a relatively ready method of testing this, although there are many recognised confounders with such comparisons.

Discussion

The studies of cardiovascular neural control contained in this thesis also suggest some further possibilities for research. In particular, it would be timely to address the issue of the interrelation between blood pressure, age and baroreflex sensitivity with a longitudinal study in human subjects at all levels of blood pressure. In the past studies of this kind have been limited by the invasive nature of intra-arterial measurement of blood pressure for the baroreflex sensitivity testing procedure. However, with the recent application of non-invasive blood pressure recording with the Finapres device and the derivation of indices of baroreflex sensitivity from sequence and spectral analysis, the repetitive measurement over time is now a realistic prospect. In this laboratory we have found baroreflex sensitivity from the Valsalva manoeuvre (using the Finapres) to be highly reproducible, with a coefficient of variation between visits of less than 7% (Dr P Weston, personal communication, 1995). This would enable the issue of whether baroreflex sensitivity declines before blood pressure rises to be addressed, and perhaps some progress made towards answering the 'chicken or egg' question in humans. Similar methods would permit the study of the effects of antihypertensive agents on changes in baroreflex sensitivity and orthostatic hypotension in elderly subjects in whom it might be assumed that advanced hypertension-related arterial damage makes such changes resistant to reversal.

6.5 CONCLUSION

The work described in this thesis represents an attempt to further clarify aspects relating to the pathophysiology of hypertension in the elderly, and in particular to two of the most important physiological changes that occur with ageing and hypertension, namely reduced baroreflex sensitivity and increased peripheral vascular resistance. It is hoped that to some extent this has been achieved, in as much as this is possible with cross-sectional work. However, as is so often the case, one of the main outcomes of this research has been to indicate other areas where more study is required. The importance of further research in hypertension in the elderly and its origins cannot be overstated, given the demographic changes that are destined to affect all developed countries and the attendant increasing burden of health care that this will bring. Hypertension represents one of the most important reversible risk factors for cardiovascular disease, with benefits for intervention in the elderly, it has recently been realised, that greatly exceed those seen in the young. A better understanding of the processes involved in the genesis and maintenance of raised blood pressure will lead to improvements in prevention and treatment that promise much in terms of a reduction both in cardiovascular morbidity and mortality in populations, and in the individual human tragedies that stem from myocardial infarction, cardiac failure and stroke.

Appendices

APPENDIX I

VALIDATION OF THE FINAPRES DEVICE AND ITS USE IN LABORATORY CARDIOVASCULAR TESTS

The requirement for invasive intra-arterial blood pressure monitoring during cardiovascular testing, which is not free from risk and discomfort, has been an obstacle to the widespread application of such methods in the clinical investigation of subjects, particularly where repeated assessments are required. The development of the non-invasive Finapres device represents an attempt to overcome some of these objections. The device (FINGER Arterial PRESSure) measures beat-to-beat blood pressure in the digital arteries by the volume-clamp method of Peñáz (1973). An inflatable cuff is placed around a finger, usually the middle or index finger or occasionally the thumb, and the pressure in the cuff is maintained by a fast-acting servo-circuit so that the transmural pressure in the digital arteries remains constant, as judged by an infra-red photoplethysmograph. Cuff pressure thus equals intra-arterial pressure, and the output from the Finapres manometer represents beat-to-beat blood pressure. The device contains an automatic calibration procedure that periodically readjusts the cuff pressure stepwise until the finger arteries are about to collapse, so as to maintain the set point about which the plethysmograph operates.

Several studies have compared non-invasive blood pressure measurement with the Finapres with that measured simultaneously in either the brachial or radial artery [Dorlas et al, 1985; Imholz et al, 1988, 1990, 1992; Parati et al, 1989; Christen et al, 1990]. These studies have examined the technique during a variety of cardio-

Appendices

vascular laboratory tests, including the Valsalva manoeuvre, standing, the cold pressor test, isometric exercise, peripheral vasoconstriction and phenylephrine, angiotensin or nitroglycerin injection, and shown that the technique accurately tracks changes recorded in intra-arterial blood pressure. In a comparative study of intra-arterial and Finapres registration of blood pressure during the Valsalva manoeuvre, Inholz et al (1988) found that group mean values agreed to within 1 mmHg, but this concealed considerable interindividual variability of between +6 mmHg to -7 mmHg during the abrupt changes in peripheral blood flow and blood pressure occurring during phase 4 of the manoeuvre. In the validation study conducted by Parati and colleagues (1989), baroreflex sensitivity indices derived from either Finapres or intra-brachial data to either a pressor (phenylephrine) or depressor (nitroglycerin) dynamic stimulus agreed very closely. Another study comparing Finapres-recorded blood pressure with pressure recorded at the aortic root during cardiac catheterisation found that the blood pressure change with phenylephrine bolus injection was less in the finger than that recorded centrally, resulting in a slight overestimation of baroreflex sensitivity based on Finapres blood pressure [Hartikainen et al, 1995]. However, these differences were reduced in subjects with lower baroreflex sensitivity, and declined with both age and systolic blood pressure level, resulting in less of an 'error' in older hypertensive subjects. Rongen et al (1995) found increased discrepancies between Finapres and intrabrachial pressures in older normotensive subjects during the Valsalva manoeuvre, whereas Virolainen (1992) found that the Finapres-intraarterial difference during Mueller's manoeuvre declined with increasing age and systolic blood pressure. Even Rongen et al, whose study perhaps represents the 'worst case' for the comparison of Finapres and intra-arterial

Appendices

recording, conclude that the advantages of non-invasive blood pressure measurement and ease of use of the Finapres outweigh the slight loss of precision seen in their study (which was itself much greater than that seen in any other comparative study) and that the device is a feasible substitute for intra-arterial measurement of blood pressure responses in the elderly.

APPENDIX II

PUBLICATIONS ARISING FROM THE WORK DESCRIBED IN THIS THESIS

James MA, Watt PAC, Potter JF, Thurston H, Swales JD

Pulse pressure and resistance artery structure in the elderly

Hypertension. 1995; 26: 301-306.

James MA, Watt PAC, Potter JF, Thurston H, Swales JD

Resistance artery endothelial function in elderly hypertensive and normotensive subjects

Journal of Hypertension. 1995; 13 (suppl 2): 2-3 (Abstract).

James MA, Robinson TG, Panerai RB, Potter JF

Arterial baroreflex sensitivity in the elderly

Age & Ageing. 1995; 24 (suppl II): P9 (Abstract).

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