



<https://theses.gla.ac.uk/>

Theses Digitisation:

<https://www.gla.ac.uk/myglasgow/research/enlighten/theses/digitisation/>

This is a digitised version of the original print thesis.

Copyright and moral rights for this work are retained by the author

A copy can be downloaded for personal non-commercial research or study,  
without prior permission or charge

This work cannot be reproduced or quoted extensively from without first  
obtaining permission in writing from the author

The content must not be changed in any way or sold commercially in any  
format or medium without the formal permission of the author

When referring to this work, full bibliographic details including the author,  
title, awarding institution and date of the thesis must be given

Enlighten: Theses

<https://theses.gla.ac.uk/>  
[research-enlighten@glasgow.ac.uk](mailto:research-enlighten@glasgow.ac.uk)

**ASPECTS OF DIABETIC AUTONOMIC NEUROPATHY**

by

**WILLIAM REID MB, ChB, MRCP (UK)**

**A thesis submitted for the degree of Doctor of Medicine in  
the University of Glasgow**

**Based on research conducted in the University Department  
of Medicine, Royal Infirmary of Edinburgh, Edinburgh, EH3  
9YW**

**Submitted June 1989**

**© William Reid, 1989**

ProQuest Number: 10970950

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10970950

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code  
Microform Edition © ProQuest LLC.

ProQuest LLC.  
789 East Eisenhower Parkway  
P.O. Box 1346  
Ann Arbor, MI 48106 – 1346

## TABLE OF CONTENTS

|   | Page No |
|---|---------|
| <b>TITLE PAGE</b>   | 1       |
| <b>ACKNOWLEDGMENTS</b>  | 10      |
| <b>DECLARATION</b>  | 12      |
| <b>PUBLICATIONS AND PRESENTATIONS<br/>CONTAINED IN THIS THESIS</b>          | 13      |
| <b>SUMMARY</b>  | 16      |
| <b>INTRODUCTION</b>   | 22      |
| <b>CHAPTER 1.1      Diabetic autonomic neuropathy</b>                       | 26      |
| Chapter 1.1.1      Historical aspects                                       | 26      |
| Chapter 1.1.2      Clinical features of diabetic<br>autonomic neuropathy    | 28      |
| Chapter 1.1.3      Natural history of diabetic<br>autonomic neuropathy      | 31      |
| Chapter 1.2      Neural control of the heart                                | 33      |
| Chapter 1.2.1      Anatomical considerations                                | 34      |
| Chapter 1.2.2      Physiological effects of afferent<br>inputs              | 35      |
| Chapter 1.2.3      Sympathetic/parasympathetic<br>interactions in the heart | 37      |
| Chapter 1.2.4      The denervated heart                                     | 39      |
| Chapter 1.2.5      Beta-adrenoreceptors and partial<br>agonist activity     | 43      |
| Chapter 1.3      Vasopressin (AVP = Arginine<br>Vasopressin)                | 46      |
| Chapter 1.3.1      Historical review  | 48      |

|                  |   |            |
|------------------|---|------------|
| Chapter 1.3.2    | Osmotic and non-osmotic release of vasopressin  | 49         |
| Chapter 1.3.3    | The roles of vasopressin  | 58         |
| Chapter 1.3.4    | Vasopressin in diabetes mellitus  | 62         |
| Chapter 1.3.5    | Vasopressin in disorders of autonomic function  | 65         |
| Chapter 1.4      | Aims of this thesis   | 76         |
| <b>CHAPTER 2</b> | <b>Methods and materials</b>  | <b>79</b>  |
| Chapter 2.1      | Subjects  | 79         |
| Chapter 2.2      | Cardiovascular autonomic reflex tests   | 79         |
| Chapter 2.3      | Heart rate, 24 hour heart rate, blood pressure, physiological tremor, tilt studies and infusion studies   | 84         |
| Chapter 2.4      | Biochemical variables   | 86         |
| Chapter 2.5      | Statistical methods   | 90         |
| Chapter 2.6      | Ethical aspects   | 92         |
| <b>CHAPTER 3</b> | <b>Effects of B-adrenoceptor blockade on heart rate and physiological tremor in diabetics with autonomic neuropathy: a comparative study of epanolol, atenolol and pindolol</b> | <b>93</b>  |
| Chapter 3.1      | Introduction  | 93         |
| Chapter 3.2      | Subjects  | 95         |
| Chapter 3.3      | Drugs used  | 95         |
| Chapter 3.4      | Procedures  | 96         |
| Chapter 3.5      | Analysis  | 96         |
| Chapter 3.6      | Results   | 97         |
| Chapter 3.7      | Discussion  | 98         |
| <b>CHAPTER 4</b> | <b>Vasopressin secretion following osmotic stimulation with hypertonic saline in diabetics with and without autonomic neuropathy</b>  | <b>104</b> |
| Chapter 4.1      | Subjects  | 104        |
| Chapter 4.2      | Protocol  | 105        |
| Chapter 4.3      | Results   | 105        |
| Chapter 4.4      | Discussion  | 107        |
| <b>CHAPTER 5</b> | <b>Vasopressin secretion in response to head-up tilt in diabetics with and without autonomic neuropathy</b>   | <b>109</b> |
| Chapter 5.1      | Subjects  | 109        |
| Chapter 5.2      | Protocol  | 110        |

|                  |  |            |
|------------------|--|------------|
| Chapter 5.3      | Results  | 110        |
| Chapter 5.4      | Discussion   | 111        |
| <b>CHAPTER 6</b> | <b>The pressor effect of arginine vasopressin in diabetic autonomic neuropathy</b> | <b>115</b> |
| Chapter 6.1      | Subjects   | 115        |
| Chapter 6.2      | Protocol   | 115        |
| Chapter 6.3      | Results  | 116        |
| Chapter 6.4      | Discussion   | 118        |
| <b>CHAPTER 7</b> | <b>General discussion and conclusions</b>  | <b>123</b> |
|                  | <b>REFERENCES</b>  | <b>133</b> |
|                  | <b>ABBREVIATIONS</b>   | <b>162</b> |

## LIST OF TABLES

### Chapter 2

|         |  |                 |
|---------|--|-----------------|
| Table I | Normal, borderline and abnormal values for cardiovascular autonomic function tests | follows page 92 |
|---------|--|-----------------|

### Chapter 3

|          |  |                  |
|----------|--|------------------|
| Table II | Age, duration of diabetes and cardiovascular reflex tests in eight diabetics studied | follows page 103 |
|----------|--|------------------|

|           |   |                  |
|-----------|---|------------------|
| Table III | Group mean resting supine heart rates at baseline, two and four hours after taking each of the drugs or placebo | follows table II |
|-----------|---|------------------|

|          |  |                   |
|----------|--|-------------------|
| Table IV | Group mean heart rates during the 24 hour ECG recordings after taking each of the drugs or placebo | follows table III |
|----------|--|-------------------|

|         |  |                  |
|---------|--|------------------|
| Table V | Group mean tremor measurements at baseline, two and four hours after taking each of the drugs or placebo | follows table IV |
|---------|--|------------------|

|          |  |                 |
|----------|--|-----------------|
| Table VI | Group mean plasma glucose values at baseline, two and four hours after taking each of the drugs or placebo | follows table V |
|----------|--|-----------------|

### Chapter 4

|           |  |                  |
|-----------|--|------------------|
| Table VII | Age, duration of diabetes, details of complications and cardiovascular autonomic reflex tests in the diabetics undergoing 5% saline infusion | follows page 108 |
|-----------|--|------------------|

Table VIIIA,b Group mean biochemical and osmolality responses during 120 minutes of 5% saline infusion in diabetics with, diabetics without autonomic neuropathy, and normal subjects follows table VII

Table IX Linear regression relationships between plasma osmolality, sodium, and AVP during 5% saline infusion in diabetics with and without autonomic neuropathy and normal subjects follows table VIIIB

#### Chapter 5

Table X Age, duration of diabetes, details of complications and cardiovascular reflex tests in the diabetics undergoing 45° head-up tilt follows page 114

Table XIa,b Group mean biochemical and osmolality responses during 120 minutes of head-up tilt to 45° in diabetics with, diabetics without, and normal subjects follows table X

Table XIIa,b Group mean biochemical and osmolality responses during 120 minutes of head-up tilt to 45° in diabetics with, diabetics without, and normal subjects follows table XIb

#### Chapter 6

Table XIII Age, duration of diabetes, details of complications and cardiovascular reflex tests in the diabetics undergoing AVP infusion follows page 122

## LIST OF FIGURES

### Introduction

Figure 1 Dynamic spheres of influence follows page 25

### Chapter 3

Figure 2 Group mean hourly heart rate during ambulatory monitoring over the 24 h after dosing with placebo, epanolol, atenolol and pindolol follows table VI

Figure 3 Group mean log tremor measurements at each frequency (range 0-30 Hz) at baseline before dosing with placebo, epanolol, atenolol and pindolol follows figure 2

Figure 4 Group mean log tremor measurements at each frequency (range 0-30 Hz) after dosing with placebo, atenolol and pindolol follows figure 3

Figure 5 Group mean ( $\pm$  SEM) change from baseline in plasma glucose 2 and 4 h after dosing with placebo, epanolol, atenolol and pindolol follows figure 4

### Chapter 4

Figure 6 Mean blood pressure and heart rate during 120 minutes of 5% saline infusion in six diabetics with, five diabetics without autonomic neuropathy, and five normal subjects follows table IX

Figure 7 Mean biochemical and osmolality responses during 120 minutes of 5% saline infusion in six diabetics with, five diabetics without autonomic neuropathy, and five normal subjects follows figure 6

## Chapter 5

- Figure 8 Mean blood pressure and heart rate during 120 minutes of head-up tilt to 45° in seven diabetics with, six diabetics without autonomic neuropathy, and six normal subjects follows table XIIb
- Figure 9 Change in systolic blood pressure during 120 minutes of head-up tilt to 45° in seven diabetics with, six diabetics without autonomic neuropathy, and six normal subjects follows figure 8
- Figure 10 Mean biochemical and osmolality responses during 120 minutes of head-up tilt to 45° in seven diabetics with, six diabetics without, and six normal subjects follows figure 9

## Chapter 6

- Figure 11 Group mean ( $\pm$  SEM) plasma AVP concentrations during intravenous infusion of incremental doses of AVP in eight diabetics with, eight diabetics without autonomic neuropathy, and six normal subjects follows table XIII
- Figure 12 Group mean ( $\pm$  SEM) blood pressure and heart rate responses during intravenous infusion of incremental doses of AVP in eight diabetics with, eight diabetics without autonomic neuropathy, and six normal subjects follows figure 11
- Figure 13 Group mean ( $\pm$  SEM) systolic blood pressure, diastolic blood pressure and heart rate during intravenous infusion of incremental doses of AVP in eight diabetics with, eight diabetics without autonomic neuropathy, and six normal subjects follows figure 12

Figure 14 Individual mean blood pressure responses, measured as 'area under the curve' during intravenous infusion of incremental doses of AVP, in eight diabetics with (DAN +ve), eight diabetics without (DAN -ve), and six normal volunteers follows figure 13

## ACKNOWLEDGMENTS

I wish to acknowledge the help of all of the people who contributed to the research incorporated in this thesis. My co-authors in the papers and presentations taken from this research are most gratefully thanked.

My most sincere thanks are reserved for Dr David J Ewing, Wellcome Trust Senior Lecturer in the University Department of Medicine, Royal Infirmary of Edinburgh, without whose support, advice and friendship none of this work would have been possible. Thanks also go to Dr Basil F Clarke, Diabetic and Dietetic Department, Royal Infirmary of Edinburgh, who allowed me to study patients under his care. Professor Stafford L Lightman, Westminster Hospital, London, provided great stimulus and enthusiasm to the studies, and I thank him for this. Dr Chris J Thompson, Lecturer in Medicine, Royal Infirmary of Edinburgh, provided much appreciated advice during the analysis of the data in the vasopressin studies. Dr Robin Prescott, Department of Medical Statistics, University of Edinburgh, gave invaluable advice in the analyses of all the data contained in this thesis.

The technical assistance of Ms Carol O'Neill and Ms Kate Henderson is also much appreciated. Particular thanks go to Mrs Elizabeth Roberts, who performed many of the

biochemical analyses in the studies, and whose far-sightedness averted disaster on one occasion. Thanks also go to the nurses in the Department of Medicine for their help in the studies.

Mrs Jean Johnstone painstakingly, accurately and efficiently typed the text contained in this dissertation. My thanks go to her for her skill and diligence in this task.

Finally, my wife, Rhonda, and my son, Harrison, saw little of me during the preparation of this thesis. It is to Rhonda, whose encouragement and support provided much-needed inspiration, that this thesis is dedicated.

## DECLARATION

The work contained in this thesis has, by necessity, involved collaboration with others, particularly in the biochemical assays, which were performed by co-workers participating in the research. I was, however, solely responsible for the majority of the data collection, and solely responsible for the statistical analyses and text contained in this thesis, with the following exceptions: the statistical analyses in chapter 3 were performed by Mr S Cook at ICI (Pharmaceuticals Division), Alderley Park, Macclesfield, Cheshire, although the methods used and the results obtained were checked by me. The six normal volunteers studied in chapter 6 underwent AVP infusion at the Westminster Hospital in London, under the supervision of Professor S L Lightman. The data obtained from these subjects were used purely for comparison with my group of diabetics in Edinburgh.

I have consulted each reference listed in the bibliography personally, except where indicated in the text.

## **PUBLICATIONS AND PRESENTATIONS CONTAINED IN THIS THESIS**

The studies presented in this thesis have formed the basis for presentations to learned societies, published abstracts and substantive publications in medical journals. I hereby enclose a list of such submissions.

### **Published papers**

Reid, W., Ewing, D.J., Harry, J.D., Smith, H.J., Neilson, J.M.M. & Clarke, B.F. (1987) Effects of beta-adrenoceptor blockade on heart rate and physiological tremor in diabetics with autonomic neuropathy. A comparative study of epanolol, atenolol and pindolol. *British Journal of Clinical Pharmacology* 23, 383-389.

Reid, W., Ewing, D.J., Lightman, S.L., Eadington, D., Williams, T.D.M., Roulston, J.E. & Clarke, B.F. (in press) Vasopressin secretion in diabetics with and without autonomic neuropathy: responses to osmotic and postural stimulation. *Clinical Science*.

### **Submitted for publication**

Reid, W., Ewing, D.J., Lightman, S.L., Williams, T.D.M., Eadington, D. & Clarke, B.F. The pressor effect of arginine vasopressin in diabetic autonomic neuropathy.

### **Published Abstracts**

Reid, W., Ewing, D.J. & Clarke, B.F. (1985) Heart rate responses to beta-blockade in diabetic autonomic neuropathy. *Journal of the Autonomic Nervous System* **14**, 102.

Reid, W., Ewing, D.J., Harry, J.D., Smith, H.J., Neilson, J.M.M. & Clarke, B.F. (1986) Heart rate and physiological tremor after B-adrenoreceptor blockade in diabetics with autonomic neuropathy. *British Journal of Clinical Pharmacology* **21**, 581P.

### **Presentations to Learned Societies**

To Clinical Autonomic Research Society, King's College Hospital, London, November, 1984. "Heart rate responses to beta-blockade in diabetic autonomic neuropathy".

To Clinical Section, British Pharmacological Society, London, December, 1985. "Heart rate and physiological tremor after B-adrenoreceptor blockade in diabetics with autonomic neuropathy".

To Clinical Autonomic Research Society, Royal Free  
Hospital, London, September, 1986. "Endogenous  
vasopressin response to head-up tilt in diabetics with  
and without autonomic neuropathy".

## SUMMARY

Diabetic autonomic neuropathy is the most commonly-occurring form of autonomic nervous system dysfunction found in clinical practice. Despite the difficulties encountered in studying single control mechanisms in the presence of this disease (**Introduction**), it may be possible to elucidate information on physiological control of some dynamic aspects of cardiovascular and neuroendocrine influences by looking at a group of severely affected diabetics and their responses.

The aims of this thesis (**Chapter 1.4**) were to examine certain aspects of Beta-adrenoreceptor function and physiology of arginine vasopressin and its effects in a group of diabetics with cardiovascular reflex test evidence of autonomic neuropathy. By comparing and contrasting these results with the existing literature in both diabetes and other forms of autonomic dysfunction, conclusions about denervation in diabetes might be reached.

Chapter 1 provides the background to the studies presented in chapters 3 - 6. An historical background to the discovery of, and the importance of autonomic neuropathy in diabetes is considered, along with sections addressing the clinical features and natural history of the disease

(Chapter 1.1.1 - 1.1.3). There follows a section on neural control of the heart, with special reference to the anatomy, physiological effects of afferent inputs and sympathetic/parasympathetic interactions within the heart. The physiological control of the heart in denervation is also considered (Chapter 1.2.1 - 1.2.4). In order to illustrate the study presented in chapter 3, I provide a background to beta-adrenoreceptors, beta-adrenoreceptor blocking agents and the property of partial agonist activity (Chapter 1.2.5).

A brief historical review (Chapter 1.3.1) on the discovery and subsequent elucidation of the roles of arginine vasopressin, is followed by a detailed review of the anatomy and physiology of its release in man, together with a discussion of the physiological roles and difficulties in investigation of these roles (Chapter 1.3.2 - 1.3.3.).

I then review the current literature on arginine vasopressin in diabetes mellitus (in the absence of autonomic neuropathy) with reference to both osmotic and non-osmotic release of the peptide (Chapter 1.3.4). In order to provide a background to the studies in chapters 4 - 6, the control of vasopressin release and the effects of exogenous vasopressin in the presence of disordered autonomic control of the circulation is considered, with

special reference to relevant animal studies, the human literature on subjects with pharmacological denervation, progressive autonomic failure, tetraplegics, cardiac transplant patients, and finally, diabetics with cardiovascular reflex test evidence of autonomic neuropathy (Chapter 1.3.5).

Chapter 2 details the methods and materials used in the studies presented in this thesis. Because different subjects were used in each study, their details are given in the individual results chapters.

In chapter 3, observations are presented from a randomised double-blind controlled study on eight diabetics with autonomic neuropathy in which cardioselective and non-cardioselective beta-adrenoceptor blocking agents both with and without partial agonist activity (PAA) (atenolol, epanolol and pindolol) were given in order to determine changes in heart rate (a Beta<sub>1</sub> effect) and changes in physiological tremor (a Beta<sub>2</sub> effect).

Relative changes in heart rate were observed which reflect the amount of partial agonism in these drugs. Pindolol, the drug with most partial agonism, raised heart rate compared with placebo at night time. Epanolol, with moderate PAA, had little effect on heart rate at night and atenolol, with no PAA, lowered heart rate compared with

placebo throughout the 24 hour period.

Changes in physiological tremor were seen which reflected the cardioselectivity of the drugs used, in that that four hours post dose, pindolol, the non-selective drug with PAA, increased tremor compared with placebo, epanolol and atenolol, none of which affected tremor values.

A significant rise in plasma glucose was seen after pindolol only, which may demonstrate a heightened sensitivity of Beta<sub>2</sub>-adrenoceptors in diabetics with autonomic neuropathy.

These results are compared and contrasted with those previously demonstrated in other forms of denervation, and suggest that denervation in autonomic neuropathy is not as complete as in either animal models or progressive autonomic failure.

In **chapter 4**, data from a study is presented in which 5 normal controls, 5 diabetics without (DAN -ve) and 6 with (DAN +ve) cardiovascular reflex test evidence of autonomic neuropathy were studied by 5% saline infusion, to test whether osmotic stimulation of AVP is disordered in diabetics with autonomic neuropathy. 5% saline infusion produced appropriate rises in plasma osmolality and AVP levels which did not differ between the three groups,

confirming that osmotic release of AVP is normal in diabetic autonomic neuropathy. The blood pressure responses to this stimulus were similar, although one DAN +ve subject displayed a marked pressor response. The reasons for this are discussed with reference to other studies on PAF and tetraplegic subjects.

**Chapter 5** looks at the cardiovascular and AVP responses to 45° head-up tilt over 120 minutes in three groups; 6 normal, 6 DAN -ve and 7 DAN +ve subjects. No differences in AVP responses were observed between the three groups, despite a major hypotensive stimulus in the group with autonomic neuropathy. This confirms that an afferent defect exists in the neural pathways which control AVP release in diabetic autonomic neuropathy. Such a pattern is also seen in patients with PAF, but contrasts with the response in subjects with selective efferent sympathetic loss secondary to spinal cord transection.

Plasma renin release to the same stimulus was found to be normal in DAN +ve subjects, in contrast to earlier work. It seems likely that deterioration in renal function causes low renin responses in diabetic autonomic neuropathy.

**Chapter 6** studies the cardiovascular responses to an incremental infusion of AVP in physiological doses in 6

normal, 8 DAN -ve and 8 DAN +ve subjects. No pressor responses were seen in the normal subjects, but marked increases in blood pressure were observed in most of the DAN +ve group and in two of the DAN -ve group. The responses tended to wane at the maximum AVP infusion rate.

As heart rate fell slightly in all three groups, the pressor response to AVP is unlikely to be secondary to the loss of baroreflex activation. More probably it represents an increased sensitivity of the peripheral vasculature to vasopressin associated with autonomic nerve damage.

**Chapter 7** presents a general discussion of these results in relation to other work on autonomic failure, both in diabetics and in other forms of denervation. The findings indicate that in these diabetics the concept of complete cardiac denervation is an untenable idea, and that the importance of neuropeptidergic and hormonal influences in maintaining homeostasis is not yet fully elucidated.

## INTRODUCTION

The concept of the autonomic nervous system was first mooted in 1898 by Langley, to encompass the unconscious function of the "sympathetic" nervous system, the cranial and sacral nerves and the local gut innervation. Since then the apparent simplicity of this system has been confounded by subdivision into parasympathetic, adrenergic and cholinergic systems (Webber, 1978), the inclusion of endocrine influences and more latterly, the recognition that neuropeptide transmitters and opioids may have profound importance in its function (Reid & Rubin, 1987).

The integrity of the autonomic nervous system is essential to the maintenance of normal cardiovascular regulation in man. For instance, the ability to stand up is largely taken for granted, but underlying this simple manoeuvre is a complex interplay between several cardiovascular reflexes, which compensates for pooling of blood in the extremities by peripheral vasoconstriction and tachycardia to maintain the constant perfusion of blood to vital organs. Disruption of normal autonomic function may involve several of these sophisticated control mechanisms, leading to large postural changes in blood pressure and consequent cerebral hypoperfusion, hypoxia and syncope.

Examination of the effects of autonomic dysfunction on

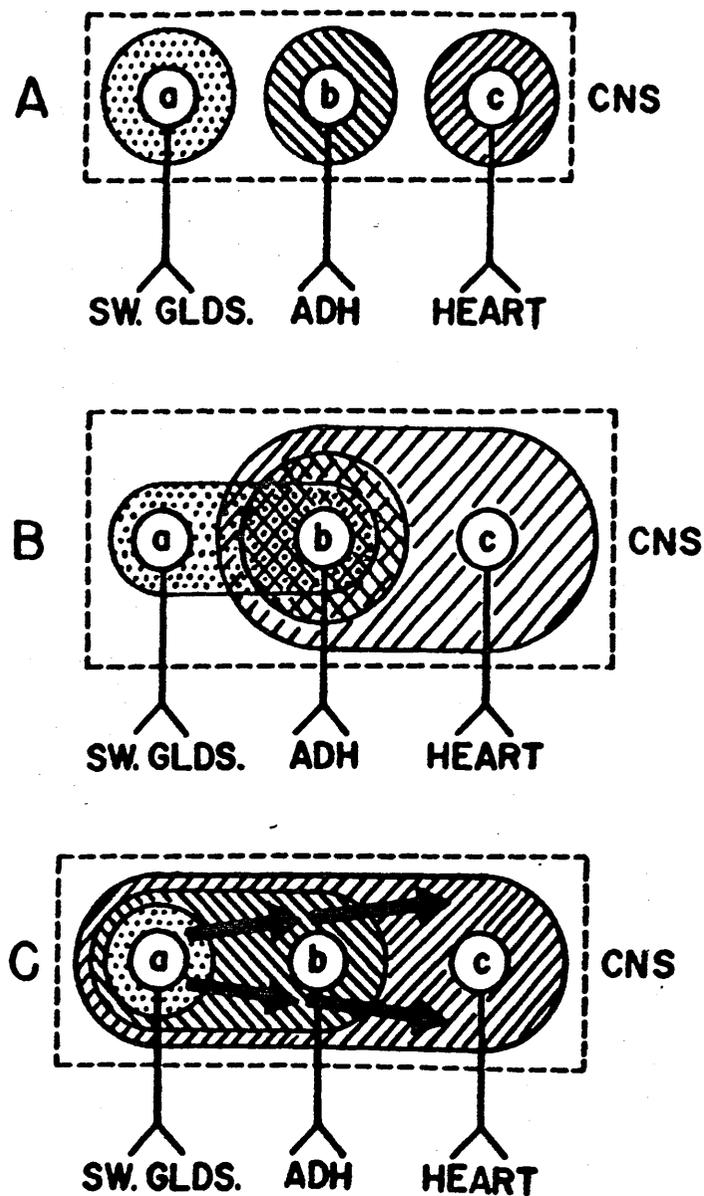
single control systems in the body can be fraught with difficulty in interpretation. It is important to bear in mind that no function of the autonomic nervous system operates in total isolation from another, that a particular function may alter according to the stimulus effected on it, and that its purpose may change secondary to a pathological process. In an elegant model of central nervous system regulation of cardiovascular function, such a concept was expressed as "dynamic spheres of influence" (Wurster, 1984) in which as an example, the separate neuronal circuits controlling sweat glands, vasopressin secretion and control of the heart were considered. What is perceived as a thermoregulatory control function to a temperature regulation physiologist may be considered as a water and electrolyte control mechanism to a renal physiologist, in other words each "separate" function overlaps with others and is dependent on the integrity of others to maintain homeostasis (figure 1).

The overview this concept provides is useful to bear in mind in this thesis. Although separate autonomic control systems will be considered in isolation, they are only isolated from each other in the context of the individual experiments, and that when autonomic control is disordered, the function and importance of the individual systems may take on a different emphasis.

It is recognised that autonomic nervous system damage may occur in a variety of systemic disorders such as tabes dorsalis, polyneuropathies, amyloidosis, porphyria, chronic renal failure and chronic alcoholism (Johnson & Spalding, 1974; Bannister, 1983). Primary disorders may also occur, such as familial dysautonomia or the Riley-Day syndrome (Riley et al, 1949; Brunt & McKusick, 1970), and idiopathic orthostatic hypotension or the Shy-Drager syndrome (Bradbury & Eggleston, 1925; Bannister, 1983; Shy & Drager, 1960). The most commonly occurring form of autonomic neuropathy encountered in medical practice, however, is that which occurs in diabetes mellitus. Evidence for autonomic dysfunction is found in between 17-40% of all diabetics (Ewing, 1984). Abnormal autonomic function combined with symptoms carries a significant morbidity and mortality (Ewing & Clarke, 1986a).

It is against the background of a commonly occurring complication of a common disease that this thesis is set. By studying separate aspects of autonomic, neuroendocrine and hormonal control in diabetic autonomic neuropathy, I hope to demonstrate that the influence each factor is thought to exert in intact man changes in the presence of the pathological state; that it is the disruption of the autonomic nervous system, rather than the diabetic state, which is responsible for the change; and that by studying

diabetics with autonomic neuropathy, paradoxically we may further the understanding of normal autonomic control mechanisms in man.



Dynamic spheres of influence

(A) **Spheres of influence.** Areas of the central nervous system involved in control of particular systems, e.g. sweat glands, antidiuretic hormone release (ADH), and the heart, as schematically represented by areas containing neurones, a, b, and c, respectively.

(B) **Overlapping spheres of influence.** Control of different functions may involve neuronal circuits may overlap sweat gland and antidiuretic hormone control areas of spheres of influence.

(C) **Dynamic spheres.** The effective overlap of control systems may vary from one time to another and from one physiological state to another depending on the intensity of the regulatory drive or stimulus. The CNS is represented by the structures within the dashed boxes.

(Adapted from Wurster, 1984 with permission)

Figure 1

## CHAPTER 1

### CHAPTER 1.1      DIABETIC AUTONOMIC NEUROPATHY

#### 1.1.1      Historical Aspects

Descriptions of clinical features which would now be recognised as being typical of diabetic autonomic neuropathy have appeared in the literature from the mid nineteenth century onwards. De Calvi, in 1864, is generally credited (in: Rundles, 1945) with the recognition that neurological features (sciatic pain, peripheral anaesthesia and neurogenic bladder disturbance) may be a consequence, rather than the causation, of diabetes mellitus, as was previously thought.

Further case histories followed; Pavy (1885) described painful neuropathic symptoms with abnormalities of sweating, Buzzard (1890) and Pryce (1893) described vasomotor foot problems, and Pitres (1902) outlined absence of pupillary reflexes and loss of testicular sensation in diabetics.

At this time, the neurological features associated with diabetes were most closely related to those occurring in chronic alcoholism and tertiary syphilis - thus "diabetic pseudotabes" was frequently referred to. The diagnosis

of "so-called" diabetic neuropathy was often in doubt because of the reluctance of clinicians to attribute the neuropathic changes to diabetes, rather than "senile" changes or arteriosclerotic disease which was commonly co-existent. Far from seeing these complications disappear after the introduction of insulin in 1922, more and more sufferers presented with diverse complaints such as gastric fullness, postural hypotension, nocturnal or episodic diarrhoea, and sweating abnormalities. It was not until the appearance of two major reviews, by Jordan in 1936 and in particular, Rundles in 1945 that the clinical features of autonomic dysfunction in diabetes mellitus were fully recognised. Further descriptive clinical reviews followed, such as those of Keen in 1959 and Jadzinsky, Faerman & Fox in 1973, but in 1960 Sharpey-Schafer and Taylor introduced the concept of applying simple cardiovascular reflex testing to demonstrate abnormalities in diabetes, using the Valsalva manoeuvre.

The use of, and subsequent expansion of these tests in 1973 by Wheeler & Watkins in London and Ewing and others in Edinburgh has allowed the systematic study of large numbers of diabetics, and a classification of progressive damage to the autonomic nervous system has evolved. Inherent in the interpretation of these tests is the maxim that when cardiovascular reflexes are abnormal,

other systems served by the autonomic nervous system are affected by neuropathic damage. This has been well validated in clinical studies (1.1.3).

With the development of these tests, over the last fifteen years there has been an explosion in interest in diabetic autonomic neuropathy. Autonomic neuropathy is now recognised as a relatively common complication of diabetes. The shift in emphasis from a symptom-based classification of diabetic autonomic neuropathy, which will only pick up late (and by implication, severe) autonomic damage, to a definition based on objective and measurable criteria has been crucial to the in-depth study of the natural history and pathophysiology of this disorder.

#### **1.1.2 Clinical features of diabetic autonomic neuropathy**

In keeping with other systemic disorders which cause neuropathies, autonomic neuropathy secondary to diabetes may cause many clinical syndromes by its widespread nature. In order to put its clinical effects into perspective, I will briefly outline the major clinical features found in this condition.

##### **Cardiovascular**

The most obvious feature of failure of cardiovascular autoregulation is of postural hypotension, first clearly

defined in diabetic autonomic neuropathy by Rundles (1945). Symptoms include lightheadedness, postural weakness, visual impairment or syncope, and these features may be worsened by insulin (Page & Watkins, 1976) owing to its effect in slightly reducing plasma volume (Gundersen & Christensen, 1977).

A resting tachycardia of between 90-100 beats per minute may be observed (Rundles, 1945; Keen, 1959; Wheeler & Watkins, 1973), but is probably not a specific marker for autonomic neuropathy, as diabetics as a group have faster heart rates than controls (Ewing, Neilson & Travis, 1984), whatever their "stage" of autonomic damage.

Painless or "silent" myocardial infarction is more prevalent in diabetics (Bradley & Schonfield, 1962) and this has been attributed to autonomic neuropathy, despite the fact that patients with known autonomic neuropathy may have typical pain of myocardial infarction (Campbell, Ewing & Clarke, 1978). Sudden death is also more common (Page & Watkins, 1978) but the mechanism of this is still unknown.

### **Gastrointestinal**

Delayed gastric emptying (Rundles, 1945), or gastroparesis diabeticorum (Kassander, 1958) is usually asymptomatic, but may lead to loss of diabetic control with

hypoglycaemia (Campbell & Conway, 1960), weight loss (Wooten & Meriweather, 1961) or episodes of nausea and vomiting (Howland & Drinkard, 1963).

"Diabetic diarrhoea" is a relapsing and remitting condition, characterised by episodes of frequent watery stools, often nocturnal, with faecal incontinence (Bargen, Bollman & Kepler, 1936).

Colonic atony has also been described (Rundles, 1945), but may be secondary to other factors such as dehydration or immobility.

#### **Genitourinary**

De Calvi (1864), Jordan (1936) and Rundles (1945) all described bladder abnormalities. Nocturia out of proportion to glycosuria was described by Rundles (1945) and "rediscovered" recently (Bell et al, 1987).

Impotence is often quoted as a feature of diabetic autonomic neuropathy, but may occur in diabetics for other reasons (Ewing, Campbell & Clarke, 1980).

#### **Vasomotor/sudomotor**

Both anhidrosis and vasomotor changes have been reviewed in diabetic autonomic neuropathy (Rundles, 1945; Martin, 1953) aside from the original descriptions of Pavy and

others (see 1.1.1). Compensatory upper body hyperhydrosis may occur (Goodman, 1966), and gustatory sweating may also be troublesome (Watkins, 1973).

### **Pupillary changes**

Pupillary abnormalities, such as small irregular pupils, absent light reflex or diminished hippus, have long been described (Pitres, 1902; Jordan, 1936; Rundles, 1945; Smith et al, 1978) and more recently have formed the basis for a simple test of autonomic function (Martyn & Ewing, 1986).

Autonomic neuropathy may also be a cause of unawareness of hypoglycaemia in insulin dependent diabetics (Ewing & Clarke, 1986a).

Even in such a brief review of clinical features, it is apparent that autonomic nerve damage in diabetes can cause significant problems. For a more complete picture the reader is referred to Rundles (1945), Clarke, Ewing & Campbell (1979), Ewing & Clarke (1986a) and Ewing & Clarke (1986b).

### **1.1.3 Natural history of diabetic autonomic neuropathy**

Abnormalities of cardiovascular autonomic function tests occur in between 17-40% of consecutive or randomly selected diabetic adults (Ewing & Clarke, 1982; Ewing,

1984). Thirty one per cent of a teenage cohort (Young, Ewing & Clarke, 1983) and 15% of younger diabetic children (Mitchell, Wealthall & Elliot, 1983) also had abnormal cardiovascular reflexes, though no symptoms of autonomic neuropathy were observed in these groups. Symptomatic autonomic neuropathy is rare in young diabetics, although it has been reported (Lloyd-Mostyn & Watkins, 1976; Blum et al, 1980). Predictably, symptoms of diabetic autonomic neuropathy increase in prevalence with longer durations of diabetes (Canal et al, 1978; Masaoka et al, 1985).

Cardiovascular reflex autonomic function tests have been found to show a gradation of damage in autonomic neuropathy (Bennett, 1983). The heart rate tests, which are thought predominantly to reflect vagal damage, are more commonly abnormal than blood pressure tests (thought to reflect predominantly sympathetic abnormalities) when groups of diabetics are studied. In interpreting the results of these tests, their sensitivities have to be borne in mind. The heart rate tests are much more sensitive than blood pressure tests and are more likely to display abnormalities. Despite this, cardiac vagal nerves appear to be affected before sympathetic nerves (Bennett, 1983), possibly because they are longer and more susceptible to damage (Ewing, Campbell & Clarke, 1981).

The tests also show deterioration with time; in a large group of diabetics whose tests were repeated three months or more apart, 71% had unchanged tests and 26% had deteriorated (Ewing et al, 1985). This phenomenon has been repeatedly observed.

Abnormal cardiovascular reflexes and symptoms of autonomic damage have been found to correlate well (Ewing et al, 1980; Mackay et al, 1980). In these studies, symptoms of autonomic neuropathy (other than impotence) were strongly related to cardiovascular reflex test abnormalities. When taken together, the presence of abnormal tests and symptoms has great prognostic significance. A mortality rate of 50% in 2.5 years was found in one study (Ewing et al, 1980) and patients with most test abnormalities were found to have the highest fatality rate (Clarke & Ewing, 1982). Higher mortality rates in diabetic autonomic neuropathy have been confirmed in other studies, most notably Watkins & Mackay (1980).

## 1.2 Neural control of the heart

This section of my dissertation will concern itself with some of the background aspects of neural control of the heart which are essential to the studies presented later in the thesis. Cardiovascular control mechanisms are, of course, very complex; the relationship between neurally mediated changes in heart rate, stroke volume, blood

pressure and respiration cannot in reality be separated from changes in neuroendocrine function either within the central nervous system (for example, catecholamines, serotonin, GABA and various neuropeptides) or in the periphery (where adrenal hormones, renin or thyroid hormone may, for example, exert powerful cardiovascular effects). The "spheres of influence" (see introduction) they exert upon each other are irrevocably linked. In order, however, to review those parts of cardiovascular control relevant to this thesis, I will consider such systems in isolation.

#### 1.2.1 Anatomical considerations

Sympathetic efferent fibres originate in the upper thoracic segment of the spinal cord. The cell bodies of these preganglionic neurones are largely concentrated in the intermediolateral cell column, and leave the spinal cord via the ventral roots. They then run for a short distance with the spinal nerves before separating to form the white rami communicantes. After running to the sympathetic ganglia, unmyelinated fibres then leave, mainly along the adventitia of the great vessels and the atrial walls to reach most parts of the heart. Noradrenaline released from the sympathetic nerves in addition to catecholamines diffusing from the bloodstream interact with adrenoreceptors on heart cells which leads to alterations in their physiological performance.

Parasympathetic fibres arise in the medulla from the vagal nuclei and make up a part of the vagus nerve. Fibres from the left and right vagus nerves enter the heart at atrial level, synapse in cardiac cholinergic ganglia and exert their effects through release of acetylcholine (ACh) from postganglionic fibres primarily in the atria, but also in the ventricles.

The major inputs to the brain which facilitate cardiovascular regulation include afferent fibres from arterial baroreceptors and chemoreceptors, and from cardiopulmonary receptors and lung stretch receptors, which run via the IX and Xth cranial nerves to the medulla, terminating in the region of the nucleus of the tractus solitarius (NTS), the primary relay for cardiovascular afferents in the brainstem. Spinal somatic and visceral afferent nerves, including sympathetic afferents from the heart and vessels are involved, in addition to receptors in joints and skeletal muscles. The sense organs, thermoreceptors and receptors innervated by the trigeminal nerve also function in cardiovascular control.

#### **1.2.2 Physiological effects of afferent inputs**

The walls of certain blood vessels (mainly the aortic arch and carotid bifurcation) contain receptors, termed

baroreceptors, which are activated by mechanical deformation secondary to stretching of the vessel wall. The nerve endings lie mainly in the adventitia of the arterial wall and form a dense arborization.

Stimulation of the arterial baroreceptors results in a bradycardia and hypotension, this being mediated by enhanced vagal discharge together with decreased sympathetic output (Kircheim, 1976).

The main effects following stimulation of arterial chemoreceptors (mainly situated in the aortic and carotid bodies) include a rise in parasympathetic and a fall in sympathetic activity to the heart, producing bradycardia, while vasoconstriction in the major vascular beds and increased secretion of adrenal catecholamines are brought about by an increased efferent sympathetic discharge, increasing total peripheral resistance to such a degree that arterial pressure increases. These effects are only seen when respiration is kept constant by artificial means, for chemoreceptor activation produces a dramatic increase in both rate and depth of respiration such that vasodilatation occurs, producing a tachycardia and increase in cardiac output. Similar effects are seen after central chemoreceptor stimulation, secondary to brainstem pH changes. Chemoreceptor mechanisms are thus "emergency" regulatory systems, unlike baroreceptor

functions, which have an on-going regulatory role.

Cardiac receptors have been found in the coronary vessels and throughout the heart, and mediate many different reflexes through both sympathetic and vagal pathways (Brown, 1979).

Lung stretch receptors are activated primarily by pulmonary inflation, and their cardiovascular effects include reflex vasodilatation in the skin, muscle and splanchnic beds due to a decrease in sympathetic efferent activity.

Spinal inputs from various receptor sources, such as from muscles and joints, skin pain receptors and various receptors in the vasculature may elicit some cardiovascular effects. Isometric muscle contraction, for example, leads to a dramatic increase in blood pressure and heart rate due to an augmented sympathetic outflow mediated by this means.

### **1.2.3 Sympathetic/parasympathetic interactions in the heart**

In general terms, neural control of cardiac function can be divided into sympathetic and parasympathetic, or alternatively, adrenergic and cholinergic effects. Both atrial and ventricular contractility are greatly enhanced

by sympathetic stimulation, resulting in increased stroke work at a given filling pressure, and greater systolic emptying (increased ejection fraction) which decreases end-systolic volume. Increased parasympathetic tone decreases atrial contractility, but has only a small negative inotropic effect on the ventricles (Higgins, Vatner & Braunwald, 1973).

Independent of innervation, the heart increases its output as venous return is increased (Frank-Starling effect). An increase in heart rate also augments the contractile force of the heart (Bowditch effect). An increase in aortic pressure (increased afterload) will also cause an increase in contractile force (Anrep effect).

Control of heart rate may be viewed in simplistic terms as having a quick-onset, vagus mediated "brake" and a sympathetic "accelerator", acting over a longer period of time. The reality is much more complex: there is a constant and varying balance between cardiac vagal and sympathetic influences that determines the heart rate at any given time or circumstance. It is clear that this process is not a simple algebraic summation of adrenergic and cholinergic effects; the vagus exerts a disproportionate inhibition of the cardiostimulatory effects of sympathetic stimulation ("accentuated antagonism"), probably by both reducing the amount of

noradrenaline released by the sympathetic nerve ends, and by a direct cholinergic attenuation of the magnitude of the intracellular response in the heart (Levy, 1971; Higgins, Vatner & Braunwald, 1973; Levy & Martin, 1984).

Conversely vagal stimuli may produce a paradoxical sympathetic response and vice-versa. This phenomenon is known as "reciprocal excitation", and may occur secondary to (a) the presence of sympathetic fibres in a predominantly parasympathetic nerve trunk, (b) cholinergic stimulation of cardiac activity evoked by noradrenergic mechanisms, and (c) cholinergic stimulation of the heart mediated by adrenergic mechanisms (Levy & Martin, 1984). Despite the complexity of these neural interactions, the working model of vagal "brake" and sympathetic "accelerator" is still a useful concept, particularly when denervation is considered (see 1.2.4).

#### **1.2.4 The denervated heart**

The heart may be deprived of neural influence in three ways; by surgical section of sympathetic and parasympathetic nerves leading to the heart, by pharmacological depletion or blockade of transmitter substances and receptors, or by the pathological processes which lead to autonomic nerve damage. Even after "denervation" is accomplished by these means, complete denervation may not occur, as neuropeptidergic or

endocrine influences may maintain other, intrinsic cardiac reflexes. In broad terms these different processes lead to a common end-effect, and may be regarded as analogous to one another, but differences exist which will be considered in view of the study presented in chapter 3.

Surgical denervation in man may be most completely achieved by removal and reimplantation of the heart, as in cardiac transplantation. In excising the heart both afferent and efferent nerves are severed. Efferent fibres of the vagus, which are preganglionic, are sectioned as they enter the atria, abolishing cholinergic effects on the heart, but leaving cardiac cholinergic neurones and postganglionic fibres intact. Efferent sympathetic nerves are postganglionic, and dividing them inactivates cardiac adrenergic neurones, leading to depletion of noradrenaline. The adrenoreceptors remain intact, but may develop supersensitivity to exogenous catecholamines, probably by changes in neuronal uptake (Kaye, 1984).

The effect of surgical denervation on heart rate in man is to produce a moderate tachycardia which does not respond to stimuli that would normally reflex the influenced heart rate (Shaver et al, 1969). Beat-to-beat variation, or sinus arrhythmia, is under vagal control, and is also lost following transplantation. After transplantation, the

atrial remnant of the recipient is left intact, but is separated from the donor heart by a suture line. In this circumstance there are two independent atrial rates, the recipient atrium beating at a slower rate than the transplanted one, as it is still subject to vagal influence, which is lacking in the transplanted donor heart. The recipient atrial remnant responds to neural control - for example, slowing of rate occurs when arterial pressure rises, but the donor heart remains uninfluenced (Kent & Cooper, 1974).

The transplanted heart increases its rate following exercise largely as a result of an increase in circulating catecholamines. This response may be blocked by beta-adrenoreceptor blocking drugs. Thus, although intrinsic mechanisms still remain to influence heart rate, denervation means that the heart is more reliant on extrinsic control to effect rate changes.

Pharmacological means may produce denervation. In animals, syringosopine will deplete catechols irreversibly. Fortunately, in human studies it is more usual to "denervate" subjects reversibly, by using intravenous atropine and propranolol. Such blockade leads to a moderate tachycardia, which is not influenced by normal cardiovascular reflexes, as in the transplanted heart. The concept of "intrinsic heart rate" was

developed by Jose & Collison in 1970 to describe a heart denervated by drugs.

Much is now known about denervation secondary to autonomic neuropathy, particularly in the most common form, occurring in diabetics. They display moderate tachycardia, loss of R-R variation and lack of response to tests of cardiovascular reflexes. In many ways the situation resembles both surgical and pharmacological denervation. Kaldor et al (1977) compared the heart rate of diabetics with autonomic neuropathy with the intrinsic heart rate of both normal subjects and diabetics without autonomic neuropathy and found them to be similar. Heart rate increases when autonomic damage first occurs, is shown to increase further as more reflex tests become abnormal, and finally drops slightly as all tests become abnormal (Ewing, Campbell & Clarke, 1981). This seems to mirror the changes seen in pharmacological blockade, where atropinisation causes a tachycardia, and propranolol leads to a small drop in heart rate thereafter (Leon, Shaver & Leonard, 1977).

Thus when 'neural' denervation occurs in man, intrinsic systems become more important in maintenance of heart rate, and extrinsic peptidergic or hormonal control may play an important part in altering cardiac function. Chapter 3 will discuss one such study in which heart rate

control in denervated diabetics is influenced by drugs.

#### 1.2.5 Beta-adrenoreceptors and partial agonist activity

Beta-adrenergic receptors are present in almost all mammalian tissues. Both beta<sub>1</sub> and beta<sub>2</sub> adrenergic receptors stimulate the membrane-bound enzyme adenylate cyclase, which leads to the intracellular accumulation of adenosine 3:5 cyclic phosphate (cyclic AMP), which acts as the "second messenger", and by phosphorylation of certain proteins influences the rate of calcium flux across cell membranes, thus altering the activity of the cells affected. The classification of beta-receptors into beta<sub>1</sub> and beta<sub>2</sub> is based on pharmacological grounds (Lands, Luduena & Buzzo, 1967), but has been confirmed by direct radioligand binding studies (Lefkowitz, Caron & Stiles, 1984).

Pharmacological classification of receptor subtype (ie beta<sub>1</sub> or beta<sub>2</sub>) depends on their responses to certain agonists: beta<sub>1</sub> receptors are defined by the potency series isoprenaline > adrenaline > noradrenaline > phenylephrine, whereas beta<sub>2</sub> receptors show a more marked response to adrenaline, ie isoprenaline > adrenaline >> noradrenaline > phenylephrine. In other words, in vascular and bronchial smooth muscle, adrenaline is approximately tenfold more potent than noradrenaline in stimulating the (beta<sub>2</sub>) receptors, whereas in the heart

and adipose tissue, adrenaline and noradrenaline are roughly equipotent at the ( $\beta_1$ ) receptors.

Agonist potency therefore became the method by which receptor subtype ( $\beta_1$  or  $\beta_2$ ) was defined, but following the development of beta-adrenoreceptor blocking agents in the late 1950's and early 1960's, it became apparent that the compounds synthesised had different blocking effects on infused agonists. Propranolol, a non-selective ( $\beta_1$  and  $\beta_2$ ) adrenoreceptor blocking agent displayed equal antagonism at both receptors, whereas practolol, a  $\beta_1$  selective antagonist failed to block the effects of  $\beta_2$  agonists to the same extent. The use of selective antagonists to define receptor populations can be criticised on the grounds that agonist potencies were originally used to define the receptors, but their use is well supported in the literature (Stiles, Caron & Lefkowitz, 1984).

The effects of the two receptors in physiological terms are quite different;  $\beta_1$  stimulation produces stimulation of rate and force of contraction in the heart, activation of lipolysis in the liver and stimulation of amylase secretion by the salivary glands all by postsynaptic effects, whereas  $\beta_2$  effects include smooth muscle relaxation in bronchi, blood vessels, and genitourinary and gastrointestinal tracts. In the liver

they facilitate noradrenaline release and increase glycogenolysis and gluconeogenesis. Glycogenolysis is increased in muscle cells. Pancreatic secretion of insulin and glucagon is facilitated, and beta<sub>2</sub> receptors in the juxtaglomerular apparatus are responsible for renin release. Beta<sub>2</sub> receptors are present both presynaptically and postsynaptically, and are also found on lymphocytes and polymorphonuclear leucocytes.

Beta-adrenoreceptor blocking agents can therefore be classified as selective or non-selective according to their relative ability to antagonise beta<sub>1</sub> adrenoreceptors in some tissues at lower doses than those required for beta<sub>2</sub> receptors in other tissues. "Cardioselective" beta-adrenoreceptor antagonists inhibit cardiac beta<sub>1</sub> adrenoreceptors but exert little effect on bronchial and peripheral vascular beta<sub>2</sub> adrenoreceptors. The "cardioselective" blockers are considered so because the heart contains predominantly beta<sub>1</sub> receptors and very few beta<sub>2</sub> receptors. "Selectivity" of these compounds is a relative term, however, for as dosage is increased, increasing effects on beta<sub>2</sub> receptors are seen in vivo (Frishman, 1981).

Beta-adrenoreceptor antagonists are all analogues of the agonist isoprenaline. Although they bind to the receptor in much the same way as isoprenaline, their structural

properties mean that stimulation of the receptors does not necessarily occur. When they combine with the receptor, they produce beta-adrenoreceptor blockade by preventing access of agonist molecules to the receptor. Such "pure" blockade occurs for example in the cases of propranolol (non-selective) and atenolol (beta<sub>1</sub> selective). Other kinds of beta-adrenoreceptor antagonists, such as pindolol, or practolol, respectively non-selective and selective, again combine with the receptor to prevent access of agonist molecules. However, these drugs, by nature of their structure, actually exert a weak agonist activity on the receptor, and produce stimulatory effects. This property was originally termed intrinsic sympathomimetic activity (ISA), but is now more commonly referred to as partial agonist activity (PAA), a more accurate terminology in pharmacological terms.

The clinical significance of drugs possessing partial agonism is not entirely clear, but since their development they have provided insights into beta-adrenoreceptor function. One such study will be presented in chapter 3.

### 1.3 Vasopressin (AVP = Arginine vasopressin)

The studies contained in chapters 4, 5 and 6 are concerned with the relationship between the posterior pituitary and the cardiovascular system in diabetics both with and

without denervation produced by autonomic neuropathy. In order to place my work in the context of the (rapidly expanding) literature on vasopressin, I will review the current knowledge of this peptide hormone. Firstly, a brief historical introduction will set the scene for a review of the physiological release mechanisms involved in both salt and water, and blood pressure homeostasis in man. A consideration of the receptors involved in producing the end organ effects will follow. The effects of exogenous vasopressin and its analogues in normal subjects will be reviewed.

The diabetic state, although little studied until recently, may produce further changes in the release of vasopressin and its actions. I will therefore consider the literature on this separately.

Denervated states have been shown to influence both secretion of vasopressin and the peripheral actions of the hormone. By reviewing what is known about AVP in other forms of autonomic neuropathy and pharmacological manoeuvres which produce 'denervation', in addition to the present knowledge of vasopressin in diabetic autonomic neuropathy, I hope to show that the studies presented in chapters 4, 5 and 6 help to answer questions raised by the literature in its present form.

### 1.3.1 Historical review

In 1895, Oliver and Schafer reported that pituitary extracts caused a pressor response when injected into anaesthetised animals. The discovery that the active pressor agent was confined to the posterior pituitary was made three years later (Howell, 1898), when vagotomised dogs were shown to have a larger rise in blood pressure than intact animals. The name "vasopressin" arose because pressor bioassay became the standardisation technique for the pharmacopoeia.

In parallel with the work on pressor responses, other extracts of "pituitrin" were being studied in the treatment of diabetes insipidus. Isolated kidney work performed by Starling and Verney (1924) established more fully the antidiuretic properties of this extract. The work of this group over the next twenty years elaborated the physiological role of antidiuretic hormone in water balance (Verney, 1947).

It was only in 1953 that Du Vigneaud and co-workers proved that the two peptides were one and the same. It was seen that vasopressin had both pressor and antidiuretic properties. The importance of vasopressin in cardiovascular regulation was largely ignored, because it seemed that only large pharmacological doses of AVP produced haemodynamic effects. However, following the

introduction of sophisticated radioimmunoassay for AVP (Robertson et al, 1973), which allowed the measurement of low, and therefore physiological levels of the peptide, its role in both salt and water homeostasis and cardiovascular control has been increasingly recognised.

### 1.3.2 Osmotic and non-osmotic release of vasopressin

The secretion of vasopressin from the hypothalamo-neurohypophyseal system, although a neural function, is very similar to endocrine secretion of hormones from glands elsewhere in the body. A nonapeptide is manufactured by proteolytic cleavage of a high molecular weight precursor. This is then released directly into the blood stream. Much has been learned of the mechanism by which this occurs by analysis of genetic material which now allows study of the human gene from which vasopressin is derived (Majzoub, 1985). The anatomical and physiological background to the release of AVP is worth considering prior to discussion of factors affecting its release.

The hypothalamus lies at the anterior end of the diencephalon. It embodies a group of nuclei that form the floor and ventrolateral walls of the triangular-shaped third ventricle, the anterior boundary of which is made up of a thin membrane called the lamina terminalis; the osmoreceptor cells are believed to be sited there in a

structure known as the organum vasculosum of the lamina terminalis (OVLT). This structure, like the pituitary gland, lacks a blood-brain barrier and is thus suited to its putative purpose of sensing osmotic changes in the blood. The supraoptic nucleus (SON) and paraventricular nucleus (PVN) lie respectively just dorsal to the optic chiasm and in the suprachiasmatic portion of the hypothalamus. They contain the majority of the large neurosecretory cells, or magnocellular cells which manufacture vasopressin and oxytocin (OT), the closely related posterior pituitary nonapeptides. These magnocellular cells project to multiple areas of the CNS, but the cells concerned with secretion of AVP and OT project to the neurohypophysis or posterior pituitary.

The vasopressin prohormone is manufactured in the magnocellular cell body at hypothalamic level, packaged into membrane-bound granules and cleaved during axonal transport to the axonal bulbs of the neurohypophysis where the storage of the final hormone takes place. It is finally released by exocytosis into the systemic circulation (Sklar & Schrier, 1983).

The "final common pathway" of vasopressin secretion is therefore stimulation of the magnocellular neurones in the hypothalamus. The stimuli to release of vasopressin may be divided into two main kinds; osmotic and non-osmotic.

### Osmotic release

Verney (1947) first proposed that release of vasopressin took place in response to various osmotic stimuli, and not purely to changes in sodium concentration, by showing in conscious dogs, that both sodium-rich and non-sodium containing hypertonic solutes produced comparable vasopressin responses.

These and subsequent investigations used indirect or urinary measurements to assess vasopressin function. Once a sensitive radioimmunoassay was established (Robertson et al, 1973), it became apparent that a close relationship existed between plasma osmolality and plasma vasopressin in healthy individuals.

By infusing human subjects with hypertonic saline, a linear relationship is seen between plasma osmolality and plasma AVP concentrations. The slope of the regression line obtained represents the sensitivity of the system in responding to osmotic change. For every unit rise in plasma osmolality, plasma vasopressin rises by  $0.63 \text{ pmol l}^{-1}$ , or to express this differently, a change in plasma osmolality of one per cent alters plasma vasopressin by  $1.8 \text{ pmol l}^{-1}$ . Maximum water diuresis is achieved at plasma AVP levels of around  $5 \text{ pmol l}^{-1}$ . The extraordinary sensitivity of this control system underlies

much of sodium and water balance in man.

The other important variable defined by the regression coefficient is the threshold or 'set-point' of the osmostat, defined by the abscissal intercept. This defines the plasma osmolality above which plasma vasopressin starts to increase. Genetic or environmental factors may be important in determining the osmotic threshold for vasopressin release in man. Most studies from Western countries suggest that the threshold is around 280-289 mOsm kg<sup>-1</sup> (Baylis, 1987; Schrier, Berl & Anderson, 1979) whereas studies in occidental man have shown the threshold to be lower, at approximately 265 mOsm kg<sup>-1</sup> (Shimamoto, Murase & Yamaji, 1976). There is also great individual variation in the osmotic threshold for AVP release, and the sensitivity, or slopes of the regression lines vary between subjects. Despite this wide range of individual variation, it is said that any specific individual demonstrates similar values when studied over a period of time (Robertson, Shelton & Athar, 1976), allowing the use of subjects as their own controls in serial studies.

From other work, reviewed by Baylis (1987), it is clear that infusions of different solutes are sensed in different ways. Hypertonic saline and mannitol produce equivalent release of AVP in subjects, but hypertonic urea provides a poor stimulus for release. The release of AVP

by hypertonic glucose will be considered elsewhere in this thesis, but in normal man it fails to stimulate AVP secretion (Zerbe & Robertson, 1983).

It appears, therefore, that solutes such as glucose, which readily penetrate cell membranes, do not greatly affect AVP release, but those which cross membranes with difficulty, for example, sodium, will set up an osmotic gradient across the cell, causing intracellular water to flow into the extracellular compartment, leading to a decrease in cellular volume by intracellular dehydration. Thus it seems that the osmoreceptor acts by changing its cellular volume (Robertson et al, 1976; Zerbe & Robertson, 1983). This hypothesis is an attractive one, but it is difficult to prove. In theory, it should be possible to demonstrate a suppression of AVP release when cell swelling occurs, but as baseline levels of AVP are low, the detection limits of current assays limit work on these lines.

Osmoreceptor function varies with age: in one study (Helderman et al, 1978), the release of AVP to osmotic stimulation was greater in an older group of subjects, with no differences in the osmotic thresholds between the groups. It appears that the sensitivity of osmotic control of vasopressin secretion improves with age, in contrast to most other physiological mechanisms in man.

In addition to age, pregnancy and the menstrual cycle can affect osmoregulation in humans (Baylis, 1987).

#### **Non-osmotic release**

It seems, therefore, that there is a sensitive mechanism by which changes in osmotic pressure regulate vasopressin release, but it also became apparent that its release could be produced by other means. Verney, in his Croonian lecture (1947), produced evidence of antidiuresis caused by the "emotional stress" of electrically stimulating the flanks of dogs during a water diuresis. This manoeuvre produced a small and transient antidiuresis, but was accompanied by a large increase in blood pressure. This antidiuresis was shown to be related to vasopressin (O'Connor & Verney, 1942).

More recent work has shown that catecholamines are implicated in this response. Schrier et al (1975) showed that both alpha-adrenergic (diuresis-producing) and B-adrenergic (anti-diuresis-producing) effects on renal water excretion are mediated mainly by alterations in the secretion of vasopressin, the mechanism acting through the baroreceptor pathways. Both the low and high pressure baroreceptors are implicated in this response (Schrier, Berl & Anderson, 1979). Changes in blood volume and blood pressure are relayed from cardiovascular receptors

in the carotid sinus and thorax, possibly the ventricles (Wang et al, 1988; Quail, Woods & Korner, 1987) via the glossopharyngeal and vagal cranial nerves to the nucleus tractus solitarius (NTS) and thence the paraventricular and supraoptic nuclei of the hypothalamus via predominantly catecholaminergic pathways.

Hypotension, with or without hypovolaemia, has been shown to be a potent stimulus to vasopressin release, hypotension acting on the high pressure (carotid sinus) baroreceptors, and hypovolaemia on the low pressure (left heart) receptors. Orthostasis, achieved by head-up tilt, has also been shown to stimulate vasopressin secretion (Davies et al, 1976). By infusing ganglion blocking drugs to lower blood pressure in a controlled manner, large quantities of vasopressin are released. In contrast to the linear relationship between osmotic stimulation and vasopressin release, the relationship between the percentage fall in mean arterial blood pressure during infusion and plasma vasopressin level is exponential. Baylis (1987) has claimed that falls in blood pressure of only 5% may produce significant changes in plasma vasopressin concentration (approximately  $1 \text{ pmol l}^{-1}$ ) within the range that influences urinary concentration. The sensitivity of baroregulatory release of AVP is, however, considerably less than that of osmoregulatory release, although it is argued that small

blood pressure changes may influence short term control of renal water excretion. There is evidence that, unlike osmotic regulation of AVP, baroreflex-mediated release becomes less sensitive with increasing age (Rowe et al, 1982).

Although two discrete pathways mediate AVP release, there is a complex and little understood interplay between osmotic and non-osmotic influences. Despite degrees of hypotension and/or hypovolaemia, which increase plasma AVP levels, osmoregulation of vasopressin remains intact, although a lower set point for vasopressin release is produced (Robertson & Athar, 1976; Baylis, 1987; Schrier et al, 1979). In addition, osmotic release of AVP may be suppressed by baroreceptor influences (Goldsmith, 1988).

Another potent stimulus to AVP release in man is nausea or emesis. Rowe et al (1979) showed that apomorphine-induced nausea or vomiting caused rises in plasma vasopressin concentrations independent of changes in blood pressure, blood volume, or intrathoracic pressure.

Drinking in humans may also play a part in non-osmotic suppression of vasopressin release. Recent work has shown that osmotic stimulation of thirst and AVP displays similar characteristics, and that transient inhibition of both thirst and AVP release may take place through

swallowing reflexes, independent of the prevailing plasma osmolality. This suggests that non-osmotic neuronal pathways from the oropharynx mediate changes in thirst perception, probably in a protective role to prevent sudden overhydration of the brain and its consequences (Thompson et al, 1987; Seckl, Williams & Lightman, 1986).

Hypoglycaemia is known to raise vasopressin levels in man, but the precise mechanism is unknown as yet. Whether it occurs as a direct effect of neuroglycopenia on the hypothalamus (Thompson et al, 1981; Fisher, Baylis & Frier, 1987), or is secondary to other considerations (Thompson et al, 1989) is not clear. It appears that there is an exaggerated response of AVP to hypoglycaemia in type I diabetics, and it is possible that through the action of AVP on hepatic glycogen phosphorylase, this may be a heightened counter-regulatory response to impaired glucose recovery in diabetes (Fisher et al, 1989), as AVP has been shown to raise blood glucose in normals (Spruce et al, 1985).

It is apparent, therefore, that apart from osmotic changes, many non-osmotic factors influence the release of AVP from the posterior pituitary. Although they are directed primarily through two separate pathways to act upon the hypothalamus, it is clear that they may act together to release AVP into the circulation. There is

obviously a complex mechanism within the autonomic nervous system by which changes in the balance of sympathetic and parasympathetic discharge regulate non-osmotic release of AVP. Thus, parasympathetic afferents which lead to the hypothalamus are influenced by changes in "tone" which occur during stress situations such as pain, rapid decreases in cardiac output, acute hypoxia, psychiatric disturbances and acute adrenal insufficiency (Schrier et al, 1979).

Considerable work will be required to elucidate the relationships between osmotic and non-osmotic release of arginine vasopressin in man. Much of it is now under way.

### 1.3.3 The roles of vasopressin

The discovery that different structural analogues of arginine vasopressin could produce widely varying actions on different tissues led to work which identified specific peripheral tissue receptors, which have since been characterised as V<sub>1</sub> and V<sub>2</sub> receptors. In addition, there is work that suggests the existence of as yet uncharacterised receptors within the central nervous system (Manning & Sawyer, 1985).

V<sub>1</sub> receptors have been found in vascular smooth muscle (Schiffrin & Genest, 1983), in liver cells (Cantau et al,

1980), and in various parts of the kidney (Dousa, 1985). V<sub>2</sub> receptors are located in the renal tubule (Guillon et al, 1982). The stimulation of V<sub>1</sub> receptors produces vasoconstriction, by stimulating phosphatidylinositol turnover (Creba et al, 1983), whereas V<sub>2</sub> receptor stimulation brings about reabsorption of free water in the renal tubule - the antidiuretic effect.

The antidiuretic response involves activation of membrane-bound adenylate cyclase and the generation of cyclic AMP as a second messenger (Roy, Barth & Jard, 1975), whereas the contractile response of vascular smooth muscle and the glycogenolytic response of hepatocytes act independently of cyclase activation via generation of intracellular calcium. This is analogous to the designation of histamine receptors as H<sub>1</sub> and H<sub>2</sub>. H<sub>1</sub> receptors do not appear to mediate the activation of adenylate cyclase, but H<sub>2</sub> receptors do.

Investigation of the role of AVP is also complicated by the fact that simply measuring plasma levels of the hormone may not reflect its activity in vivo. It is now clear that AVP containing neurones have a variety of central projections that impinge on important cardiovascular regulatory areas (Swanson & Sawchenko, 1980) and thus AVP may influence cardiovascular functions in circumstances under which plasma AVP levels are

unaffected. To further complicate matters, the discovery of an AVP-like peptide localised to peripheral sympathetic nerve cell bodies, intraganglionic axons and terminal fibres in various viscera (Hanley et al, 1984) may imply that peptidergic nerves store a biologically active substance which behaves like AVP, yet is undetectable by normal assays. In addition to this, it may be possible that under some circumstances, AVP neurones might establish new neuronal pathways which influence cardiovascular regulation (Silverman & Zimmerman, 1982).

The effects of vasopressin on the kidney are relatively well known, but it is in the field of cardiovascular regulation that its role has become recognised in the last fifteen years. Once again, however, it is clear that there are pitfalls in interpretation of experimental data involving both animals and man.

Bennett & Gardiner (1986) in a critical review of the literature on the effects of exogenous AVP on baroreflex mechanisms, pointed out the wide inter-species variation in the results obtained, and in their interpretation by investigators. Most of the work reviewed involved modifications of some kind to the autonomic nervous system, the renin angiotensin system or to AVP levels themselves. In a separate review, the same authors (1985) remind us that cardiovascular regulation involves

subtle interactions between these three control systems, and that interference with any of these may merely prompt the remaining mechanisms to adjust to the interference, perhaps producing spurious results.

It is with this background that its effects are reviewed. Experimental work has shown that AVP is a powerful vasoconstrictor (Altura & Altura, 1984), but that it exerts differential effects and different vascular beds (Monos, Cox & Peterson, 1978; Liard et al, 1982). Despite this, studies in both animals and man show wide inter-species variation in AVP concentrations required to produce a pressor response (Bennett & Gardiner, 1986). Normal human subjects seem to be particularly insensitive to its pressor effects (Williams et al, 1986; Aylward et al, 1986; Simpson et al, 1986; Möhring et al, 1980; Padfield et al, 1976). It is clear, therefore, that mechanisms which buffer the blood pressure changes in these subjects come into play. Animal work suggested that baroreflex gain was facilitated by AVP (Cowley et al, 1984), but inconsistent results have been obtained in human subjects (Ebert, Cowley & Skelton, 1986; Aylward et al, 1986). It is clear however, that small changes in systemic vascular resistance may occur after infusion of small doses of AVP (Ebert et al, 1986), and that posture may affect these changes (Simpson et al, 1986). Whether these buffering changes are neurally mediated, or mediated

by vasodilatation in other vascular beds, perhaps by activation of V<sub>2</sub> receptors (Williams, Lightman & Leadbetter, 1986) is not clear.

#### 1.3.4 Vasopressin in diabetes mellitus

Although an elevation of blood glucose concentration in normal man is an ineffective stimulus to thirst or vasopressin secretion (Zerbe & Robertson, 1983), it is apparent that in poorly controlled insulin dependent diabetes mellitus, characterised by polyuria, polydipsia and raised plasma osmolality, marked increases in plasma vasopressin are observed in diabetic ketoacidosis (Walsh, Baylis & Malins, 1979), non-ketotic hyperglycaemia (Zerbe, Vinicor & Robertson, 1979) and during acute insulin withdrawal (Milles, Baylis & Wright, 1981). The cause of this hypervasopressinaemia may in part be secondary to non-osmotic factors such as hypovolaemia or nausea, but its discovery has stimulated work on the physiology of both osmotic and non-osmotic vasopressin release in the diabetic state.

##### **Osmotic release**

Zerbe, Vinicor & Robertson (1985) performed both hypertonic saline and hypertonic glucose infusions in insulin-dependent diabetics and found that the threshold plasma sodium level at which plasma vasopressin levels rise was lower than in normals. They also found that

hypertonic glucose had no effect on AVP levels in both groups, despite higher basal levels in the diabetics. Thompson et al (1988), however, found no such shift in the plasma sodium threshold. Their diabetics however, were euglycaemic during the saline infusion, and it seems clear that the differences obtained were probably because of this. Acute moderate hyperglycaemia during this study had no effect on AVP levels, suggesting that other factors must influence the elevation of AVP in uncontrolled diabetes.

A more recent study by the same group (Thompson, Davis & Baylis, 1989) looked at this question, by performing hypertonic saline infusions in diabetics rendered either euglycaemic or hyperglycaemic by a clamping technique. They found that the hyperglycaemic state was indeed responsible for the shift in plasma sodium threshold to the left. It is known that hypovolaemia lowers the osmotic threshold for AVP release (Robertson, Aycinena & Zerbe, 1982), but there was no evidence for this in their data.

A further explanation for this phenomenon could be the theory that insulin deficiency alters the function of osmoreceptor cells. It is thought that insulin promotes transport of glucose into the osmoreceptor cells, preventing the development of an osmotic gradient

necessary to change cellular volume and stimulate AVP release (Baylis & Robertson, 1980). If insulin levels are low, high glucose levels may be able to create an osmotic gradient which then stimulates AVP release. Evidence supporting this view comes from a study (Vokes, Aycinena & Robertson, 1987) in which insulin-deficient diabetic subjects had greater incremental rises of plasma AVP to osmotic stimuli than healthy controls. The differences appear to occur only during severe insulinopenia, and result from an increase in sensitivity of the osmoreceptors, rather than the set-point. In a group of 10 type I diabetics, Grimaldi et al (1988) found that elevating blood glucose from a mean of 12.4 mmol l<sup>-1</sup> to 47.0 mmol l<sup>-1</sup> raised plasma AVP from a (high) basal value of 5.6 pg ml<sup>-1</sup> to 7.7 pg ml<sup>-1</sup>. They have postulated a direct effect of glucose on osmoreceptors, but the fact that both basal and post-infusion blood glucose levels were much higher than in other studies implies that non-osmotic influences were probably responsible for the raised plasma AVP levels.

In general, therefore, the evidence suggests that hypothalamic control of osmoregulated vasopressin secretion appears to be normal in diabetics without evidence of autonomic neuropathy. I will present evidence in chapter 4 on whether this is so in the presence of clear autonomic dysfunction.

### **Non-osmotic release**

What evidence there is in diabetics without autonomic neuropathy suggests that postural and hypovolaemic stimuli to AVP release are normal. In a well defined group of insulin-dependent diabetics without autonomic neuropathy, volume depletion by frusemide appropriately raised AVP levels by over fifty per cent (Grimaldi et al, 1985). Another study found that in four out of five such diabetics plasma AVP levels rose after standing for two hours (Cignarelli et al, 1986). In a more recent paper, using rather less well characterised type II diabetics who were also hypertensive, no significant changes in plasma AVP levels were seen after thirty minutes of standing (Saad et al, 1988).

The only other non-osmotic stimulus to AVP release in diabetics yet studied is that of insulin-induced hypoglycaemia. Plasma AVP levels rose significantly higher in a group of type I diabetics than in matched normal controls (Thompson et al, 1989). As mentioned in 1.3.2, the mechanism by which hypoglycaemia releases AVP is not yet known

### **1.3.5 Vasopressin in disorders of autonomic function**

Early in the course of investigation into vasopressin, it was apparent that interference with the autonomic nervous

system produced effects. Verney (1947) demonstrated a heightened antidiuretic effect of electrical flank stimulation in a dog which had been surgically denervated. The antidiuresis could be countered by an infusion of tyramine or noradrenaline. Later work (Schrier & Berl, 1972), again in dogs, showed that bilateral cervical vagotomy caused an AVP-mediated antidiuresis secondary to interruption of afferent neural pathways, since efferent blockade of parasympathetic pathways with atropine did not influence renal water excretion. Thus increased AVP levels were produced by damage to the parasympathetic pathways.

In the intervening period, it was shown that in human subjects, the pressor effect of AVP could be demonstrated in the presence of autonomic problems. Wagner & Braunwald, in 1956, caused blood pressure to rise in both patients with idiopathic orthostatic hypotension and in subjects pretreated with a ganglion-blocking agent.

It is clear, therefore, that in crude terms both the release of AVP and its effects in vivo are affected by autonomic dysfunction. In this section I will attempt to outline the current knowledge of how AVP release and its actions differ from the normal state in the presence of damage to the autonomic nervous system, with reference to some of the relevant animal work, to work on subjects with

primary disorders of the autonomic system, to tetraplegics, subjects with cardiac transplants, and finally in diabetics with autonomic neuropathy.

### **Animal considerations**

Recent work in animals has provided evidence that AVP may play a central role in control of blood pressure despite its lack of pressor effect in physiological doses, and that it exerts powerful effects on central nervous mechanisms concerned with cardiovascular regulation. Montani et al (1980) showed in dogs that mean arterial pressure (MAP) did not change when AVP was infused at physiological doses because, despite large increases in total peripheral resistance (TPR), cardiac output fell to compensate. The effects were in part attributed to a direct action of AVP on baroreflex activation. Cowley et al (1984) performed elegant studies on surgically denervated dogs which suggested that AVP enhanced carotid reflex gain by more than two fold when intrasinus pressure fell below the equilibrium point. A vagally mediated bradycardia was produced by AVP which buffered the increase in pressure by reducing cardiac output. Further evidence for an important action of AVP in denervation came from work which showed that both AVP and the sympathetic nervous system interact in the presence of vagal deafferentation to produce a pressor response (Hasser et al, 1984). In the presence of bilateral vagal

cold block, conscious dogs both with and without denervation of the carotid sinus were given both a ganglion blocking agent and a specific vasopressin antagonist. It was shown that in 'intact' dogs the AVP antagonist augmented the hypotensive effect of the ganglion blocker but that no intrinsic hypotensive effect was produced. When the sinoaortic denervated animals were studied, the AVP antagonist produced a consistent hypotensive effect, which was heightened by ganglion blockade. The authors suggested that AVP thus modulates the haemodynamic changes associated with vagal deafferentation. It is also clear that in dogs, levels of AVP which have no effect on arterial pressure, may have profound effects on central venous pressure which may affect cardiopulmonary afferents which act to offset a rise in arterial pressure (Bie & Warberg, 1983). Further work in both dogs and other animals suggests an interplay between the renin-angiotensin, sympathetic nervous system and AVP in the presence of denervation. In dogs, data suggests that AVP plays an important role in blood pressure maintenance following haemorrhage. Despite activation of the renin-angiotensin system following mild haemorrhage, a dramatic fall in blood pressure was seen after administration of a vasopressin antagonist (Schwartz & Reid, 1981). AVP release following haemorrhage in cardiac denervated dogs has also been shown to be reduced (Wang et al, 1983), suggesting an important role for

cardiac volume receptors in AVP release. No significant changes in haemodynamics were seen between the denervated dogs and controls, suggesting that sensitivity to AVP may be heightened under these circumstances.

Total ablation of the CNS (Cowley & Barber, 1983) and total chemical autonomic blockade (Pullan et al, 1980) have also been shown to result in increased pressor sensitivity to AVP.

Therefore, in the dog, the role for AVP appears to have been well established. In rats, there are considerably more technical problems in both methods used to study them and in interpreting the results obtained, although much of the anatomical work delineating central pathways for AVP release has been gathered from them (Sawchenko & Swanson, 1981; Sawchenko & Swanson, 1982). The difficulties inherent in interpreting the studies on animals are outwith the scope of this thesis, and have been comprehensively reviewed elsewhere (Bennett & Gardiner, 1985; Bennett & Gardiner, 1986).

#### **Denervation in humans**

In 1956 Wagner & Braunwald showed in man that infusion of AVP could indeed have major pressor effects in the presence of autonomic problems. Both volunteers, pretreated with ganglion blockers, and patients suffering

from idiopathic orthostatic hypotension demonstrated this effect. Later work confirmed this. This condition is also known as Shy-Drager syndrome, or progressive autonomic failure (PAF) with or without multiple system atrophy (MSA). These patients have degeneration of the sympathetic nervous system and often have gross pathological and neurochemical damage (Spokes, Bannister & Oppenheimer, 1979) to the locus coeruleus in the brainstem (Bannister, 1983). Work in rats has demonstrated that the noradrenergic projection from the locus coeruleus to the periventricular zone of the paraventricular nucleus is of great importance in the arginine vasopressin response to cardiovascular stimuli (Lightman, Todd & Everitt, 1984).

Two studies in patients with PAF have found defective release of AVP to the stimulus of head-up tilt, despite marked hypotension which would normally cause a marked rise in AVP levels (Puritz et al, 1983; Zerbe, Henry & Robertson, 1983). These patients have been demonstrated to have normal release of AVP to osmotic stimulation by hypertonic saline infusion (Williams, Lightman & Bannister, 1985), indicating that an afferent defect below the hypothalamic level, perhaps at the locus coeruleus is responsible for the subnormal release of AVP. Interestingly, a significant rise in mean blood pressure was seen in this group, which at least in part may have

been caused by sensitivity to the pressor effects of AVP. Certainly since Wagner & Braunwald's original report, other studies have confirmed the phenomenon, which also occurs in response to lysine vasopressin (Bannister, Ardill & Fentem, 1969). Möhring et al (1980), in two such patients found that physiological levels of AVP produced a pressor response which was maximal early in the infusion. Williams et al (1986) performed stepwise infusion of AVP in eight subjects with PAF and found marked pressor effects at levels within the physiological range. In contrast to the animal work, no effects of interaction with the cardiac baroreflex were observed. The authors suggested that receptor changes may occur in the presence of denervation and that studies in groups of patients with denervation of different aetiologies may help to illuminate whether this is so.

One such group of patients are tetraplegics, who like the PAF patients have an interruption of their sympathetic outflow as well as a spinal cord lesion, but in contrast have normally functioning glossopharyngeal and vagal cranial nerves and normal central catecholaminergic pathways. Three papers provide much insight into this matter. Di Pette et al (1984) looked at changes in AVP, blood pressure and heart rate for twenty minutes following the injection of a hyperosmolar radiocontrast agent for intravenous urography. They found a rise in blood

pressure and AVP levels in a tetraplegic group, but not in their control subjects. The changes were maximal in the first five minutes and returned towards normal at the end of the study. They therefore had heightened AVP release to an osmotic stimulus (though non-osmotic influences may have contributed to this) and perhaps an increased pressor responsiveness to AVP. Sved, McDowell & Blessing (1985) studied the release of AVP to head-up tilt in their patients, and found a four-fold increase in plasma AVP levels to rapid tilt to 70°, in keeping with the marked hypotension observed during the procedure. In both reports, basal AVP levels were normal. The most compelling work (Poole et al, 1987) studied eight tetraplegics by hypertonic saline infusion, head-up tilt and stepwise infusion of AVP. In contrast to Di Pette et al, they found no significant differences compared with controls in plasma AVP levels following hypertonic saline, but found a marked rise in mean arterial pressure. Following head-up tilt, AVP levels rose much higher than controls because of the hypotensive stimulus. Infusion of arginine vasopressin at physiological levels produced a marked rise in blood pressure in the tetraplegics. In contrast to the patients with PAF, the tetraplegics showed a marked bradycardia, presumably mediated via the baroreflex and intact vagal outflow, an action of AVP known to occur in animals (Courtice et al, 1984).

Cardiac transplant patients are a little studied group. Only one study has looked at AVP in such patients (Drieu et al, 1986). As hypertonic saline infusion was considered unethical in these mainly hypertensive patients, water loading was performed and was suggestive of normal osmotic control. Basal AVP levels, however, were raised, and no increase in plasma AVP levels was seen after moderate volume depletion with frusemide. This suggests that cardiac receptors and innervation play a dominant role in the AVP response to volume depletion in man.

Surprisingly, little work on AVP release or its effects has been performed in diabetics with autonomic neuropathy. No work has addressed whether osmotic release of AVP in these patients is normal or not. Autonomic neuropathy in diabetics is considered to be a disease which does not affect the central nervous system, although vascular changes are well documented in the brains of longstanding diabetics (Reske-Nielsen, Lundbaek & Rafaelsen, 1965). It may well be important therefore to see whether osmotic release of AVP following hypertonic saline infusion is abnormal or not, as hypothalamic damage could affect its secretion. No studies in the literature have addressed this question, but I will present my findings from such a study in chapter 4.

The responses of AVP to orthostatic and/or volume stimuli in diabetic autonomic neuropathy is another area in which there is a paucity of data. Zerbe, Henry & Robertson (1983) studied two patients with diabetic autonomic neuropathy (DAN +ve) amongst a group of eighteen subjects with various causes of orthostatic hypotension following the stimulus of head-up tilt, initially to sixty degrees, and progressively to ninety degrees. Their results were conflicting. One diabetic responded appropriately to tilt with a rise in plasma AVP, and the other, despite a sixty per cent fall in mean arterial pressure, had only a small increase in AVP levels.

Grimaldi et al (1985) used volume depletion induced by intravenous frusemide as a stimulus for AVP release in a well characterised group of sixteen DAN +ve patients and compared their responses with seventeen DAN -ve patients. In both groups plasma volume (estimated from the rise in plasma protein) fell by approximately twelve per cent, while MAP fell only in the DAN +ve patients (by thirteen and a half per cent). Plasma AVP levels remained unchanged after sixty minutes in the DAN +ve subjects, but had risen by over fifty per cent in the DAN -ve group. Interestingly, one cardiac transplant patient was also studied. No rise in plasma AVP was seen, despite a similar fall in plasma volume. This subject maintained

his blood pressure throughout the study.

Further evidence for an afferent defect in the pathways controlling AVP release in diabetic autonomic neuropathy comes from Cignarelli et al (1986). In four out of nine diabetics affected by autonomic neuropathy, plasma AVP failed to increase after five minutes of standing, although two hours after standing plasma AVP levels had risen in one of them. Two of the diabetics had no blood pressure fall on standing, and may therefore not have provided a sufficient hypotensive stimulus to AVP release. Evidence for an afferent defect in AVP release therefore does exist in diabetics with autonomic neuropathy, but responses seem to be variable, perhaps for methodological reasons. In chapter five I will present further evidence for an afferent defect, using the well established technique of head-up tilt.

No studies exist in the human literature examining the effects of infused AVP, either in physiological or pharmacological doses in diabetics with autonomic neuropathy, although one study in rats rendered diabetic by streptozotocin has suggested a decreased pressor responsiveness to vasopressin following ganglion blockade (Hebden, Bennett & Gardiner, 1987).

Only one study has indirectly suggested that AVP may play

an important role in blood pressure maintenance in diabetics with autonomic neuropathy (Saad et al, 1988). The only evidence of autonomic dysfunction in this group of type II diabetics with hypertension was postural hypotension. After dosing with a  $V_1$  receptor antagonist, a sub group had a very marked hypotensive response. No details of renal function or other diabetic complications were given and their conclusions are perhaps overstated from the data presented.

There is therefore no clear evidence for the role that AVP may play in diabetics with autonomic neuropathy. If the theory devised from work on tetraplegics and patients with progressive autonomic failure, that autonomic damage leads to changes in  $V_1$ -receptor sensitivity is true, one might

expect diabetics with autonomic dysfunction to display similar changes, in keeping with work on supersensitivity to catecholamines (Hilsted et al, 1987). I will present evidence to confirm this theory in chapter six.

#### 1.4 AIMS OF THIS THESIS

As already discussed in this introductory chapter, this thesis presents studies on different aspects of denervation in diabetic autonomic neuropathy. In chapter 3 data is presented from a study in which autonomic

neuropathy in diabetes is used as a model to study heart rate changes ( $B_1$ -adrenoreceptor mediated) and changes in physiological tremor ( $B_2$ -adrenoreceptor mediated) which occur after dosing with beta-adrenoreceptor blocking drugs which possess partial agonist activity. As well as determining the cardioselectivity of the drugs' partial agonism, the changes in heart rate should give an indication of the denervated state which prevails in the diabetics studied. By comparing these changes with those which occur both in normal subjects and in other forms of denervation, it may be possible to infer whether the heart rate changes in diabetic autonomic neuropathy reflect  $B_1$ -adrenoceptor denervation supersensitivity, as may be found in both animals (Barrett & Carter, 1970) and in autonomic failure (Man in't Veld & Schalekamp, 1981).

Chapters 4, 5 and 6 are studies which follow on from work performed in progressive autonomic failure and tetraplegia. Chapter 4 examines whether autonomic neuropathy affects osmotic control of vasopressin secretion in diabetes. This is an important point to address, as the interpretation of the results presented in chapter 5, where endogenous AVP secretion is stimulated by head-up tilt, and in chapter 6, where physiological doses of AVP are infused to assess pressor responsiveness, may be affected if abnormal osmotic secretion is present.

By comparing and contrasting these studies with work performed in the other forms of autonomic dysfunction, it should be possible to obtain information about the effect of diabetic autonomic damage on the neural pathways involved in vasopressin release, on hypothalamic and posterior pituitary function, and on end-organ (vascular) effects of vasopressin. By placing this work in the perspective of the current literature, I hope to provide some insight into the degree of autonomic nervous damage that diabetes can cause.

## CHAPTER 2

### METHODS AND MATERIALS

#### 2.1 Subjects

The diabetic subjects studied in this thesis were drawn from the diabetic and dietetic outpatient department in the Royal Infirmary of Edinburgh, which looks after patients from the city of Edinburgh and other parts of Lothian region. Because of the research interests of the clinic, large numbers of patients each year are screened for signs and symptoms of peripheral and autonomic neuropathy. With the exception of the patients mentioned in my declaration, all the subjects were seen by me after screening and entered into the studies.

As different subjects were used in the different studies, for convenience details are given in the individual chapters.

#### 2.2 Cardiovascular autonomic reflex tests

The classification of autonomic status in all the subjects studied in this thesis depends upon a battery of five cardiovascular autonomic reflex tests which are simple, non-invasive, reproducible and distinguish clearly between normal and abnormal results. Three tests are based on

measurements of heart rate responses: the Valsalva manoeuvre, deep breathing and standing. The other two are measurements of blood pressure responses to standing and to sustained handgrip. The heart rate responses are indicative of cardiac parasympathetic integrity, while the blood pressure changes are only abnormal with more extensive and widespread (extracardiac) sympathetic damage (Ewing, 1983; Watkins & Edmonds, 1983). The autonomic pathways involved in these reflexes are complex. Both parasympathetic and sympathetic innervation plays some part in all five tests, and while a division into parasympathetic and sympathetic tests is clinically convenient, this does not strictly reflect all the complex underlying physiological mechanisms. In this thesis, therefore, I will refer only to heart rate and blood pressure tests, rather than being dogmatic about the precise autonomic innervation of each reflex test. These tests are further outlined in Ewing (1978), Ewing & Clarke (1982), Ewing (1983) and Ewing et al (1985).

#### **1. Valsalva manoeuvre (Valsalva ratio)**

The subject sits quietly and then blows into a mouthpiece at a pressure of 40 mmHg for 15 seconds. The heart rate normally increases during the manoeuvre, followed by a rebound bradycardia after release. The ratio of the longest R-R interval shortly after the manoeuvre to the shortest R-R interval during the manoeuvre is then

measured. The result is routinely expressed as the Valsalva ratio, as the mean ratio from three successive Valsalva manoeuvres.

## **2. Heart rate response to standing up (30:15 ratio)**

The subject lies quietly on a couch and then stands up unaided. Normally an immediate increase in heart rate occurs that is maximal at about the 15th beat after starting to stand, followed by a relative bradycardia, maximal around the 30th beat. This can be quantified as the 30:15 ratio, which is the ratio of the longest R-R interval around the 30th beat to the shortest R-R interval around the 15th beat.

## **3. Heart rate response to deep breathing (R-R variation)**

The subject sits quietly and then breathes deeply and evenly at 6 breaths per minute. The maximum and minimum heart rates during three successive breathing cycles are taken to give the maximum-minimum heart rate.

## **4. Blood pressure response to standing up**

The blood pressure is measured using a standard sphygmomanometer while the subject is lying down, and again after standing up. The difference in systolic blood pressure is taken as the measure of postural blood pressure change.

## 5. Blood pressure response to sustained handgrip

Handgrip is maintained at 30% of the maximum voluntary contraction using a handgrip dynamometer (Tephcotronics Limited, Edinburgh) up to a maximum of five minutes, and the blood pressure measured each minute. The difference between the diastolic blood pressure just before release of handgrip, and before starting, is taken as the measure of response.

Tests 1, 2 and 3 reflect heart rate responses and were previously referred to as "parasympathetic" (PS) tests, while tests 4 and 5 measure blood pressure responses and were previously referred to as "sympathetic" (S) tests.

The results of these tests are grouped into three categories - normal, borderline or abnormal. If all tests are normal or one borderline, they are referred to as normal (N); early involvement (E) if one of the three heart rate tests are abnormal or two borderline; definite involvement (D) if two or more of the heart rate tests are abnormal; and severe (S) where two or more of the heart rate tests are abnormal, plus one or both of the blood pressure tests are abnormal or both borderline (Ewing et al, 1985). Each subject can also be given an "autonomic score" by scoring each test as normal (0), borderline (1), or abnormal (2), giving a total score for the five tests of 0 (normal) to 10 (most abnormal). The repeatability

of these tests has been well established (Ewing et al, 1985).

Where a test is omitted, for example the Valsalva ratio when there is untreated proliferative retinopathy, the score is given out of eight.

These tests are summarised in table I.

During the period of time this work was performed, these tests were carried out by using an on-line BBC microcomputer linked to an ECG recorder, using software specifically designed for the purpose ("AUTOCAFT" - UnivEd Technologies Limited, Edinburgh). This system allows individual R-R intervals to be measured to an accuracy of  $\pm 10$  ms, and thus allows very precise measurement of both R-R variation, and indeed heart rate.

Retinopathy was assessed by indirect ophthalmoscopy, after mydriasis by Tropicamide 0.5% eyedrops, and graded as absent (-), background (B), exudative (E) or proliferative (P).

Proteinuria was assessed by Albustix (Ames Division, Miles Limited, Stoke Poges, UK) and classified according to the manufacturer's instructions.

2.3 **Heart rate** in all studies performed in Edinburgh in this thesis was recorded using the microcomputer system to average heart rate over a period of time (five minutes in chapter 3, ten seconds for each measurement point in chapters 4, 5, and 6).

**24 hour heart rate** (chapter 3) was measured using a standard ambulatory ECG technique ("Tracker" - Reynolds Medical Company Limited), which allows 'real-time' recordings to be made, with an event button to mark specific points in the recording. The tapes thus obtained were replayed through a Pathfinder high speed arrhythmia analyser (Reynolds Medical Company Limited) at exactly 60 times the original recording speed to generate an output representing heart rate.

**Blood pressure** (chapters 4, 5 and 6) All blood pressure recordings in Edinburgh were made using a Hawksley random zero sphygmomanometer, Korotkoff phase V being taken as the diastolic pressure. Mean blood pressure was calculated by adding one-third of the pulse pressure to the diastolic pressure. The normal controls in chapter six had blood pressure measurements made with an automatic sphygmomanometer (Sentron, Bard Biomedical).

**Physiological tremor** (chapter 3) Changes in physiological tremor produced by beta-adrenoreceptor

blocking or stimulating agents can be used as a model of beta<sub>2</sub>-adrenoreceptor activity. B<sub>2</sub>-adrenoreceptor agonists such as isoprenaline increase tremor and differences between B<sub>1</sub>-selective and non-selective B-adrenoreceptor blocking drugs can be detected in man (Arnold & McDevitt, 1984).

Physiological tremor was measured using a piezo-electric accelerometer (Vibro-Meter Corp, Boston, USA - sensitivity in the vertical plane 10.05 mVg<sup>-1</sup>) attached by a tubigauze (Seton Medical, UK) bandage to the outstretched index finger of the right hand with the arm supported at the elbow on a firm laboratory bench. The output from the transducer was recorded onto magnetic tape via the vibration module of an Instrumentation Cassette Recorder (Type DA 1442 Data Acquisition Limited, Stockport, UK). The magnetic tape signal was subjected to a fast Fourier Transformation using a PDP11 computer and a power spectrum in the frequency 0 - 30 Hz obtained.

**Tilt studies** (chapter 5) were performed using a modified operating table with a footrest at the base. Tilt was achieved manually over a period of 15 seconds.

**Infusion studies** (chapters 4 and 6) were performed using a standard intravenous infusion pump (IMED 960, Abingdon, England).

## 2.4 BIOCHEMICAL VARIABLES

**Arginine vasopressin** - All samples for plasma AVP levels were collected into lithium heparin tubes, immediately centrifuged, and the separated plasma deep frozen for later analysis at the Westminster Hospital. Samples were stored for less than two months at  $-20^{\circ}\text{C}$  before SEP-Pak extraction and estimation of AVP by radioimmunoassay (Williams, Carter & Lightman, 1985).

Plasma samples (1 ml) were acidified with 3 ml 0.1M formic acid and extracted using a modification of the SEP-Pak method. Octadecasilyl silica columns (C18 Sep-Paks, Waters Associates, Norwich, England) attached to a multichannel pump (Watson-Marlow, Falmouth, England) were first regenerated with 95% methanol-5% 0.1M formic acid (3 ml) followed by 0.1M formic acid (3 ml), and the acidified samples were passed on to the columns at 1 ml per minute and washed with 0.1M formic acid (3 ml). Elution with 95% methanol-5% 0.1M formic acid (3 ml) was followed by solvent extraction in a vacuum centrifuge (Speed Vac Concentrator, Uniscience, London, England). The residue was taken up in 500  $\mu\text{l}$  RIA buffer (0.1M Tris acidified to pH 7.4 with HCL and containing  $2\text{g l}^{-1}$  BSA) for subsequent assay of AVP in 100  $\mu\text{l}$  duplicates. Extraction yield for AVP, calculated from extracting  $^{125}\text{I}$ -labelled AVP added to

plasma was  $94 \pm 2\%$  (mean  $\pm$  SD,  $n = 10$ ). AVP was measured by a highly specific and sensitive RIA using 100  $\mu$ l each of: (1) First International Standard for AVP, (2)  $^{125}\text{I}$ -labelled AVP (3000-4000 cpm), and (3) specific AVP antiserum at a final dilution of 1:300,000. Incubation was carried out for 48 h. Bound tracer was separated from the free fraction by precipitating with chilled ethanol, centrifuging, and decanting the supernatant. Fifty per cent inhibition of tracer binding occurred with 2.5 fmol standard AVP.

The coefficients of variation for control AVP samples (2 fmol) were 6% (intra-assay) and 15% (inter-assay).

**Plasma adrenaline and noradrenaline** - These samples were also immediately centrifuged, the plasma deep frozen and stored in liquid nitrogen, and later assayed by the radioenzymatic method of Da Prada and Zurcher (1976). This assay is based on the 3-O-methylation of catecholamines by the enzyme catechol-O-methyl-transferase (COMT) in the presence of  $^3\text{H}$ -S-adenosylmethionine ( $^3\text{H}$ -SAM). Labelled O-methylated products are transformed to less polar complexes by adding sodium tetraphenylborate (TPB) and thereafter extracted into diethyl ether. The products of the reaction are separated by thin-layer chromatography. Methoxytyramine (MT) derived from Dopamine 3-O-methylation, is eluted and the radioactivity

measured. Metanephrine and normetanephrine is further oxidised to vanillin and its radioactivity is counted after extraction. Aliquots of pooled normal plasma were analysed concurrently (mean  $2.5 \text{ nmol l}^{-1}$ , CV 5.4%).

**Plasma renin activity** was measured by a modification (Roulston & MacGregor, 1978) of the method of Boyd et al (1969). Blood is sampled and put into tubes containing EDTA (2.5 mg EDTA per ml blood). The tubes are centrifuged at  $2000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$ . The supernatant plasma is then frozen at  $-20^{\circ}\text{C}$  until assay.

The plasma was thawed, but the temperature not allowed to exceed  $4^{\circ}\text{C}$ . It was then mixed with an equal volume of 0.1 molar potassium citrate/citric acid buffer at pH 5.7 followed by 0.46 molar 8-hydroxyquinolone (40 ul per ml of plasma) and 80 ul per ml of plasma of a saturated ethanolic solution of phenylmethylsulfonylfluoride (PMSF). After thorough mixing, one portion of the sample was incubated at  $37^{\circ}\text{C}$  for three hours to generate angiotensin I whilst the other portion was maintained at  $0^{\circ}\text{C}$  in an ice bath. Radioimmunoassay of angiotensin I was then performed by the method of Boyd et al on both the incubated and unincubated portions; plasma renin activity (or angiotensin I generation rate) being calculated as a direct fraction of the difference in angiotensin I concentration in the two portions (after correction for

incubation time and plasma dilution). Interassay coefficients of variation (CV) were between 9 and 12.6% (Roulston & MacGregor, 1978).

**Plasma aldosterone** was measured by a commercially available radioimmunoassay kit (CIS UK Limited, High Wycombe, Bucks). The principle of this assay is based on the competition between the labelled aldosterone and aldosterone contained in standards or specimens to be assayed, for a fixed and limited number of antibody binding sites. After incubation, the amount of labelled aldosterone bound to the antibody is inversely related to the amount of unlabelled aldosterone present in the sample. The method adopted for B/F separation is based on the use of the antibody-coated tubes, where the antibody is fixed on the tube walls by Catt's method. The interassay CVs were between 8 and 10% (data on file, CIS (UK) Limited).

**Plasma glucose** was measured by a standard glucose oxidase technique. **Plasma sodium** was measured by flame photometry (Corning 435 flame photometer) and **plasma osmolality** by an Advanced Digimatic Osmometer model 3DII. **Haematocrit** was obtained from the mean of three micro-haematocrit measurements.

## 2.5 STATISTICAL METHODS

### Chapter 3

The 24 hour heart rate measurements were analysed over three periods. Mean hourly heart rates and mean heart rate (mean of the mean hourly heart rates) within each of the three periods have been used in the analysis. The physiological tremor data was transformed into natural logarithms for statistical analysis as its distribution was non-normal and the transformed data was displayed as power spectra over the range 0 - 30 Hz. To give an overall measure of tremor for each patient, the log transformed readings were summed over the frequency range 0 - 30 Hz ('area under the curve') and this measurement was used in the analysis. These results and the resting heart rate and glucose measurements were subjected to an analysis of variance technique. Overall treatment significance was assessed by an F test after which, if significant differences were found ( $p < 0.05$ ), pairs of treatments were compared using a two sided Student's t-test with the residual mean square estimating the standard error. The level of significance for the t-tests was  $p < 0.05$ , calculated from the least square estimates of the means.

## Chapters 4, 5 and 6

Data analysis was carried out using a statistical software package (Minitab Statistical Package). In chapters 4 and 5, paired Student's t tests were performed on all baseline values within each group. As there were no significant differences between the -10, -5 and 0 minute measurements within the three groups, baseline measurements were taken as the 0 minute readings. Areas above and below baseline values over the next 120 minutes were then calculated for each variable, and used to determine differences from baseline. An analysis of variance with a simultaneous comparison of the three groups was first performed on the baseline and the area under the curve values. Where significant differences were observed, a further analysis was performed using a Student's t test to detect which pairs of groups differed significantly. Significant changes from baseline during the 120 minute infusion period in chapter 4 were tested by calculating a 't' statistic for each area under the curve value for each of the groups. Linear regression equations were calculated for each individual for the relationships between plasma osmolality and AVP, and plasma sodium and AVP, during the saline infusion. The individual regression coefficients, slopes and intercepts were then used in a subsequent analysis to compare groups.

In chapter 6, baseline values at -10, -5 and 0 minutes

were compared within each group using an analysis of variance. The AVP levels at the different infusion rates were compared as follows. Regression coefficients from the logarithmically transformed plasma AVP levels on log infusion rates over the range of  $0.2 \text{ pmol min}^{-1}\text{kg}^{-1}$  to  $5 \text{ pmol min}^{-1}\text{kg}^{-1}$  were calculated for each subject, and the derived variables compared between the three treatment groups using an analysis of variance.

In view of the slightly different intermediate infusion rates used in the normal and diabetic groups, it was considered more appropriate to make statistical comparison of the results using 'area under the curve' measurements rather than maximum blood pressure responses. 'Areas under the curve' (AUC) were therefore calculated for the heart rate and mean blood pressure responses for each subject over the same range of infusion levels, and the differences between the three groups compared using an analysis of variance. Omission of the lowest AVP infusion rate meant that this small AVP dose was not given a disproportionate emphasis in the analysis.

## 2.6 Ethical aspects

Permission for all the studies presented in this thesis was granted by the appropriate local Hospital Ethical Advisory Committees, and all subjects gave their written informed consent before taking part.

Normal                      Borderline                      Abnormal  
 ('score' = 0)                      ('score' = 1)                      ('score' = 2)

Heart rate tests

|   |                      |   |
|---|----------------------|---|
| 1. Heart rate response to Valsalva manoeuvre (Valsalva ratio)                             | 1.21 or more         | 1.20 or less                              |
| 2. Immediate heart rate response to standing (30:15 ratio)                                | 1.04 or more         | 1.01 - 1.03<br>1.00 or less               |
| 3. Heart rate (R-R interval) variation during deep breathing (maximum-minimum heart rate) | 15 beats/min or more | 11 - 14 beats/min<br>10 beats/min or less |

Blood pressure tests

|   |                 |                                 |
|---|-----------------|---------------------------------|
| 4. Blood pressure response to standing (fall in systolic BP)                      | 10 mmHg or less | 11 - 29 mmHg<br>30 mmHg or more |
| 5. Blood pressure response to sustained handgrip (increase in diastolic pressure) | 16 mmHg or more | 11 - 15 mmHg<br>10 mmHg or less |

Table I

NORMAL, BORDERLINE AND ABNORMAL VALUES FOR CARDIOVASCULAR AUTONOMIC FUNCTION TESTS

## CHAPTER 3

### **EFFECTS OF B-ADRENOCEPTOR BLOCKADE ON HEART RATE AND PHYSIOLOGICAL TREMOR IN DIABETICS WITH AUTONOMIC NEUROPATHY: A COMPARATIVE STUDY OF EPANOLOL, ATENOLOL AND PINDOLOL**

#### **3.1 INTRODUCTION**

As outlined in chapter 1.2.5, some B-adrenoceptor blocking agents are known to be partial agonists at the B-adrenoceptors, for example practolol and pindolol. It is, however, unclear, especially in man, whether the stimulant effect of a non-selective B-adrenoceptor blocking drug with PAA is at the B<sub>1</sub>-adrenoceptor, B<sub>2</sub>-adrenoceptor, or both, or whether a B<sub>1</sub>-selective partial agonist produces stimulation only at the B<sub>1</sub>-adrenoceptor. To answer this question a study measuring both B<sub>1</sub>- and B<sub>2</sub>-adrenoceptor function is required in which a non-selective and a B<sub>1</sub>-selective adrenoceptor blocking agent, both with PAA, are compared in the same subjects.

The demonstration of partial agonism of B-adrenoceptor blocking drugs at the B<sub>1</sub>-adrenoceptor in animal models requires that the heart is surgically denervated and the animals depleted of catecholamines by syrosingopine

(Barrett & Carter, 1970). This situation may be analogous in man to diabetic patients with severe diabetic autonomic neuropathy (Watkins & Edmonds, 1983). Certainly in autonomic failure from other causes an increase in heart rate has been noted when B-adrenoceptor blocking agents with PAA were given (Man in't Veld & Schalekamp, 1981). If B-adrenoceptor blocking agents with PAA are given to such diabetics, increases in heart rate might occur due to B<sub>1</sub>-adrenoceptor stimulation.

Physiological tremor has been shown to involve peripheral B-adrenoceptors (Marsden et al, 1967). More recently, increase in physiological tremor induced by sympathomimetic amines has been thought to be due to B<sub>2</sub>-adrenoceptor stimulation (Arnold & McDevitt, 1984), and provides a model for measurement of B<sub>2</sub>-adrenoceptor function.

Diabetics with extensive autonomic neuropathy may therefore provide a model in man for investigating B-adrenoceptor blocking drugs with PAA. The aim of this study was to determine the effects of three different B-adrenoceptor blocking drugs: epanolol (B<sub>1</sub>-selective with PAA (Pringle et al, 1986)), atenolol (B<sub>1</sub>-selective with no PAA) and pindolol (non-selective with PAA) on heart rate and physiological tremor in diabetics with autonomic neuropathy. Their selectivity or otherwise has been

established in man (Harry, 1977; Aellig, 1982; Norris et al, 1984; Pringle et al, 1986). In animals, atenolol has been shown to have no PAA, while epanolol and pindolol do (Harry, Knapp & Linden, 1974; Bilski, Robertson & Wale, 1979; Smith et al, 1983). Pindolol stimulated the heart rate of catecholamine depleted rats (a B<sub>1</sub> effect) to a maximum of 120 beats min<sup>-1</sup> (Bilski et al, 1979) whereas epanolol stimulated it only to 80 beats min<sup>-1</sup> (Data for clinical investigators on epanolol - ICI document), and therefore pindolol has more PAA than epanolol.

### 3.2 Subjects

Eight diabetic men (six taking insulin and two on oral hypoglycaemic agents) aged 34 - 58 (mean 48.5) years and mean duration of diabetes 6 - 28 (mean 17) years with symptomatic autonomic neuropathy and markedly abnormal cardiovascular reflex function with involvement of both heart rate and blood pressure tests (table II) were studied. No subject had an abnormal 12-lead electrocardiograph (ECG) or evidence of airways obstruction. The insulin or tablet regimes were not changed in any patient during the study.

### 3.3 Drugs used

The drugs used were epanolol (200 mg), atenolol (50 mg), pindolol (5 mg) and placebo, and the doses chosen were the 'unit doses' used in clinical practice. At these doses

each drug has a 24 hour duration of action in man (Aellig, 1976; Harry, 1977; Floras et al, 1982; Pringle et al, 1986). The drugs were given as single oral doses in a double-blind randomised order with at least one week between dosing. Each patient received each drug and was therefore studied on four occasions.

#### 3.4. Procedures

On each study day, the subjects were fitted with an ambulatory ECG tape recorder in the late morning, following which they were given their midday meal. Baseline measurements of resting heart rate, physiological tremor and blood glucose were made between 1200 hours and 1300 hours. The patients then received their single randomised dose of drug. Two and four hours after taking the tablets further measurements of heart rate, tremor and blood glucose were made. The patients were then allowed home to their usual daily activities while ambulatory monitoring was continued until lunchtime the following day.

#### 3.5 Analysis

Missing data - there was a technical fault in part of the 24 hour ECG recording of patient 1 after placebo, and this data has been omitted from analysis. The tremor recording 4 hours after atenolol on patient 8 was also technically unsatisfactory. The only glucose results

obtained from patient 3 were at baseline and 2 hours after pindolol. No blood glucose results were obtained on patient 4 after atenolol. All other data obtained from all the subjects was used in the statistical analysis of the results.

The twenty four hour heart rate measurements were analysed over three periods: a first waking period of 1400 hours - 2300 hours; a second period of 2300 hours - 0800 hours, when the subjects were assumed to be asleep; and 0800 hours - 1300 hours the following morning.

### 3.6 RESULTS

#### Heart rate

Mean supine resting heart rates at baseline, and 2 and 4 hours after each drug are shown in table III. Atenolol lowered heart rate significantly, whereas at 4 hours the heart rate after pindolol was higher than placebo, epanolol and atenolol. The heart rate following epanolol was similar to placebo but significantly higher than atenolol at 2 and 4 hours. The 24 hour mean heart rates are shown in table IV and figure 2. During the 'waking' hours (1400 - 2300 hours), the mean heart rates on epanolol and atenolol were less than placebo or pindolol with atenolol lower than epanolol. By contrast during the sleeping hours 2300 - 0800 hours the heart rate on

pindolol was significantly higher than placebo, epanolol and atenolol, while the heart rate on atenolol was lower than on epanolol or placebo.

#### **Physiological tremor**

There were no significant changes seen in baseline physiological tremor over the four different study days thus showing that the tremor measurements were reproducible (table V, figure 3). At 4 hours post dose pindolol produced a significant increase in physiological tremor when compared with placebo, epanolol and atenolol. There were no differences between the tremor measurements on placebo, epanolol or atenolol (table V, figure 4).

#### **Plasma glucose**

Plasma glucose did not change significantly during the 4 hour study period after placebo, epanolol or atenolol (table VI, figure 5). However, there was a rise after pindolol, which was significantly different from both epanolol and atenolol.

### **3.7 DISCUSSION**

In this study, despite looking at a group of diabetics in whom there was clear evidence of both cardiac parasympathetic and sympathetic dysfunction, no direct stimulating effects of B-adrenoreceptor blocking drugs

with PAA were shown. However, relative changes in heart rate emerged which were more marked during sleep, and which could be considered to be dependent upon the different degrees of PAA in the drug used. If there had been complete absence of any sympathetic neural influence, as in the animal model, no drop in heart rate after atenolol would have been observed, and an increased heart rate after the drugs with partial agonism would have been seen, most marked on pindolol. From this evidence it might be inferred that complete sympathetic denervation was not present in these diabetics with severe autonomic neuropathy. These results contrast with those reported by Man in't Veld, Boomsa & Schalekamp (1982), who studied four patients with autonomic failure (two with amyloidosis and two with idiopathic orthostatic hypotension) after treatment with pindolol, both acutely by intravenous injection and chronically by oral administration. A rise in heart rate was demonstrated on both occasions. It seems likely that sympathetic denervation in these subjects was more complete than in the diabetics. Although my diabetics were severely affected, with grossly abnormal tests of cardiovascular reflex function, it is apparent that complete denervation was not present in them. It is interesting to speculate on whether or not it occurs in the later stages of the disease, when renal insufficiency intervenes (Ewing et al, 1980), or whether 'complete' denervation occurs at all in the disease. It

would also be useful to know whether heart rate falls in PAF subjects after dosing with atenolol. I am not aware of any literature on this subject.

The diurnal variation in heart rate in these diabetics was affected by the B-adrenoceptor blocking agents used in this study in proportion to the amount of partial agonism present. The resting supine heart rate fell during the 4 hours after dosage even on placebo, reflecting the normal afternoon heart rate pattern, and both epanolol and atenolol lowered the heart rate significantly more than pindolol, with atenolol producing the lowest heart rate. Pindolol, by contrast, lowered heart rate significantly less and at 4 hours after dosing the heart rate was significantly higher than on placebo. This pattern of heart rate change was also reflected in the 24 hour ECG tape recording. At night on pindolol, the drug in this study with most PAA, the heart rate was significantly higher than on placebo. On epanolol, which has less PAA than pindolol, the heart rate was similar to that on placebo, whilst after atenolol, with no PAA, the heart rate was significantly lower.

I have assumed that the heart rate changes after dosing with these drugs are mediated through cardiac B<sub>1</sub>-adrenoceptors. Epanolol and atenolol are B<sub>1</sub>-selective at the doses used and would appear to affect heart rate by

this means. It could, however, be argued, from animal experiments, that pindolol, as well as acting on B<sub>1</sub>-adrenoceptors, might also be directly stimulating cardiac B<sub>2</sub>-adrenoceptors to effect a relative increase in heart rate (Clark, Menninger & Bertholet, 1982). As yet there is no convincing support for this contention in man, although there is suggestive evidence that B<sub>2</sub>-adrenoceptors may be present in the atria (Brown, MacLeod & Shand, 1983; Brodde et al, 1983). Irrespective of the mechanism of the stimulant effect of pindolol it is clear that the heart rate results are consistent with epanolol and pindolol possessing partial agonism with respect to heart rate and that pindolol appears to have a greater effect on heart rate than epanolol.

The changes in physiological tremor produced by B-adrenoceptor blocking or stimulating agents can be used as a model of B<sub>2</sub>-adrenoceptor activity. B<sub>2</sub>-adrenoceptor agonists such as isoprenaline increase tremor, and differences between B<sub>1</sub>-selective and non-selective B-adrenoceptor blocking drugs can be detected in man (Arnold & McDevitt, 1983). As epanolol and atenolol are both selective B<sub>1</sub>-adrenoceptor blocking drugs at this dosage, it was not surprising that physiological tremor was unchanged after these drugs. By contrast there was a significant increase in tremor values after the non-cardioselective drug pindolol which is known to have PAA.

The effect of pindolol on tremor has not been previously demonstrated. These observations suggest that of the drugs studied, only pindolol has B<sub>2</sub>-adrenoceptor stimulating properties.

The surprising finding that pindolol, in contrast to the two cardioselective B-adrenoceptor blockers, increased blood glucose values also suggests that B<sub>2</sub>-adrenoceptors are stimulated by this drug. Isoprenaline (a B<sub>1</sub>- and B<sub>2</sub>-adrenoceptor agonist), when given intravenously to man, increased blood glucose which was reduced by ICI 118,551 (a B<sub>2</sub>-selective antagonist) and not by atenolol (Arnold et al, 1985). This suggests that sympathomimetic amine-induced increases in glucose are due to stimulation of B<sub>2</sub>-adrenoceptors probably promoting glycogenolysis in the liver (Weiner & Taylor, 1985; Rizza et al, 1980). The rise in glucose after pindolol in this study could therefore be explained by stimulation of B<sub>2</sub>-adrenoceptors. This effect is probably mediated by denervation supersensitivity as DAN +ve patients also exhibit enhanced metabolic responses to catecholamines compared to normals and DAN -ve patients (Hilsted et al, 1987).

The results presented in this study clearly demonstrate that epanolol and pindolol show evidence of partial agonism with their effects on heart rate. By contrast, stimulation of tremor and blood glucose were seen only

with pindolol. Pindolol thus appears to have PAA at both B<sub>1</sub>- and B<sub>2</sub>-adrenoceptors, at least in the doses used in this study. Similar results might be seen in normal subjects and indeed some preliminary evidence (McCaffrey, Riddell & Shanks, 1986) supports this view. Despite the limitations of this study therefore, using diabetics with severe autonomic neuropathy as a model of denervation, changes in both B<sub>1</sub>- and B<sub>2</sub>-adrenoceptor function have been observed which probably reflect the amount and selectivity of partial agonist activity in the drugs used.

| Age<br>(yrs)  | Duration<br>(yrs) | Valsalva<br>ratio | 30:15<br>ratio | Heart rate<br>variation<br>(beats min <sup>-1</sup> ) | Systolic<br>BP<br>fall on<br>standing<br>(mmHg) | Diastolic<br>BP<br>rise during<br>sustained<br>handgrip<br>(mmHg) | Autonomic<br>'score' |   |
|---------------|-------------------|-------------------|----------------|---|---|---|----------------------|---|
| 1             | 34                | 17                | 1.04           | 0.97  | 5   | 78  | 26                   | 8 |
| 2             | 51                | 28                | 1.00           | 1.00  | 3   | 50  | 28                   | 8 |
| 3             | 51                | 18                | 1.01           | 1.03  | 2   | 26  | 26                   | 6 |
| 4             | 52                | 22                | 1.06           | 1.00  | 1   | 22  | 14                   | 8 |
| 5             | 51                | 11                | 1.41           | 1.00  | 8   | 30  | 21                   | 6 |
| 6             | 58                | 12                | 1.07           | 0.97  | 17  | 70  | 14                   | 7 |
| 7             | 50                | 6                 | 1.80           | 0.93  | 3   | 36  | 15                   | 7 |
| 8             | 44                | 24                | 1.14           | 0.97  | 0   | 26  | 19                   | 7 |
| Normal values |                   | > 1.21            | > 1.04         | > 15  | < 10  | > 16  |                      |   |

(Chapter 3)

AGE, DURATION OF DIABETES AND CARDIOVASCULAR REFLEX TEST RESULTS  
IN EIGHT DIABETICS STUDIED

TABLE II

|          | <u>Baseline</u> | <u>2 hours</u> | <u>4 hours</u> |
|----------|-----------------|----------------|----------------|
| PLACEBO  | 83.5 ± 4.8      | 80.0 ± 3.8     | 77.0 ± 3.3     |
| EPANOLOL | 83.3 ± 4.1      | 76.4 ± 3.9     | 74.4 ± 3.1     |
| ATENOLOL | 84.6 ± 3.6      | 68.8 ± 2.2     | 67.5 ± 2.2     |
| PINDOLOL | 84.0 ± 3.6      | 80.0 ± 2.4     | 80.9 ± 2.6     |

SIGNIFICANCE VALUES

|                      | <u>2 hours</u> | <u>4 hours</u> |
|----------------------|----------------|----------------|
| placebo vs epanolol  | NS             | NS             |
| placebo vs atenolol  | p < 0.01       | p < 0.001      |
| placebo vs pindolol  | NS             | p < 0.05       |
| epanolol vs atenolol | p < 0.05       | p < 0.01       |
| epanolol vs pindolol | NS             | p < 0.01       |
| atenolol vs pindolol | p < 0.001      | p < 0.001      |

(Chapter 3)

GROUP MEAN RESTING SUPINE HEART RATES (beats min<sup>-1</sup>) AT  
 BASELINE, TWO AND FOUR HOURS AFTER TAKING EACH OF  
 THE DRUGS OR PLACEBO (MEAN ± SEM) (n = 8)

TABLE III

|          | <u>14.00 - 23.00 h</u> | <u>23.00 - 08.00 h</u> | <u>08.00 - 13.00 h</u> |
|----------|------------------------|------------------------|------------------------|
| PLACEBO  | 89.0 ± 1.3             | 76.6 ± 1.2 (n = 7)     | 91.4 ± 1.6 (n = 7)     |
| EPANOLOL | 81.5 ± 1.0             | 76.5 ± 0.9             | 86.0 ± 1.2             |
| ATENOLOL | 72.9 ± 0.8             | 68.0 ± 0.9             | 78.1 ± 1.2             |
| PINDOLOL | 85.8 ± 0.9             | 84.4 ± 1.4             | 90.1 ± 1.5             |

SIGNIFICANCE VALUES

|                      | <u>14.00 - 23.00 h</u> | <u>23.00 - 08.00 h</u> | <u>08.00 - 13.00 h</u> |
|----------------------|------------------------|------------------------|------------------------|
| placebo vs epanolol  | p < 0.05               | NS                     | p < 0.05               |
| placebo vs atenolol  | p < 0.001              | p < 0.01               | p < 0.001              |
| placebo vs pindolol  | NS                     | p < 0.05               | NS                     |
| epanolol vs atenolol | p < 0.05               | p < 0.05               | NS                     |
| epanolol vs pindolol | p < 0.05               | p < 0.01               | NS                     |
| atenolol vs pindolol | p < 0.001              | p < 0.001              | p < 0.01               |

(Chapter 3)

GROUP MEAN HEART RATES (beats min<sup>-1</sup>) DURING THE 24 HOUR ECG RECORDINGS

AFTER TAKING EACH OF THE DRUGS OR PLACEBO (MEAN ± SEM)

(n = 8 except where indicated)

TABLE IV

|          | <u>Baseline</u> | <u>2 hours</u> | <u>4 hours</u>       |
|----------|-----------------|----------------|----------------------|
| PLACEBO  | 418.7 ± 14.8    | 409.0 ± 16.3   | 395.7 ± 14.8         |
| EPANOLOL | 419.8 ± 10.3    | 406.0 ± 16.2   | 402.0 ± 16.4         |
| ATENOLOL | 418.6 ± 13.7    | 402.9 ± 12.9   | 394.0 ± 13.8 (n = 7) |
| PINDOLOL | 417.1 ± 16.1    | 422.6 ± 17.8   | 425.8 ± 17.6         |

SIGNIFICANCE VALUES

|                      | <u>2 hours</u> | <u>4 hours</u> |
|----------------------|----------------|----------------|
| placebo vs epanolol  | NS             | NS             |
| placebo vs atenolol  | NS             | NS             |
| placebo vs pindolol  | NS             | p < 0.01       |
| epanolol vs atenolol | NS             | NS             |
| epanolol vs pindolol | NS             | p < 0.05       |
| atenolol vs pindolol | NS             | p < 0.05       |

(Chapter 3)

GROUP MEAN TREMOR MEASUREMENTS (LOG TREMOR READINGS SUMMED  
OVER FREQUENCY) AT BASELINE, TWO AND FOUR HOURS AFTER  
TAKING EACH OF THE DRUGS OR PLACEBO (MEAN ± SEM)  
(n = 8 except where indicated)

TABLE V

|          | <u>Baseline</u>  | <u>2 hours</u>   | <u>4 hours</u>   |
|----------|------------------|------------------|------------------|
| PLACEBO  | 11.1 ± 1.5 (n=7) | 11.6 ± 1.9 (n=7) | 11.6 ± 1.3 (n=7) |
| EPANOLOL | 14.4 ± 3.5 (n=7) | 14.6 ± 2.5 (n=7) | 13.3 ± 2.5 (n=7) |
| ATENOLOL | 10.1 ± 2.7 (n=6) | 11.2 ± 2.1 (n=6) | 10.5 ± 1.7 (n=6) |
| PINDOLOL | 11.2 ± 2.3 (n=8) | 15.1 ± 2.1 (n=8) | 16.3 ± 2.3 (n=7) |

SIGNIFICANCE VALUES

|                      | <u>2 hours</u> | <u>4 hours</u> |
|----------------------|----------------|----------------|
| placebo vs epanolol  | NS             | NS             |
| placebo vs atenolol  | NS             | NS             |
| placebo vs pindolol  | p < 0.05       | NS (p=0.059)   |
| epanolol vs atenolol | NS             | NS             |
| epanolol vs pindolol | p < 0.05       | p < 0.01       |
| atenolol vs pindolol | p < 0.01       | p < 0.01       |

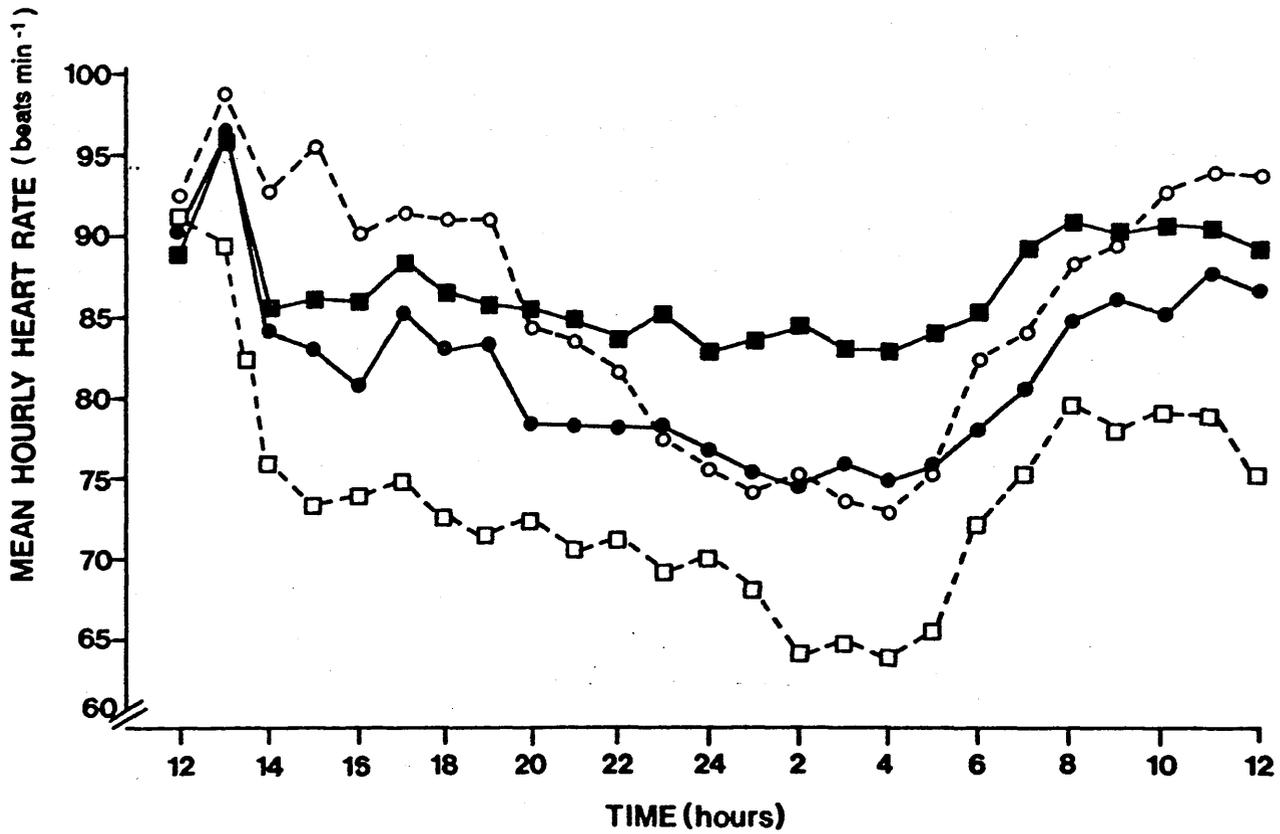
(Chapter 3)

GROUP MEAN PLASMA GLUCOSE VALUES (mmol L<sup>-1</sup>) AT BASELINE, TWO AND FOUR

HOURS AFTER TAKING EACH OF THE DRUGS OR PLACEBO (MEAN ± SEM)

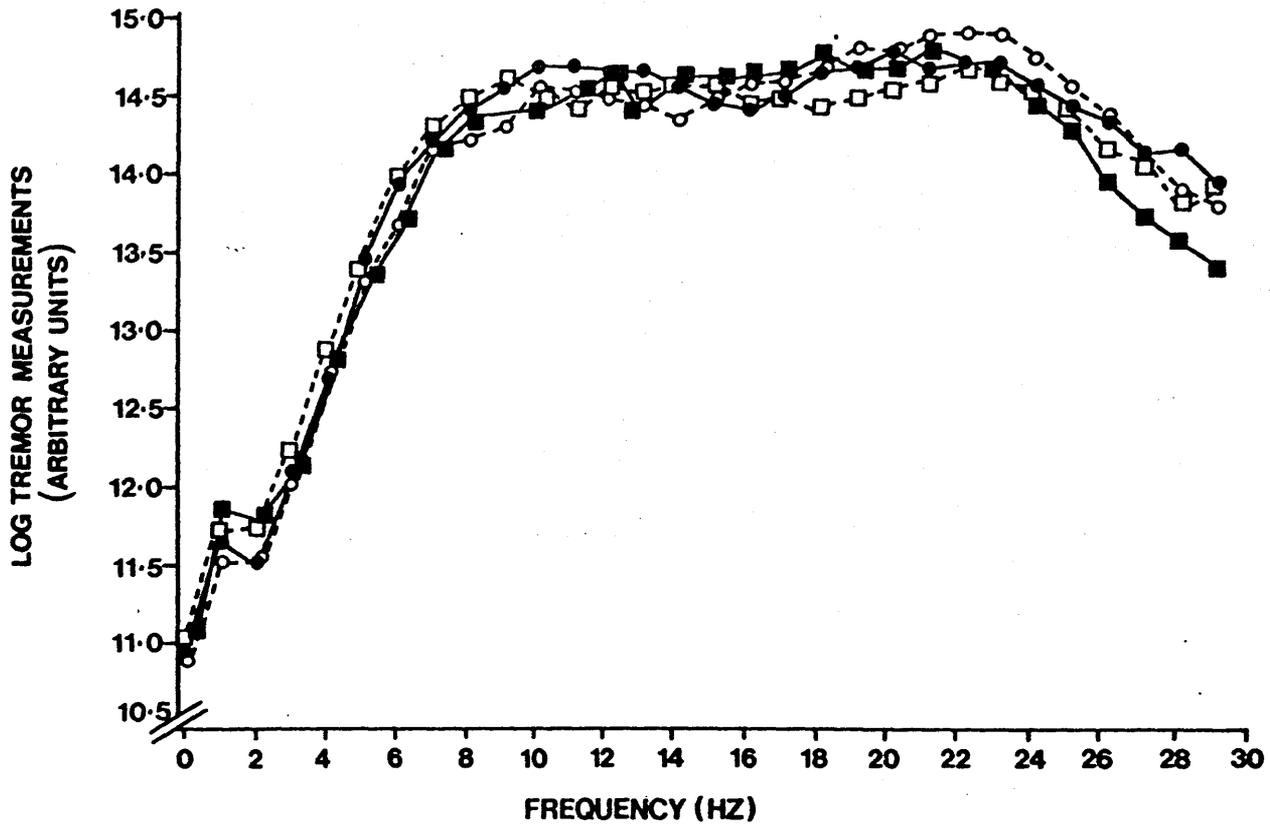
(numbers of subjects are indicated in brackets)

TABLE VI



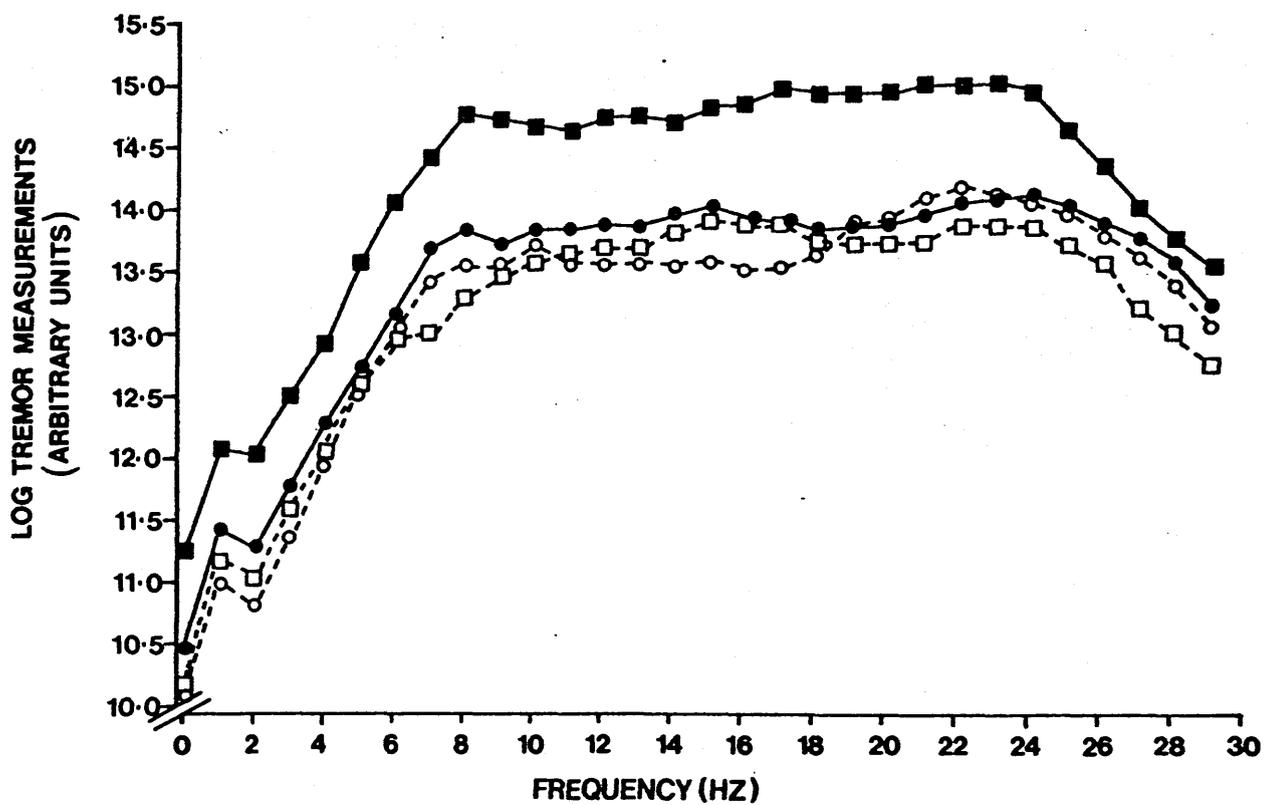
Group mean hourly heart rate during ambulatory monitoring over the 24 h after dosing with placebo (o), epanolol (●), atenolol (□) and pindolol (■).

Figure 2



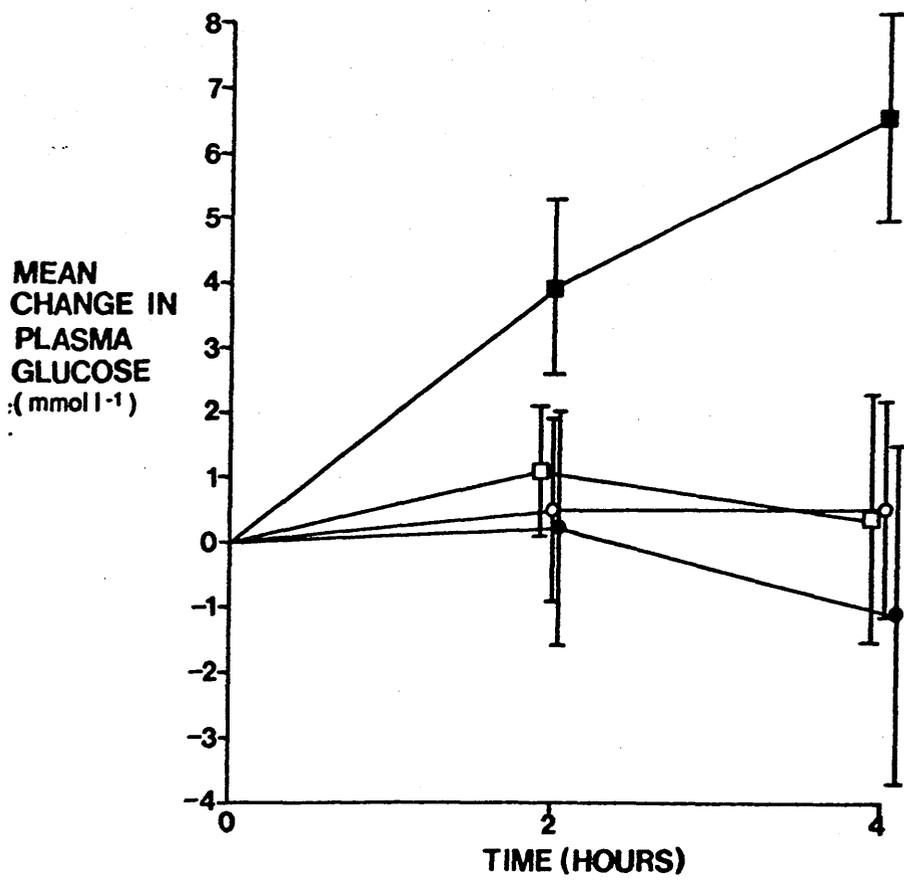
Group mean log tremor measurements at each frequency (range 0-30 Hz) at baseline before dosing with placebo (o), epanolol (●), atenolol (□) and pindolol (■).

Figure 3



Group mean log tremor measurements at each frequency (range 0-30 Hz) 4 h after dosing with placebo (o), epanolol (●), atenolol (□) and pindolol (■).

Figure 4



Group mean ( $\pm$  SEM) change from baseline in plasma glucose 2 and 4 h after dosing with placebo (o), epanolol (●), atenolol (□) and pindolol (■).

Figure 5

**VASOPRESSIN SECRETION FOLLOWING OSMOTIC STIMULATION  
WITH HYPERTONIC SALINE IN DIABETICS WITH AND  
WITHOUT AUTONOMIC NEUROPATHY**

**4.1. SUBJECTS**

Eleven male insulin-dependent diabetics were studied: five without (DAN -ve) and six with (DAN +ve) cardiovascular reflex test evidence of autonomic neuropathy. Within the two groups there was a similar distribution of ages and duration of diabetes (table VII). All six DAN +ve subjects had one or more symptoms suggestive of autonomic neuropathy together with clinical evidence of somatic nerve involvement. Proteinuria (albusix +ve) was present in 1 DAN -ve and 1 DAN +ve patients, while retinopathy was present in 2 DAN -ve (1 background, 1 proliferative) and all 6 DAN +ve patients (1 background, 5 proliferative) patients. Plasma creatinine values ranged from 86 to 123 (mean DAN -ve 99, DAN +ve 104)  $\mu\text{mol l}^{-1}$  in the eleven subjects in whom it was measured.

The studies were conducted in a quiet room under standard conditions. In addition to the diabetics, five normal

volunteers aged 35 - 48 (mean 43) years took part, using an identical protocol. The subjects fasted overnight and (the diabetics) delayed their usual morning insulin dose until after the study was completed. They were asked to abstain from alcohol, caffeine and smoking for at least twelve hours prior to the study, which commenced at around 0900 hours. No subjects were receiving any drugs other than insulin. None complained of nausea (a powerful stimulus to AVP secretion) during the studies.

#### 4.2 Protocol

Each subject lay supine while an intravenous cannula was inserted into each antecubital fossa: the left for sampling and the right for infusion. After thirty minutes rest, an infusion of 5% saline was given at a rate of  $0.05 \text{ mg kg body weight}^{-1} \text{ min}^{-1}$  over the next 120 minutes. Blood was withdrawn at -10, -5, 0, +30, +60, +90 and +120 minutes for estimation of haematocrit, plasma osmolality, sodium, glucose and AVP. Heart rate and blood pressure were measured at -10, -5 and 0 and then at 10 minute intervals throughout the study. Further details of analysis are given in chapter 2.

#### 4.3 RESULTS

There were no significant differences in baseline values between the groups with respect to plasma osmolality,

sodium, plasma AVP and haematocrit. Plasma glucose was similarly raised in the two diabetic groups (table VIIIb). Baseline blood pressure and heart rate were slightly elevated in the DAN +ve group (figure 6).

During the 120 minute 5% saline infusion, plasma osmolality and sodium rose appropriately in parallel, with no significant differences in response between the three groups (table VIIIa, figure 7). An elevation of plasma AVP accompanied the rises in osmolality and sodium, so that there was a very close linear relationship between both plasma osmolality and AVP, and plasma sodium and AVP, in each of the groups (table IX). There were no significant differences in the plasma AVP area under the curve responses between the three groups.

Haematocrit fell slightly in all three groups, while plasma glucose remained unchanged (table VIIIb, figure 7). There were no significant differences in the mean blood pressure and heart rate responses between the three groups, although blood pressure rose most in the DAN +ve group. One DAN +ve subject (no 8) had a 30 mmHg rise in systolic blood pressure at 110 minutes, and for safety reasons the study was stopped at this point. Samples taken then were considered equivalent to 120 minutes.

#### 4.4 DISCUSSION

This work demonstrates that osmotic release of AVP after infusion of hypertonic saline appears to be intact in diabetics both with and without autonomic neuropathy. Similar rises in plasma osmolality after five per cent saline infusion produced appropriate rises in plasma AVP in the diabetic and normal subjects, with a very close correlation between plasma osmolality and AVP, and plasma sodium and AVP during the infusion period. This relationship has been well recognised previously (Zerbe, Vinicor & Robertson, 1985; Vokes et al, 1987; Thompson et al, 1988), and there were no significant differences in AVP response between the three groups.

Diabetics with and without autonomic neuropathy therefore appear to respond in a normal fashion to an osmotic stimulus. This effectively excludes any significant abnormality of hypothalamic or posterior pituitary function and demonstrates the integrity of the neural pathways involved in osmotic control of AVP. Other groups with abnormalities of autonomic function have also been shown to respond normally, for example, patients with primary autonomic failure (Williams, Lightman & Bannister, 1985) and subjects with spinal cord transection (Poole et al, 1987).

Although one of the DAN +ve subjects had a marked hypertensive response to hypertonic saline infusion, no significant group changes were observed in the diabetics. This is in contrast to those results found in patients with PAF (Williams, Lightman & Bannister, 1985) and in tetraplegics (Poole et al, 1987), in both of whom hypertonic saline produced a significant pressor response, which at least in part seems to be secondary to increased sensitivity to AVP at vascular level. Chapter 6 will address this issue further in a diabetic group.

| Patient No           | Age (yrs) | Duration of diabetes (yrs) | Retinopathy status (B=background (E=exudative (P=proliferative) | Proteinuria (albustix) | Plasma creatinine ( $\mu\text{mol l}^{-1}$ ) | Valsalva ratio | 30:15 ratio | Heart rate variation (beats $\text{min}^{-1}$ ) | Systolic BP fall on standing (mmHg) | Diastolic BP rise during grip (mmHg) | Autonomic score (0-10) |
|----------------------|-----------|----------------------------|---|------------------------|--|----------------|-------------|---|-------------------------------------|--------------------------------------|------------------------|
| 1                    | 29        | 27                         | P   | +                      | 92   | 1.34           | 1.27        | 14  | 2                                   | 21                                   | 1                      |
| 2                    | 45        | 24                         | -   | -                      | 86   | 1.66           | 1.60        | 38  | 4                                   | 30                                   | 0                      |
| 3                    | 58        | 15                         | -   | -                      | -  | 1.35           | 1.31        | 15  | +4                                  | 25                                   | 0                      |
| 4                    | 48        | 15                         | -   | -                      | 123  | 2.39           | 1.79        | 33  | +8                                  | 22                                   | 0                      |
| 5                    | 38        | 22                         | B   | -                      | 95   | 1.28           | 1.25        | 14  | 22                                  | 18                                   | 2                      |
| <b>DAN +ve</b>       |           |                            |   |                        |  |                |             |   |                                     |                                      |                        |
| 6                    | 51        | 28                         | P   | -                      | 103  | 1.00           | 1.00        | 3   | 50                                  | 28                                   | 8                      |
| 7                    | 48        | 18                         | B   | -                      | 97   | 1.03           | 1.00        | 3   | 50                                  | 12                                   | 9                      |
| 8                    | 53        | 7                          | P   | -                      | 110  | 1.09           | 1.00        | 1   | 36                                  | 25                                   | 8                      |
| 9                    | 70        | 34                         | P   | ++                     | 103  | -              | 1.03        | 1   | 30                                  | 14                                   | (6/8)                  |
| 10                   | 58        | 16                         | P   | -                      | 98   | -              | 1.14        | 4   | 20                                  | 10                                   | (5/8)                  |
| 11                   | 40        | 20                         | P   | -                      | 115  | -              | 1.00        | 8   | 60                                  | 18                                   | (6/8)                  |
| <b>Normal values</b> |           |                            |   |                        |  |                |             |   |                                     |                                      |                        |
|                      |           |                            |   |                        | < 130  | > 1.21         | > 1.04      | > 15  | < 10                                | > 16                                 |                        |

(Chapter 4) AGE, DURATION OF DIABETES, DETAILS OF COMPLICATIONS AND CARDIOVASCULAR REFLEX TESTS  
IN THE DIABETICS UNDERGOING 5% SALINE INFUSION

|  | TIME (min)     |                 |                |                |                 | Area under curve 0-120 min (Arbitrary units) | Difference from baseline |
|--|----------------|-----------------|----------------|----------------|-----------------|--|--------------------------|
|  | Baseline       | 30              | 60             | 90             | 120             |  |                          |
| <b>PLASMA OSMOLALITY</b><br>(mosm kg <sup>-1</sup> ) |                |                 |                |                |                 |  |                          |
| DAN +ve<br>(n = 6)                                   | 291.7<br>(4.8) | 295.3<br>(5.0)  | 299.3<br>(5.4) | 302.2<br>(4.4) | 306.0<br>(4.8)  | 870<br>(121)                                 | p < 0.001                |
| DAN -ve<br>(n = 5)                                   | 286.4<br>(6.1) | 289.2<br>(6.0)  | 294.8<br>(4.3) | 298.4<br>(3.5) | 302.0<br>(4.0)  | 930<br>(238)                                 | p < 0.001                |
| Normals<br>(n = 5)                                   | 285.0<br>(3.4) | 287.6<br>(4.0)  | 290.6<br>(4.5) | 293.4<br>(4.8) | 295.0<br>(5.0)  | 648<br>(198)                                 | p < 0.005                |
| F value  | 2.90 (NS)      |                 |                |                |                 | 3.18 (NS)                                    |                          |
| <b>PLASMA SODIUM</b><br>(mmol L <sup>-1</sup> )      |                |                 |                |                |                 |  |                          |
| DAN +ve  | 136.3<br>(3.2) | 138.2<br>(3.5)  | 140.3<br>(3.6) | 142.7<br>(3.1) | 144.3<br>(2.6)  | 485<br>(73)                                  | p < 0.001                |
| DAN -ve  | 138.0<br>(1.6) | 139.6<br>(1.5)  | 140.6<br>(1.3) | 143.2<br>(2.0) | 145.0<br>(2.0)  | 387<br>(63)                                  | p < 0.001                |
| Normals  | 139.0<br>(1.2) | 140.4<br>(2.7)  | 143.2<br>(1.8) | 144.2<br>(1.9) | 145.8<br>(1.9)  | 426<br>(118)                                 | p < 0.005                |
| F value  | 1.94 (NS)      |                 |                |                |                 | 1.78 (NS)                                    |                          |
| <b>PLASMA AVP</b><br>(pmol L <sup>-1</sup> )         |                |                 |                |                |                 |  |                          |
| DAN +ve  | 1.26<br>(0.78) | 1.63<br>(0.96)  | 2.21<br>(1.38) | 3.76<br>(2.38) | 5.24<br>(3.24)  | 174<br>(109)                                 | p < 0.02                 |
| DAN -ve  | 1.80<br>(0.64) | 2.78*<br>(1.23) | 3.38<br>(2.04) | 3.80<br>(1.5)  | 5.32<br>(2.99)  | 168<br>(225)                                 | NS                       |
| Normals  | 0.94<br>(0.91) | 1.48<br>(0.85)  | 2.33<br>(0.79) | 2.76<br>(0.59) | 5.02*<br>(2.03) | 155<br>(50)                                  | p < 0.005                |
| F value  | 1.54 (NS)      |                 |                |                |                 | 0.02 (NS)                                    |                          |

\* n = 4

(Chapter 4)

GROUP MEAN (SD) BIOCHEMICAL AND OSMOLALITY RESPONSES DURING 120 MINUTES OF

5% SALINE INFUSION IN DIABETICS WITH (DAN +VE), DIABETICS WITHOUT

(DAN -ve) AUTONOMIC NEUROPATHY, AND NORMAL SUBJECTS

TABLE VIIIa

TABLE VIIIa

|   | Baseline      | TIME (min)    |               |               |               | Area under<br>curve 0-120 min<br>(Arbitrary units) | Difference<br>from<br>baseline |
|---|---------------|---------------|---------------|---------------|---------------|--|--------------------------------|
|   |               | 30            | 60            | 90            | 120           |  |                                |
| <b>HAEMATOCRIT (%)</b>                          |               |               |               |               |               |  |                                |
| DAN +ve   | 40.5<br>(2.9) | 40.0<br>(3.2) | 39.8<br>(3.7) | 39.0<br>(3.6) | 38.7<br>(3.4) | -107<br>(70)                                       | p < 0.05                       |
| DAN -ve   | 41.2<br>(2.4) | 40.6<br>(2.9) | 39.8<br>(3.4) | 40.0<br>(2.7) | 39.6<br>(3.0) | -120<br>(78)                                       | p < 0.02                       |
| Normals   | 41.4<br>(2.4) | 40.2<br>(2.6) | 39.4<br>(2.4) | 39.0<br>(2.5) | 38.2<br>(2.8) | -216<br>(55)                                       | p < 0.001                      |
| F value   | 0.18 (NS)     |               |               |               |               | 3.93 (p<0.05)                                      |                                |
| <b>PLASMA GLUCOSE<br/>(mmol L<sup>-1</sup>)</b> |               |               |               |               |               |  |                                |
| DAN +ve   | 10.3<br>(4.8) | 10.3<br>(4.6) | 10.4<br>(4.9) | 10.8<br>(5.7) | 10.8<br>(5.8) | 27<br>(67)   | NS                             |
| DAN -ve   | 10.1<br>(5.2) | 9.9<br>(4.9)  | 10.4<br>(4.6) | 10.6<br>(4.3) | 10.8<br>(4.3) | 31<br>(80)   | NS                             |
| Normals   | 5.5<br>(0.7)  | 5.6<br>(0.6)  | 5.6<br>(0.6)  | 5.3<br>(0.6)  | 5.3<br>(0.7)  | 3<br>(38)  | NS                             |
| F value   | 2.20 (NS)     |               |               |               |               | 0.28 (NS)  |                                |

(Chapter 4)

GROUP MEAN (SD) BIOCHEMICAL AND OSMOLALITY RESPONSES DURING 120 MINUTES OF  
5% SALINE INFUSION IN DIABETICS WITH (DAN +ve), DIABETICS WITHOUT  
(DAN -ve) AUTONOMIC NEUROPATHY, AND NORMAL SUBJECTS

TABLE VIIIb

|  | Correlation<br>Coefficient | Slope | x intercept |
|--|----------------------------|-------|-------------|
|--|----------------------------|-------|-------------|

PLASMA OSMOLALITY

vs

PLASMA AVP

|                    |                |                |              |
|--------------------|----------------|----------------|--------------|
| DAN +ve<br>(n = 6) | 0.84<br>(0.10) | 0.28<br>(0.18) | 287<br>(8)   |
| DAN -ve<br>(n = 5) | 0.91<br>(0.13) | 0.22<br>(0.15) | 272<br>(9)   |
| Normals<br>(n = 5) | 0.89<br>(0.08) | 0.33<br>(0.10) | 283<br>(2)   |
| F value            | 0.51 (NS)      | 0.62 (NS)      | 6.1 (p<0.05) |

PLASMA SODIUM

vs

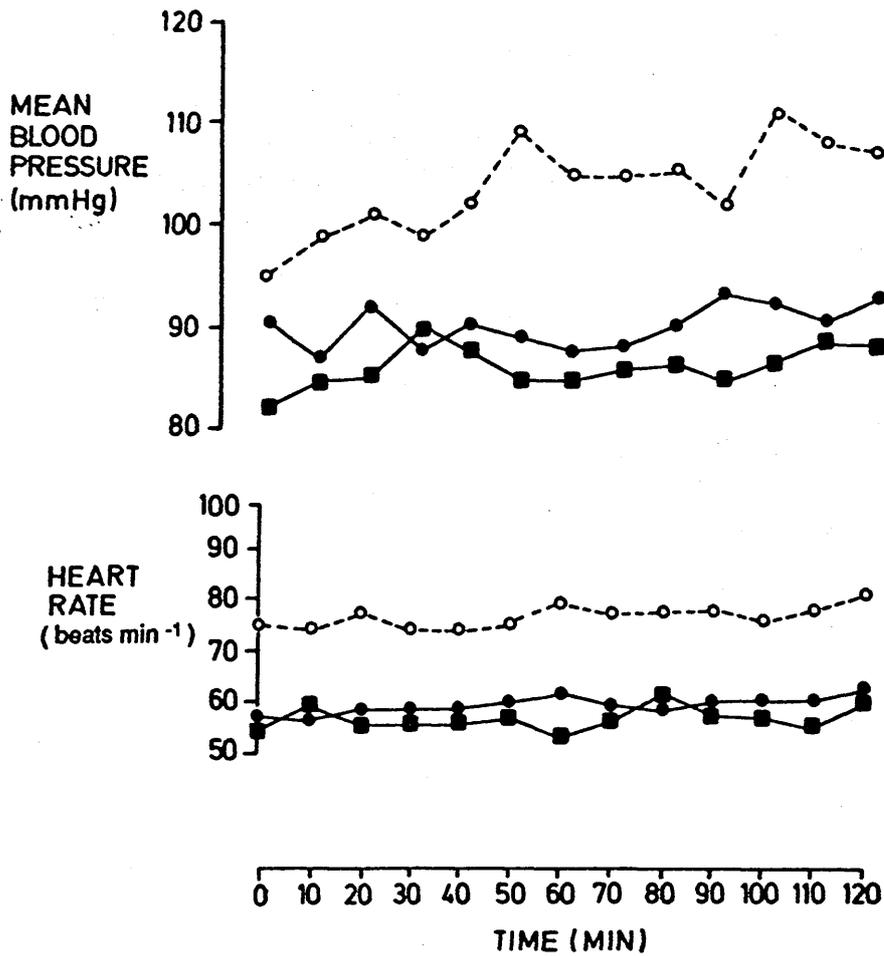
PLASMA AVP

|         |                |                |            |
|---------|----------------|----------------|------------|
| DAN +ve | 0.84<br>(0.10) | 0.50<br>(0.38) | 133<br>(5) |
| DAN -ve | 0.91<br>(0.13) | 0.53<br>(0.30) | 134<br>(2) |
| Normals | 0.89<br>(0.08) | 0.45<br>(0.11) | 137<br>(1) |
| F value | 0.62 (NS)      | 0.08 (NS)      | 1.66 (NS)  |

(Chapter 4)

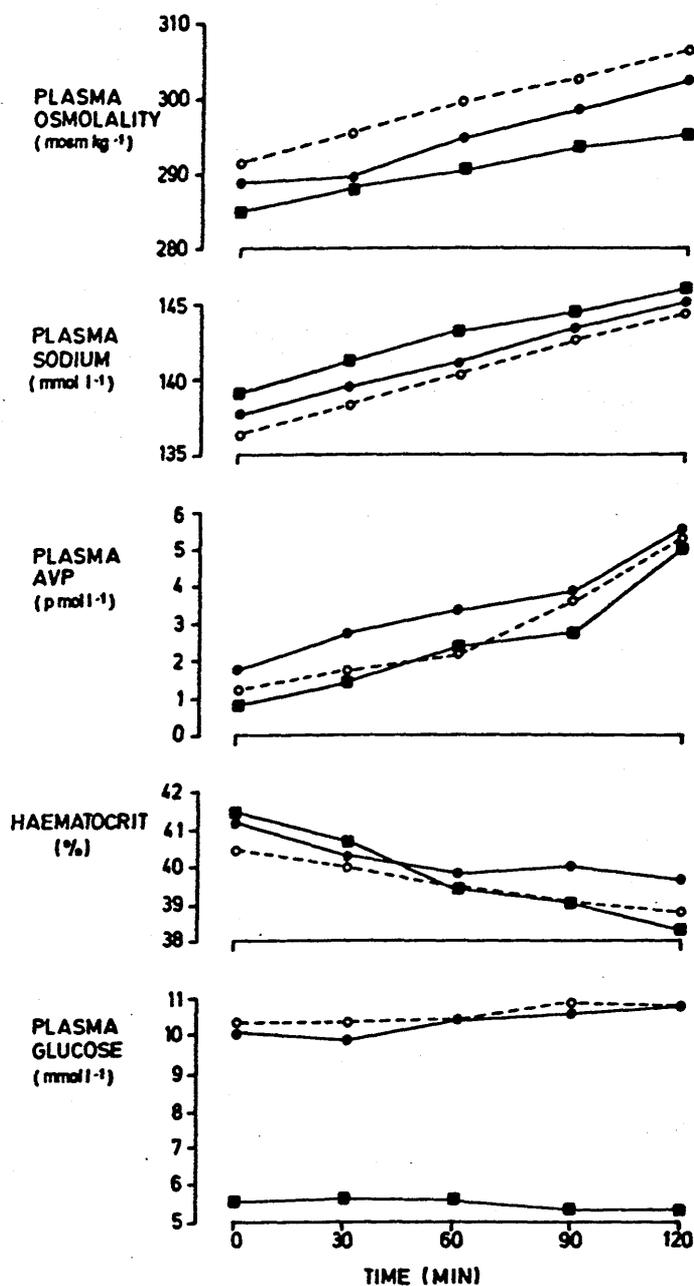
LINEAR REGRESSION RELATIONSHIPS BETWEEN PLASMA OSMOLALITY,  
SODIUM, AND AVP DURING 5% SALINE INFUSION IN DIABETICS WITH  
(DAN +ve) AND WITHOUT (DAN -ve) AUTONOMIC NEUROPATHY  
AND NORMAL SUBJECTS (MEAN (SD))

TABLE IX



Mean blood pressure and heart rate during 120 minutes of 5% saline infusion in six diabetics with (o---o), five diabetics without (●—●) autonomic neuropathy, and five normal subjects (■—■).

Figure 6



Mean biochemical and osmolality responses during 120 minutes of 5% saline infusion in six diabetics with (o---o), five diabetics without (●—●) autonomic neuropathy and five normal subjects (■—■).

Figure 7

## CHAPTER 5

### VASOPRESSIN SECRETION IN RESPONSE TO HEAD-UP TILT IN DIABETICS WITH AND WITHOUT AUTONOMIC NEUROPATHY

#### 5.1 SUBJECTS

Thirteen male insulin-dependent diabetics were studied: six without (DAN -ve) and seven with (DAN +ve) cardiovascular reflex test evidence of autonomic neuropathy. The two groups were similar in age and duration of diabetes (table X). All seven DAN +ve patients had symptoms of autonomic neuropathy and clinical evidence of somatic nerve involvement. Proteinuria (albustix +ve) was present in 1 DAN -ve and 1 DAN +ve patient, while retinopathy was present in 1 DAN -ve (proliferative) and all seven DAN +ve (2 background, 1 exudative, 4 proliferative) patients. Plasma creatinine values ranged from 81 - 123 (mean DAN -ve  $98.6 \pm 17$ ,  $n = 5$ , DAN +ve  $97.5 \pm 10$ ,  $n = 7$ )  $\mu\text{mol l}^{-1}$  in the subjects in whom it was measured.

In addition, six normal volunteers aged 35 - 48 (mean 42) years underwent tilt.

The same precautions regarding tobacco, caffeine and alcohol were observed as in chapter 4. Insulin was

withheld until after the study was completed.

## 5.2 Protocol

Each subject lay supine on a tilt table with a footrest, while an intravenous cannula was inserted into a left antecubital vein. After 30 minutes rest, the subject was tilted at 45° head-up over 15 seconds, and remained in this position for 120 minutes. Blood was withdrawn at -10, -5, 0, +30, +60, +90 and +120 minutes for haematocrit, plasma osmolality, sodium, glucose, AVP, plasma renin activity (PRA), aldosterone, and catecholamines (noradrenaline and adrenaline). Heart rate and blood pressure measurements were made at -10, -5 and 0 and then at 10 minute intervals throughout the study.

## 5.3 RESULTS

Baseline values for plasma sodium, AVP, adrenaline and aldosterone were not significantly different between the three groups (tables XIa,b, XIIa,b). Osmolality was higher and noradrenaline lower in the DAN +ve group and PRA higher in the normal group. Baseline plasma glucose values were elevated in both diabetic groups, but there were no significant differences in baseline glucose values between the two diabetic groups. Blood pressure and heart rate at baseline were higher in the DAN +ve group

(figure 8).

Immediately after tilting, blood pressure fell significantly and continued to fall throughout the 120 minute period in the DAN +ve group, (systolic blood pressure drop shown in figure 9), whereas there was a slight, but non significant rise in the DAN -ve and normal groups. Heart rate rose slightly but significantly in all three groups (figure 8).

Biochemical variables are shown in tables XIa,b and XIIa,b. Plasma osmolality and sodium altered slightly during the tilt study, while haematocrit rose similarly and significantly in all three groups. After tilt, small rises in plasma AVP, which were significant only in the normal group were seen. There were no significant differences in AVP responses between the three groups (figure 10). During the 120 minute tilt, plasma glucose increased in both diabetic groups, whereas the normal group showed no change. PRA and plasma aldosterone rose appropriately during the study in all groups, whereas plasma noradrenaline and adrenaline showed little change.

#### 5.4 DISCUSSION

There was no difference in AVP response during tilt between diabetic and normal subjects. However, the

failure of a significant AVP rise in the autonomic neuropathy group, despite a major hypotensive stimulus suggests that in this group there is, in fact, a defect in cardiovascular control of AVP release. The secretion of AVP in response to cardiovascular stimuli is dependent on intact intrathoracic receptors, afferent fibres in the IXth and Xth nerves and brain stem pathways to the hypothalamus. Subjects with progressive autonomic failure, with defective catecholamine pathways in the brain stem, have a loss of AVP response to head-up tilt in spite of the added stimulus of postural hypotension (Puritz et al, 1983). This contrasts with the amplified AVP response found in patients with selective loss of sympathetic pathways due to cervical cord transection (Sved et al, 1985; Poole et al, 1987). These results in diabetics with autonomic neuropathy reveal a subnormal AVP response to the postural fall in blood pressure that occurred on tilting. In a previous study looking at AVP levels before and after standing for two hours, four of nine diabetics with autonomic neuropathy had diminished AVP responses, but only two had postural hypotension (Cignarelli et al, 1986). In another study, abnormal AVP responses were found to volume depletion in diabetics with abnormal cardiovascular heart rate responses (Grimaldi et al, 1985).

Considerable evidence now exists to suggest that AVP

release after head-up tilt serves as an index of the integrity of afferent cardiovascular pathways (Puritz et al, 1983; Poole et al, 1987; Zerbe et al, 1983). This study suggests that diabetics with autonomic neuropathy, like subjects with PAF, also have defective cardiovascular release of AVP. It might be argued that an alternative explanation of the low AVP response in the DAN +ve group was their higher blood glucose (see 1.3.4). In this study, while the mean glucose values were higher in the DAN +ve group, they were not significantly different from the DAN -ve group.

Similarly, although the DAN +ve group were slightly older (mean 45 years) than the DAN -ves (mean 40 years) or normals (mean 42 years), the evidence for an age-related effect producing the low AVP response in these patients is poor, considering that this effect is not viewed as a general feature of ageing (Rowe et al, 1982).

Despite lower baseline plasma renin activity, the diabetic autonomic neuropathy group showed a significant rise after tilt. This is in contrast to previous studies showing low renin production in diabetic autonomic neuropathy (Campbell et al, 1976; Christlieb, Munichoodappa & Braaten, 1974), but is in keeping with more recent work (Hilsted et al, 1981a; Winocour, Dahr & Anderson, 1986).

The presence of nephropathy and possible disruption of the juxta-glomerular apparatus in end-stage diabetes, may be more likely to be responsible for a low renin response.

Plasma noradrenaline levels were lower in the autonomic neuropathy group at baseline. Previous reports have shown inconsistent patterns of resting supine noradrenaline (Cryer et al, 1978; Hilsted et al, 1981b; Ewing et al, 1986; Dejgård, Hilsted & Christensen, 1986; Matthews et al, 1987).

In conclusion, this study shows that diabetics with cardiovascular reflex evidence of autonomic neuropathy show a defective AVP response to the cardiovascular stimulus of head-up tilt. Such a pattern is also seen in patients with progressive autonomic failure but contrasts with the response in subjects with selective efferent sympathetic loss secondary to spinal cord transection. This suggests that afferent cardiovascular pathways are also damaged in these diabetic subjects.

| Patient No           | Age (yrs) | Duration of diabetes (yrs) | Retinopathy status (B=background, E=exudative, P=proliferative) | Proteinuria (albustix) | Plasma creatinine ( $\mu\text{mol l}^{-1}$ ) | Valsalva ratio | 30:15 ratio | Heart rate variation (beats $\text{min}^{-1}$ ) | Systolic BP fall on standing (mmHg) | Diastolic BP rise during grip (mmHg) | Autonomic score (0-10) |
|----------------------|-----------|----------------------------|---|------------------------|--|----------------|-------------|---|-------------------------------------|--------------------------------------|------------------------|
| <b>DAN -ve</b>       |           |                            |   |                        |  |                |             |   |                                     |                                      |                        |
| 1                    | 29        | 27                         | P   | +                      | 92   | 1.34           | 1.27        | 14  | 2                                   | 21                                   | 1                      |
| 2                    | 29        | 5                          | -   | -                      | 110  | 1.85           | 1.33        | 40  | 20                                  | 37                                   | 1                      |
| 3                    | 45        | 24                         | -   | -                      | 86   | 1.66           | 1.60        | 38  | 4                                   | 30                                   | 0                      |
| 4                    | 58        | 15                         | -   | -                      | -  | 1.35           | 1.31        | 15  | +4                                  | 25                                   | 0                      |
| 5                    | 48        | 15                         | -   | -                      | 123  | 2.39           | 1.79        | 33  | +8                                  | 22                                   | 0                      |
| 6                    | 31        | 24                         | -   | -                      | 82   | 1.37           | 1.24        | 32  | +8                                  | 31                                   | 0                      |
| <b>DAN +ve</b>       |           |                            |   |                        |  |                |             |   |                                     |                                      |                        |
| 7                    | 52        | 22                         | E   | +                      | 90   | 1.10           | 1.04        | 3   | 22                                  | 18                                   | 5                      |
| 8                    | 51        | 28                         | P   | -                      | 103  | 1.00           | 1.00        | 3   | 50                                  | 28                                   | 8                      |
| 9                    | 44        | 24                         | B   | -                      | 98   | 1.12           | 1.01        | 4   | 46                                  | 20                                   | 7                      |
| 10                   | 32        | 17                         | P   | -                      | 81   | 1.19           | 0.98        | 4   | 40                                  | 15                                   | 9                      |
| 11                   | 32        | 10                         | P   | -                      | 104  | 1.04           | 1.03        | 3   | 28                                  | 1                                    | 9                      |
| 12                   | 48        | 18                         | B   | -                      | 97   | 1.03           | 1.00        | 3   | 50                                  | 12                                   | 9                      |
| 13                   | 53        | 7                          | P   | -                      | 110  | 1.09           | 1.00        | 1   | 36                                  | 25                                   | 8                      |
| <b>Normal values</b> |           |                            |   |                        |  |                |             |   |                                     |                                      |                        |
|                      |           |                            |   |                        | < 130  | > 1.21         | > 1.04      | > 15  | < 10                                | > 16                                 |                        |

(Chapter 5) AGE, DURATION OF DIABETES, DETAILS OF COMPLICATIONS AND CARDIOVASCULAR REFLEX TESTS IN THE DIABETICS UNDERGOING 45° HEAD-UP TILT

TABLE X

|  | TIME (min)     |                |                |                |                | Area under curve 0-120 min (Arbitrary units) | Difference from baseline |
|--|----------------|----------------|----------------|----------------|----------------|--|--------------------------|
|  | Baseline       | 30             | 60             | 90             | 120            |  |                          |
| <b>PLASMA OSMOLALITY</b><br>(mosm kg <sup>-1</sup> ) |                |                |                |                |                |  |                          |
| DAN +ve<br>(n = 7)                                   | 292.3<br>(3.7) | 293.3<br>(3.9) | 293.9<br>(2.8) | 294.3<br>(3.5) | 295.0<br>(3.2) | 178<br>(144)                                 | p < 0.02                 |
| DAN -ve<br>(n = 6)                                   | 283.0<br>(4.8) | 283.2<br>(5.2) | 283.3<br>(4.5) | 283.0<br>(5.1) | 283.8<br>(5.1) | 27<br>(198)                                  | NS                       |
| Normals<br>(n = 6)                                   | 283.7<br>(2.0) | 283.8<br>(1.9) | 284.0<br>(2.6) | 284.7<br>(2.1) | 285.0<br>(2.0) | 65<br>(93)                                   | NS                       |
| F value  | 13.20 (p<0.01) |                |                |                |                | 1.70 (NS)                                    |                          |
| <b>PLASMA SODIUM</b><br>(mmol L <sup>-1</sup> )      |                |                |                |                |                |  |                          |
| DAN +ve  | 137.8<br>(2.9) | 137.6<br>(2.9) | 137.0<br>(3.3) | 137.1<br>(3.1) | 136.7<br>(3.5) | -73<br>(78)                                  | p < 0.05                 |
| DAN -ve  | 138.3<br>(1.7) | 137.8<br>(1.6) | 137.1<br>(1.9) | 137.0<br>(1.4) | 137.2<br>(1.7) | -107<br>(72)                                 | p < 0.02                 |
| Normals  | 139.2<br>(1.7) | 139.0<br>(1.9) | 139.0<br>(1.9) | 138.8<br>(1.8) | 138.7<br>(2.0) | -27<br>(74)                                  | NS                       |
| F value  | 0.55 (NS)      |                |                |                |                | 1.73 (NS)                                    |                          |
| <b>PLASMA AVP</b><br>(pmol L <sup>-1</sup> )         |                |                |                |                |                |  |                          |
| DAN +ve  | 1.39<br>(1.17) | 1.22<br>(0.81) | 1.64<br>(1.14) | 1.64<br>(1.34) | 2.00<br>(1.16) | 19<br>(78)                                   | NS                       |
| DAN -ve  | 1.58<br>(0.43) | 1.48<br>(0.41) | 1.98<br>(1.13) | 1.86<br>(0.47) | 2.26<br>(1.00) | 28<br>(30)                                   | NS                       |
| Normals  | 0.96<br>(1.16) | 0.98<br>(1.11) | 1.38<br>(1.30) | 2.08<br>(1.89) | 1.09<br>(0.70) | 48<br>(43)                                   | p < 0.05                 |
| F value  | 0.60 (NS)      |                |                |                |                | 0.46 (NS)                                    |                          |

(Chapter 5)

GROUP MEAN (SD) BIOCHEMICAL AND OSMOLALITY RESPONSES DURING 120 MINUTES OF HEAD-UP  
TILT TO 45° IN DIABETICS WITH (DAN +ve), DIABETICS WITHOUT (DAN -ve),  
AND NORMAL SUBJECTS

TABLE XIa

|   | TIME (min)    |               |               |               |               | Area under curve 0-120 min (Arbitrary units) | Difference from baseline |
|---|---------------|---------------|---------------|---------------|---------------|--|--------------------------|
|   | Baseline      | 30            | 60            | 90            | 120           |  |                          |
| <b>HAEMATOCRIT (%)</b>                      |               |               |               |               |               |  |                          |
| DAN +ve                                     | 41.8<br>(6.1) | 43.4<br>(7.8) | 44.6<br>(7.7) | 43.7<br>(7.6) | 43.4<br>(7.3) | 208<br>(170)                                 | p < 0.02                 |
| DAN -ve                                     | 40.8<br>(3.0) | 41.8<br>(3.4) | 41.8<br>(3.5) | 42.7<br>(3.2) | 42.3<br>(3.1) | 137<br>(53)                                  | p < 0.005                |
| Normals                                     | 41.0<br>(2.0) | 41.8<br>(2.0) | 42.5<br>(1.9) | 43.7<br>(2.1) | 43.8<br>(2.3) | 192<br>(93)                                  | p < 0.005                |
| F value                                     | 0.11 (NS)     |               |               |               |               | 0.60 (NS)                                    |                          |
| <b>PLASMA GLUCOSE (mmol L<sup>-1</sup>)</b> |               |               |               |               |               |  |                          |
| DAN +ve                                     | 14.9<br>(7.5) | 15.4<br>(7.3) | 15.9<br>(7.5) | 16.4<br>(7.4) | 17.0<br>(7.0) | 122<br>(54)                                  | p < 0.001                |
| DAN -ve                                     | 8.5<br>(3.9)  | 8.8<br>(3.8)  | 9.5<br>(3.7)  | 10.4<br>(3.8) | 11.2<br>(4.1) | 138<br>(88)                                  | p < 0.02                 |
| Normals                                     | 5.3<br>(0.7)  | 5.4<br>(0.8)  | 5.3<br>(0.7)  | 5.1<br>(0.7)  | 4.9<br>(0.6)  | -12<br>(26)                                  | NS                       |
| F value                                     | 5.94 (p<0.05) |               |               |               |               | 11.21 (p<0.01)                               |                          |

(Chapter 5)

GROUP MEAN (SD) BIOCHEMICAL AND OSMOLALITY RESPONSES DURING 120 MINUTES OF HEAD-UP

TILT TO 45° IN DIABETICS WITH (DAN +ve), DIABETICS WITHOUT (DAN -ve),

AND NORMAL SUBJECTS

TABLE Xib

|   | TIME (min)      |                 |                 |                 |                 | Area under curve 0-120 min (Arbitrary units) | Difference from baseline |
|---|-----------------|-----------------|-----------------|-----------------|-----------------|--|--------------------------|
|   | Baseline        | 30              | 60              | 90              | 120             |  |                          |
| <b>PRA</b><br>(ng mL <sup>-1</sup> hr <sup>-1</sup> ) |                 |                 |                 |                 |                 |  |                          |
| DAN +ve   | 0.44<br>(0.21)  | 1.31<br>(0.95)  | 1.85<br>(1.54)  | 1.85<br>(1.23)  | 2.08<br>(1.65)  | 123<br>(113)                                 | p < 0.05                 |
| DAN -ve   | 0.40<br>(0.18)  | 0.72<br>(0.50)  | 0.94<br>(0.77)  | 1.24<br>(1.20)  | 1.57<br>(1.41)  | 69<br>(85)                                   | NS                       |
| Normals   | 1.72<br>(1.20)  | 2.72<br>(0.90)  | 3.30<br>(1.90)  | 3.07<br>(1.40)  | 3.24<br>(1.40)  | 141<br>(107)                                 | p < 0.05                 |
| F value   | 7.43 (p<0.01)   |                 |                 |                 |                 | 0.80 (NS)                                    |                          |
| <b>PLASMA ALDOSTERONE</b><br>(pg mL <sup>-1</sup> )   |                 |                 |                 |                 |                 |  |                          |
| DAN +ve   | 111.1<br>(58.1) | 125.7<br>(79.2) | 152.6<br>(79.4) | 177.1<br>(81.8) | 179.7<br>(74.8) | 4689<br>(3892)                               | p < 0.02                 |
| DAN -ve   | 72.5<br>(41.2)  | 79.3<br>(26.8)  | 99.0<br>(48.0)  | 111.5<br>(40.6) | 138.5<br>(45.3) | 3160<br>(3343)                               | NS                       |
| Normals   | 97.5<br>(37.0)  | 122.7<br>(42.0) | 176.2<br>(71.0) | 168.3<br>(57.0) | 161.0<br>(73.0) | 6192<br>(6512)                               | NS                       |
| F value   | 1.10 (NS)       |                 |                 |                 |                 | 0.62 (NS)                                    |                          |

(Chapter 5)

GROUP MEAN (SD) BIOCHEMICAL AND OSMOLALITY RESPONSES DURING 120 MINUTES OF HEAD-UP TILT TO 45° IN DIABETICS WITH (DAN +ve), DIABETICS WITHOUT (DAN -ve), AND NORMAL SUBJECTS

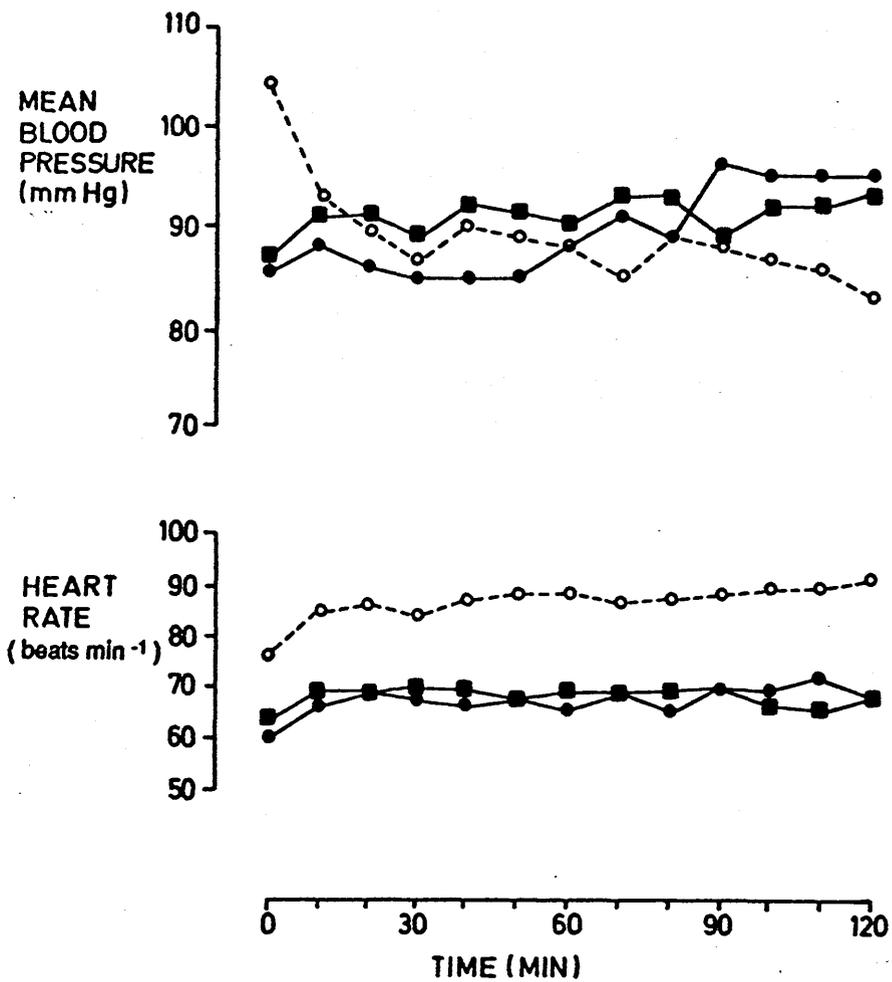
TABLE XIIa

|  | TIME (min)     |                |                |                |                | Area under curve 0-120 min (Arbitrary units) | Difference from baseline |
|--|----------------|----------------|----------------|----------------|----------------|--|--------------------------|
|  | Baseline       | 30             | 60             | 90             | 120            |  |                          |
| <b>PLASMA NORADRENALINE</b><br>(nmol l <sup>-1</sup> ) |                |                |                |                |                |  |                          |
| DAN +ve<br>(n = 7)                                     | 0.83<br>(0.25) | 0.96<br>(0.34) | 0.98<br>(0.36) | 0.97<br>(0.35) | 0.88<br>(0.24) | 657<br>(532)                                 | p < 0.02                 |
| DAN -ve<br>(n = 6)                                     | 1.48<br>(0.27) | 1.59<br>(0.38) | 1.65<br>(0.42) | 1.66<br>(0.36) | 1.51<br>(0.42) | 723<br>(1255)                                | NS                       |
| Normals<br>(n = 6)                                     | 1.36<br>(0.31) | 1.49<br>(0.27) | 1.41<br>(0.23) | 1.36<br>(0.31) | 1.39<br>(0.25) | 186<br>(380)                                 | NS                       |
| F value  | 10.17 (p<0.01) |                |                |                |                | 0.81 (NS)                                    |                          |
| <b>PLASMA ADRENALINE</b><br>(nmol l <sup>-1</sup> )    |                |                |                |                |                |  |                          |
| DAN +ve  | 0.16<br>(0.07) | 0.19<br>(0.05) | 0.19<br>(0.10) | 0.15<br>(0.07) | 0.14<br>(0.07) | 80<br>(209)                                  | NS                       |
| DAN -ve  | 0.22<br>(0.03) | 0.25<br>(0.07) | 0.26<br>(0.06) | 0.23<br>(0.07) | 0.20<br>(0.04) | 84<br>(250)                                  | NS                       |
| Normals  | 0.21<br>(0.06) | 0.22<br>(0.08) | 0.22<br>(0.06) | 0.22<br>(0.10) | 0.20<br>(0.06) | 36<br>(219)                                  | NS                       |
| F value  | 2.60 (NS)      |                |                |                |                | 0.09 (NS)                                    |                          |

(Chapter 5)

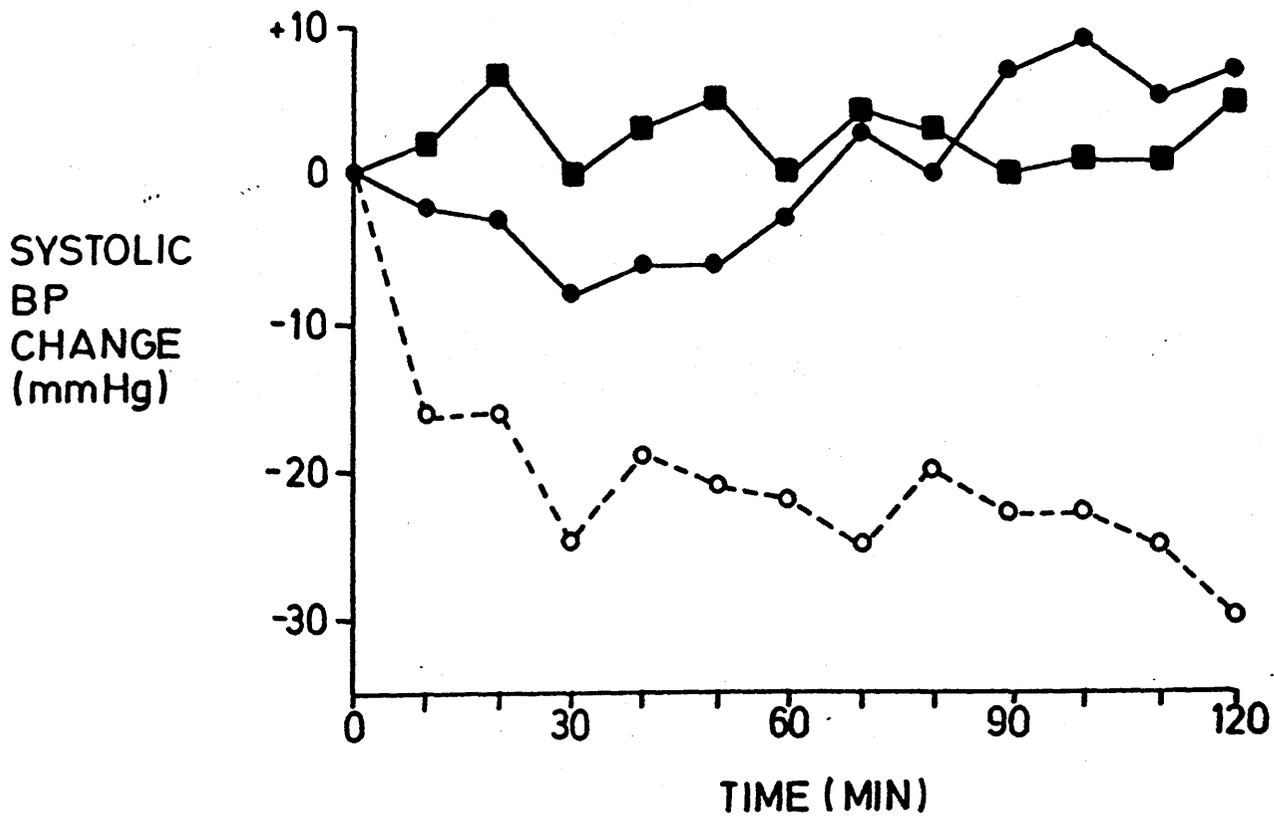
GROUP MEAN (SD) BIOCHEMICAL AND OSMOLALITY RESPONSES DURING 120 MINUTES OF HEAD-UP TILT TO 45° IN DIABETICS WITH (DAN +ve), DIABETICS WITHOUT (DAN -ve), AND NORMAL SUBJECTS

TABLE XIIB



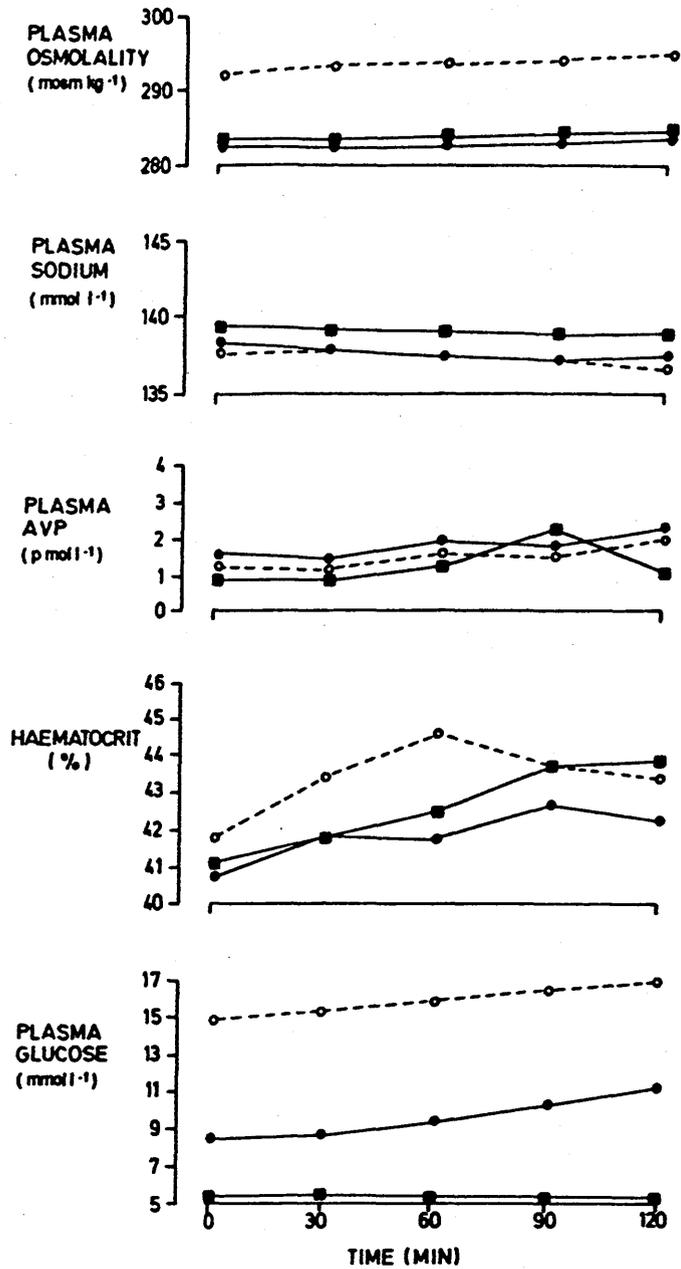
Mean blood pressure and heart rate during 120 minutes of head-up tilt to 45° in seven diabetics with (o---o), six diabetics without (●—●) autonomic neuropathy, and six normal subjects (■—■).

Figure 8



Change in systolic blood pressure during 120 minutes of head-up tilt to 45° in seven diabetics with (o---o), six diabetics without (●—●) autonomic neuropathy, and six normal subjects (■—■).

Figure 9



Mean biochemical and osmolality responses during 120 minutes of head-up tilt to 45° in seven diabetics with (o---o), six diabetics without (●—●), and six normal subjects (■—■).

Figure 10

## CHAPTER 6

### THE PRESSOR EFFECT OF ARGININE VASOPRESSIN IN DIABETIC AUTONOMIC NEUROPATHY

#### 6.1 SUBJECTS

Sixteen insulin-taking diabetic men were studied. They were divided into two groups: 8 DAN -ve and 8 DAN +ve, assessed by cardiovascular reflex testing. [Details are given in table XIII]. All 8 DAN +ve subjects had one or more symptoms suggestive of autonomic neuropathy. Proteinuria (albugin +ve) was present in 2 DAN -ve and 1 DAN +ve patients, while retinopathy was present in 3 DAN -ve (2 background, 1 proliferative) and 8 DAN +ve (2 background, 6 proliferative) patients. The fasting and abstention requirements in chapters 4 and 5 were also observed.

Six normal male volunteers aged 26 - 57 (mean 37) years were studied at the Westminster Hospital, London, using a similar protocol.

#### 6.2 Protocol

Each subject lay supine while intravenous cannulae were inserted into each antecubital fossa: the left for sampling and the right for infusion. After 30 minutes

rest, an infusion of AVP was started at  $0.05 \text{ pmol min}^{-1} \text{ kg}$  body weight<sup>-1</sup> and increased incrementally every 5 minutes to 0.2, 0.5, 1.0, 2.5 and  $5 \text{ pmol min}^{-1} \text{ kg}^{-1}$  in the two diabetic groups. In the normal volunteer group the AVP infusion was started at  $0.2 \text{ pmol min}^{-1} \text{ kg}^{-1}$  and increased every 5 minutes to 0.5, 1.0, 2.0 and  $5 \text{ pmol min}^{-1} \text{ kg}^{-1}$ . Heart rate (HR) and arterial blood pressure (BP) were measured at -10, -5, and 0 minutes, and then during the infusion at 2 minute intervals in the diabetic subjects and at 5 minute intervals in the normal subjects. Blood was withdrawn every 5 minutes for estimation of plasma AVP and glucose concentrations.

### 6.3 RESULTS

Appropriate plasma AVP concentrations were achieved in all subjects at each incremental AVP infusion rate (figure 11). There were no significant differences in mean plasma AVP responses between the three groups over the infusion period ( $f = 0.6$ , NS). Marked facial pallor was seen in all subjects during the infusions.

Blood pressure and heart rate values at baseline did not differ significantly within each group between the -10, -5 and 0 minute measurements. Baseline blood pressure was higher in the DAN +ve group. Group mean blood pressure (MBP) responses to AVP infusion are shown in figure 12,

and systolic and diastolic blood pressure and heart rate responses in figure 13. There were no significant changes in the normal group or in the DAN -ve group. In the DAN +ve group there were mean rises of 11 mmHg in systolic blood pressure, and 10 mmHg in diastolic blood pressure by the  $2.5 \text{ pmol min}^{-1} \text{ kg}^{-1}$  infusion rate. Because of the wide distribution of individual responses in the diabetic groups, there were no significant differences between the groups in overall MBP responses during the infusion (AUC, normals,  $4.9 \pm 3.8$  (SD), DAN -ve  $3.5 \pm 7.4$ , DAN +ve  $9.7 \pm 5.9$ ,  $f = 2.3$ , NS).

Examination of the individual responses, however, showed that 6 of the 8 DAN +ve subjects (numbers 9, 10, 11, 14, 15, 16) and 2 of the 8 DAN -ve subjects (numbers 5 and 7) had overall blood pressure responses greater than any of the normal subjects and clearly different from the other diabetic subjects (figure 14). One of the 2 DAN -ve subjects (number 7), with a marked pressor response to AVP infusion was found subsequently to have a low 24 hour heart rate variation count (Ewing et al, 1984), suggesting early autonomic involvement. Maximum mean blood pressure rises in these subjects were 13, 21, 11, 14, 17 and 12 mmHg (DAN +ve) and 22 and 19 mmHg (DAN -ve). This contrasts with maximum mean blood pressure rises of 2 and 8 mmHg (DAN +ve numbers 12 and 13) and 8, 6, 0, 1, 3 and 0 mmHg (DAN -ve numbers 1, 2, 3, 4, 6 and 8). Additionally

in all but one of the subjects with pressor responses, the greatest blood pressure rises occurred at intermediate AVP infusion levels, with an attenuated blood pressure response at the 5 pmol min<sup>-1</sup> kg<sup>-1</sup> infusion rate.

Although there was a faster baseline heart rate in the DAN +ve group (as might be expected), there were no significant differences in the heart rate responses between the groups. Heart rate fell on average 4-8 beats per minute in all three groups during the infusion period, but rose again at the highest AVP infusion rate (figures 13 and 14). There were no significant differences between the diabetic groups in plasma glucose, either at baseline or during the study.

#### 6.4 DISCUSSION

Arginine vasopressin is a very powerful vasoconstrictor which in experimental animals has been shown to exert differential effects on different vascular beds (Monos, Cox & Peterson, 1978; Liard et al, 1982). In normal man infusion of small doses of AVP results in a small rise in systemic vascular resistance (Ebert et al, 1986), but higher doses have little, or only transient pressor effects (Williams et al, 1986; Graybiel and Glendy, 1941; Simpson et al, 1986). Increased pressor sensitivity to exogenous AVP has, however, been reported in patients with

abnormalities of the autonomic nervous system, including patients with progressive autonomic failure (Wagner & Braunwald, 1956; Mohring et al, 1980; Williams et al, 1986), and tetraplegics (Poole et al, 1987). This increased sensitivity to the pressor effects of AVP might represent either increased vascular sensitivity to AVP or diminished baroreflex function, or a combination of both, although the results in the tetraplegics (Poole et al, 1987) would favour the first alternative.

This study confirms that in diabetes mellitus, another disorder producing autonomic nerve damage, an enhanced pressor response to infused vasopressin may be seen. Six of the 8 DAN +ve and 2 of the DAN -ve patients showed marked pressor responses in excess of any of the normal subjects. Indeed, evidence from 24 hour ECG tape recording suggested that one of the DAN -ve patients had early autonomic damage not yet detectable by standard cardiovascular reflex tests.

It might be argued that as there were no statistically significant differences demonstrated between the blood pressure results of the two diabetic and the normal groups, the apparent pressor responses were not real. In fact, the problem with statistical analysis of this data arises because of the great variability in both diabetic groups, with apparent "responders" and "non-responders" in

each group. When the results are examined on an individual rather than a group basis, it is clear that most, but not all, of those with cardiovascular reflex evidence of autonomic neuropathy, and a few of those with apparently normal cardiovascular reflexes, had enhanced blood pressure responses during AVP infusion. There is increasing evidence to suggest that early autonomic damage may be present in diabetic subjects despite normal standard cardiovascular reflex tests (Ewing & Clarke, 1986). While, too, the mean age of the diabetic group with autonomic neuropathy was slightly higher than either the normal or the other diabetic group, I know of no evidence to suggest that pressor responsiveness to vasopressin increases with age.

The pressor response to infused AVP in the diabetic subjects is unlikely to be secondary to the loss of baroreflex activation, as the heart rate changed similarly in all three groups. It seems more probable that it represents increased sensitivity of the peripheral vasculature to vasopressin. Indeed, the fact that subjects with different types of autonomic nervous system involvement, whether associated with brainstem degeneration as in progressive autonomic failure (Williams et al, 1986), at cervical cord level as in tetraplegia (Poole et al, 1987), or peripherally as in diabetes, all respond to AVP infusion with marked pressor responses

suggest that sympathetic damage itself, at whatever level, results in an alteration of vascular vasopressin responses.

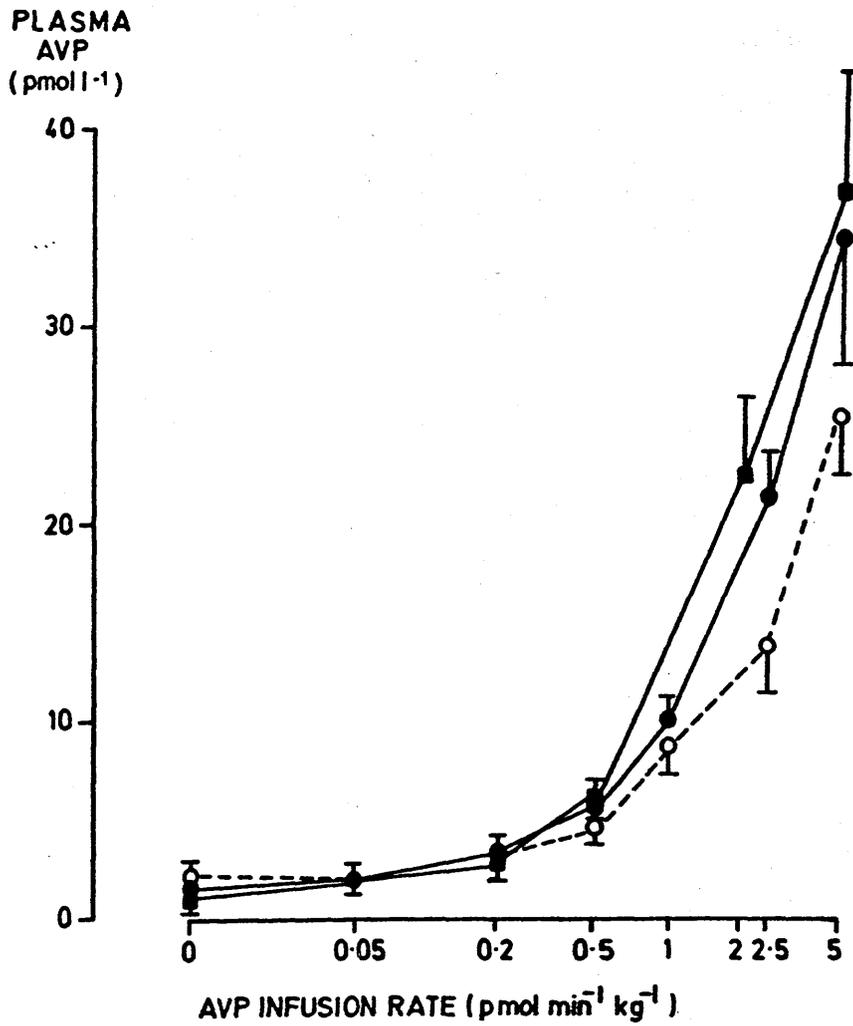
It is difficult to explain the waning of the pressor effect at the highest AVP infusion level, or the return of heart rate towards baseline values. It has, however, been noted by others during prolonged AVP infusion (Bennett & Gardiner, 1986), and might represent an adaptive neural mechanism causing less vasoconstriction, secondary release of some humoral dilator substance, or alternatively the recruitment of  $V_2$  receptors in different vascular beds. Indeed, when the  $V_2$  agonist DDAVP is infused in man, facial flushing occurs together with a fall in diastolic blood pressure (Williams, Lightman & Leadbetter, 1986).

Vasopressin has different pressor effects on different vascular beds. Activation of classical  $V_1$  receptors (Creba et al, 1983) causes vasoconstriction, while activation of  $V_2$  receptors (Roy et al, 1975) results in vasodilatation. Damage to the autonomic nervous system may alter both the sensitivity and the distribution of  $V_1$  and  $V_2$  receptors. There is some evidence for a vasopressin-like peptide in sympathetic neurones (Hanley et al, 1984), and autonomic nervous damage may result in "up regulation" of post synaptic vasopressin receptors.

Finally, there is also evidence from noradrenaline/vasopressin interactions in man which suggests that decreased sympathetic activity alone results in increased dependence of vascular tone on circulating vasopressin (Ribiero et al, 1986). In addition to this some recent work is suggestive of AVP having a role in the maintenance of blood pressure in DAN +ve patients (Saad et al, 1988). Clearly much of the work looking at AVP vascular responses in diabetics with autonomic neuropathy has yet to be done.

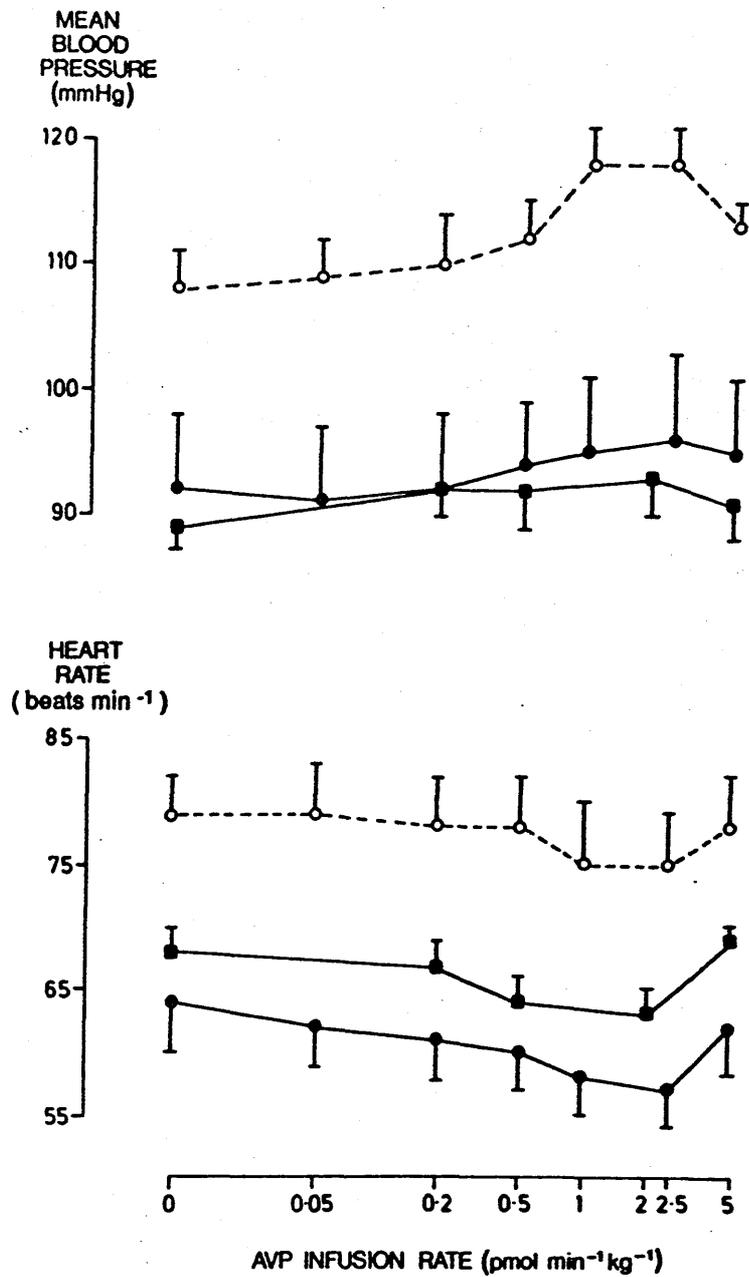
| Patient No           | Age (yrs) | Duration of diabetes (yrs) | Retinopathy status (B=background (E=exudative (P=proliferative) | Proteinuria (albutix) | Plasma creatinine ( $\mu\text{mol l}^{-1}$ ) | Valsalva ratio | 30:15 ratio | Heart rate variation (beats $\text{min}^{-1}$ ) | Systolic BP fall on standing (mmHg) | Diastolic BP rise during grip (mmHg) | Autonomic score (0-10) |
|----------------------|-----------|----------------------------|---|-----------------------|--|----------------|-------------|---|-------------------------------------|--------------------------------------|------------------------|
| 1                    | 29        | 27                         | P   | +                     | 92   | 1.34           | 1.27        | 14  | 2                                   | 21                                   | 1                      |
| 2                    | 29        | 5                          | -   | -                     | 110  | 1.85           | 1.33        | 40  | 20                                  | 37                                   | 1                      |
| 3                    | 58        | 15                         | -   | -                     | -  | 1.35           | 1.31        | 15  | +4                                  | 25                                   | 0                      |
| 4                    | 45        | 24                         | -   | -                     | 86   | 1.66           | 1.60        | 38  | 4                                   | 30                                   | 0                      |
| 5                    | 48        | 15                         | -   | -                     | 123  | 2.39           | 1.79        | 33  | +8                                  | 22                                   | 0                      |
| 6                    | 31        | 24                         | -   | -                     | 82   | 1.37           | 1.24        | 32  | +8                                  | 31                                   | 0                      |
| 7                    | 49        | 12                         | B   | -                     | -  | 1.43           | 1.15        | 19  | 0                                   | 28                                   | 0                      |
| 8                    | 38        | 22                         | B   | +                     | 95   | 1.28           | 1.25        | 14  | 22                                  | 18                                   | 2                      |
| <b>DAN +ve</b>       |           |                            |   |                       |  |                |             |   |                                     |                                      |                        |
| 9                    | 51        | 28                         | P   | -                     | 103  | 1.00           | 1.00        | 3   | 50                                  | 28                                   | 8                      |
| 10                   | 48        | 18                         | B   | -                     | 97   | 1.03           | 1.00        | 3   | 50                                  | 12                                   | 9                      |
| 11                   | 53        | 7                          | P   | -                     | 110  | 1.09           | 1.00        | 1   | 36                                  | 25                                   | 8                      |
| 12                   | 40        | 20                         | P   | -                     | 115  | -              | 1.00        | 8   | 60                                  | 18                                   | (6/8)                  |
| 13                   | 58        | 16                         | P   | -                     | 98   | -              | 1.14        | 4   | 20                                  | 10                                   | (5/8)                  |
| 14                   | 36        | 20                         | P   | -                     | 81   | -              | 0.97        | 4   | 36                                  | 7                                    | (8/8)                  |
| 15                   | 57        | 19                         | B   | -                     | -  | 1.03           | 1.03        | 5   | 30                                  | 11                                   | 8                      |
| 16                   | 70        | 34                         | P   | ++                    | 103  | -              | 1.03        | 1   | 30                                  | 14                                   | (6/8)                  |
| <b>Normal values</b> |           |                            |   |                       |  |                |             |   |                                     |                                      |                        |
|                      |           |                            |   |                       | < 130  | > 1.21         | > 1.04      | > 15  | < 10                                | > 16                                 |                        |

(chapter 6) AGE, DURATION OF DIABETES, DETAILS OF COMPLICATIONS AND CARDIOVASCULAR REFLEX TESTS IN THE DIABETICS UNDERGOING AVP INFUSION



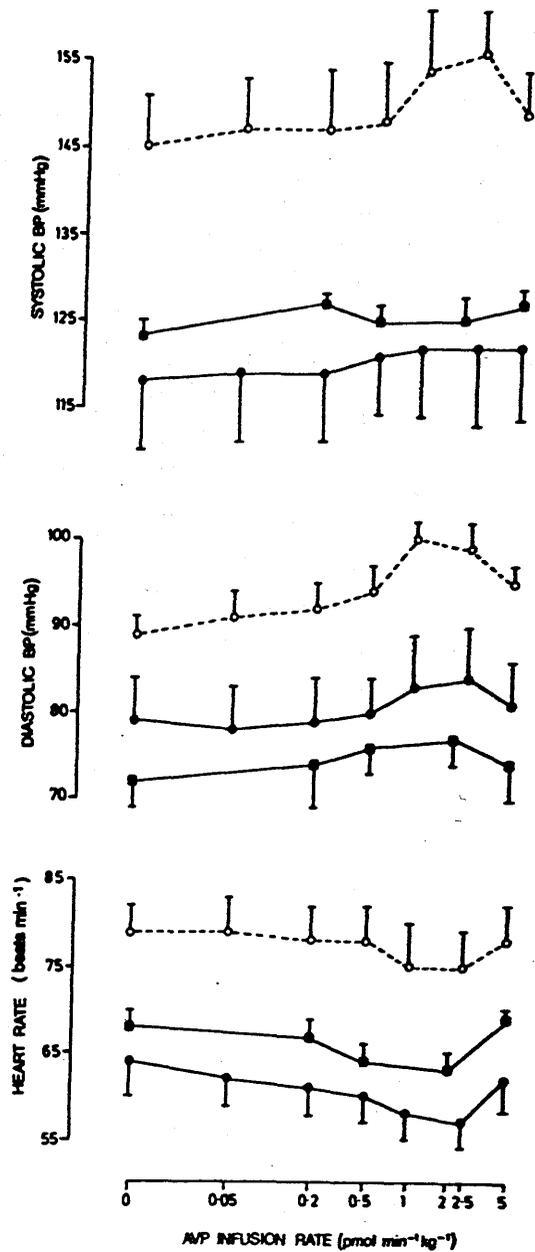
Group mean ( $\pm$  SEM) plasma AVP concentrations during intravenous infusion of incremental doses of AVP in eight diabetics with (o---o), eight diabetics without ( $\bullet$ — $\bullet$ ) autonomic neuropathy and six normal subjects ( $\blacksquare$ — $\blacksquare$ ).

Figure 11



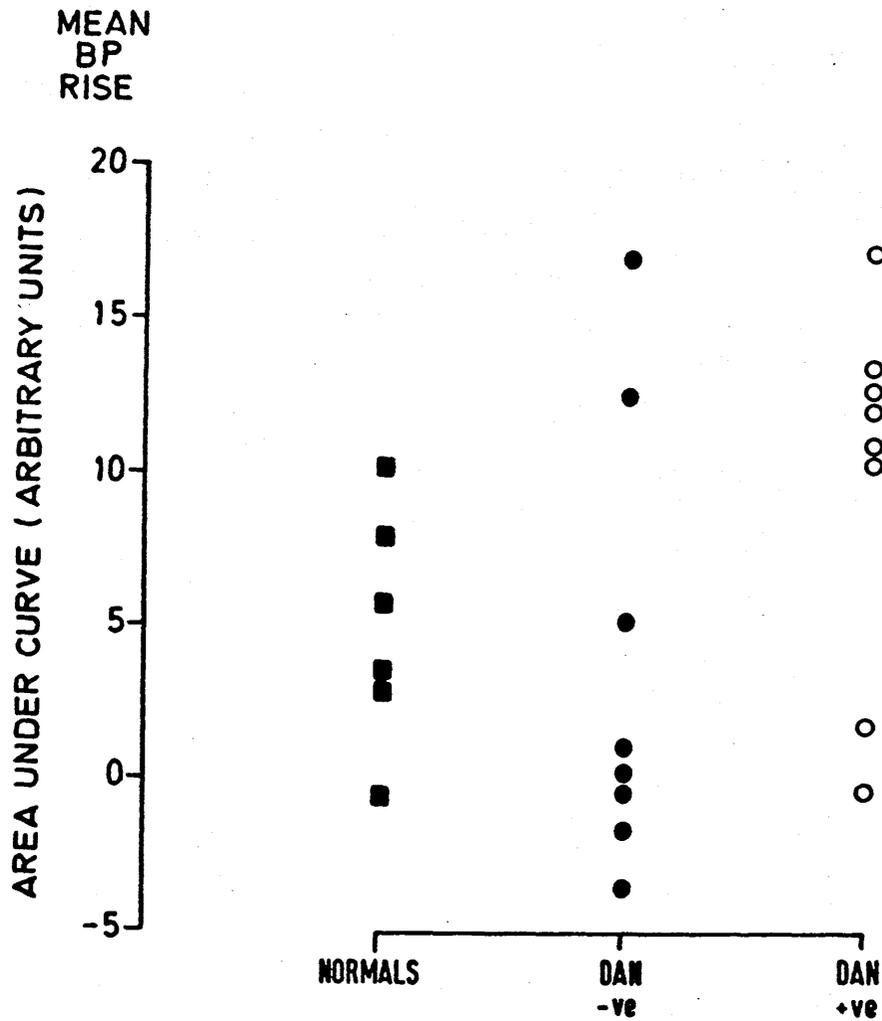
Group mean ( $\pm$  SEM) blood pressure and heart rate responses during intravenous infusion of incremental doses of AVP in eight diabetics with (o---o), eight diabetics without ( $\bullet$ — $\bullet$ ) autonomic neuropathy, and six normal subjects ( $\blacksquare$ — $\blacksquare$ ).

Figure 12



Group mean ( $\pm$  SEM) systolic blood pressure, diastolic blood pressure and heart rate during intravenous infusion of incremental doses of AVP in eight diabetics with (o---o), eight diabetics without (●—●) autonomic neuropathy, and six normal subjects (■—■).

Figure 13



Individual mean blood pressure responses, measured as 'area under the curve' during intravenous infusion of incremental doses of AVP, in eight diabetics with (DAN +ve), eight diabetics without (DAN -ve) and six normal subjects.

Figure 14

## CHAPTER 7

### GENERAL DISCUSSION AND CONCLUSIONS

In this thesis I have considered the effects of autonomic dysfunction, as encountered in diabetic autonomic neuropathy, in four separate results chapters, which have addressed aspects of control of heart rate, osmotic control of endogenous AVP release, the endogenous AVP responses to orthostasis and the cardiovascular responses to exogenous AVP administration. Each study has therefore considered separate systems in isolation from one another in an attempt to test individual hypotheses. Such work forms the cornerstone of the scientific method, where the gathering of small pieces of information eventually add together in a jigsaw puzzle to provide the complete answer. In the presence of autonomic damage, the 'dynamic spheres of influence' (see introduction) which are brought into play help to maintain homeostasis, but are impossible to fully delineate. This work, in an attempt to broaden the knowledge of the effects of diabetic autonomic neuropathy in man, has tried to provide perhaps one or two jigsaw pieces to place in the puzzle.

The subjects studied in chapters 3 to 6 represent the severe end of the spectrum of autonomic neuropathy caused by diabetes. They were categorised by well proven

methods, drawn from a clinic with perhaps the best and longest experience of dealing with the condition, and were well characterised for other features of the disease, yet differences were observed in these studies between their responses and the responses previously observed in other forms of autonomic failure. The reasons for this will be addressed in this chapter.

Chapter three, using diabetics with autonomic neuropathy as a model analogous to that in denervated animals, considered whether or not the partial agonist activity of beta-adrenoreceptor blocking drugs could be directly shown in man, and whether the partial agonist activity in these drugs displayed cardioselectivity in man. In contrast to work in other forms of autonomic failure in man (Man in't Veld et al, 1981; 1982) heart rate was raised compared with placebo in the drug with the greatest partial agonism, pindolol, although no direct effect of the drug was seen in terms of an increase in heart rate from baseline values. In addition to this, relative effects on heart rate proportional to the amount of PAA in the drugs were seen on 24 hour heart rate monitoring, whereby heart rate at night on pindolol was significantly higher than on placebo, no differences between placebo and epanolol were seen at night, and atenolol, with no PAA, lowered heart rate throughout the 24 hour period. Although there were changes observed, they were not as

striking as those seen in PAF patients.

Using measurement of changes in physiological tremor as a model of B<sub>2</sub>-adrenoceptor function, this work indeed showed that the selectivity of partial agonism in these drugs conforms to the cardioselectivity of the parent drug. By showing no change in physiological tremor after atenolol and epanolol, the B<sub>1</sub>-selectivity of these drugs was confirmed. In contrast, a rise in physiological tremor was observed after dosing with pindolol, confirming that its partial agonism acts on both B<sub>1</sub>- and B<sub>2</sub>-adrenoceptors. This study was the first to demonstrate this effect in man, although the denervated state is not necessary for its demonstration (McCaffrey et al, 1986).

There was evidence, from plasma glucose levels, that there may be some supersensitivity of B<sub>2</sub>-adrenoceptors in the liver. Plasma glucose rose significantly from baseline after pindolol, and not after the other drugs. Again this effect had not previously been shown, but is in keeping with subsequent work on the effects of infused catecholamines in diabetics with autonomic neuropathy (Hilsted et al, 1987), the B<sub>2</sub>-partial agonism perhaps stimulating hepatic receptors and promoting glycogenolysis. No studies have to my knowledge been performed in diabetics without autonomic neuropathy to examine whether the metabolic effects of this drug are

specific to DAN +ve subjects.

Chapter four looked at osmotic stimulation of AVP secretion in a group of normal controls, a group of DAN -ve subjects, and for the first time in the human literature, diabetics with cardiovascular reflex evidence of autonomic neuropathy. Using the well established technique of hypertonic saline infusion, the data obtained show that, consistent with other groups in whom autonomic dysfunction occurs (Williams et al, 1985; Poole et al, 1987), the hypothalamic-neurohypophyseal axis responds normally to an osmotic stimulus. There were, however, differences in the way blood pressure responded to hypertonic saline infusion. The mean blood pressure changes calculated by AUC in the DAN +ve subjects during this study were not significantly different from the other two groups, in contrast to the studies of Williams et al (1985) and Poole et al (1987) who in PAF subjects and tetraplegics respectively, found that blood pressure rose significantly during hypertonic saline infusion, and that the pressor response may in part be secondary to increased sensitivity of vascular  $V_1$  receptors in the presence of the denervated state. It is interesting that one of the DAN +ve subjects had a 30 mmHg systolic blood pressure rise during the study, which may have been mediated by the same mechanism. The apparent lack of group blood pressure change may also have other roots: either a type

II error in the numbers in the study occurred, which is unlikely in view of the other positive data accrued; or the vascular V<sub>1</sub> receptors, as with the B-adrenoceptors in chapter three, fail to 'upregulate' or increase their sensitivity, because denervation in diabetic autonomic neuropathy is not as 'complete' as in the other forms of autonomic dysfunction.

In addition to this, close correlations were seen between plasma osmolality and AVP, and plasma sodium and AVP. The abscissal intercept in the DAN -ve subjects was significantly lower than either DAN +ve or normal groups, but this is in keeping with the wide inter-individual variation already known to occur (Schrier et al, 1979), and no other firm conclusions can be drawn from the data.

Chapter five addressed the question of whether cardiovascular release of AVP is abnormal in diabetic autonomic neuropathy. The finding that, despite a major drop in blood pressure in the DAN +ve subjects, AVP levels did not increase relative to normals and DAN -ve subjects, is in keeping with the interruption of afferent pathways found in PAF subjects (Williams et al, 1985), but contrasts with work in tetraplegics (Sved et al, 1985; Poole et al, 1987), in whom an exaggerated rise in AVP levels was found. Tetraplegics have selective efferent loss of sympathetic pathways, and my study confirms that

AVP response to head-up tilt tests the integrity of afferent pathways (Puritz et al, 1983; Poole et al, 1987; Zerbe et al, 1983).

Studies have confirmed this finding in DAN +ve subjects by using volume depletion (Grimaldi et al, 1985) and (less convincingly) orthostasis (Cignarelli et al, 1986).

The results in chapter five also confirm that in the absence of severe nephropathy (assessed by plasma creatinine levels), plasma renin release by orthostasis is normal in diabetic autonomic neuropathy, in keeping with the more recent literature (Hilsted et al, 1981; Winocour et al, 1986).

The significance of subnormal AVP release in diabetic autonomic neuropathy is as yet not fully explored. If denervation supersensitivity indeed exists, even low plasma levels of AVP may help to maintain blood pressure in the presence of orthostatic hypotension, and may have implications for treatment of this distressing condition in man. With the development of more specific V<sub>1</sub> agonists (Manning & Sawyer, 1985) such therapeutic advances may be possible in the future.

In chapter six blood pressure responses were measured after infusion of physiological doses of AVP in normals,

DAN -ve and DAN +ve subjects. There were no significant changes in blood pressure in the normal group. Six DAN +ve and two DAN -ve subjects showed marked rises in blood pressure during the infusion period, with maximum rises in mean blood pressure of 11 - 22 mmHg. The responses waned at the maximum AVP infusion rate in seven of the eight subjects. Despite the lack of statistical differences between the three groups, it is apparent that the 'responders' clearly displayed abnormal sensitivity to the effects of AVP at physiological levels. If this is the correct interpretation of the data, the diabetics display increased pressor sensitivity to AVP in much the same way as PAF subjects and tetraplegics (Williams et al, 1986; Poole et al, 1987). Also, in keeping with the other groups of patients with autonomic dysfunction and in normal human subjects (Williams et al, 1986; Poole et al, 1987; Ebert et al, 1986; Aylward et al, 1986), there appears to be little evidence that AVP influences baroreflexes significantly, as measured by the effects on heart rate. This is in contrast to the bulk of work in animals (Bennett & Gardiner, 1986).

Therefore, as in other groups of patients with both central and peripheral lesions affecting the autonomic nervous system, it appears that there is increased sensitivity to the pressor effects of infused vasopressin in diabetics with autonomic neuropathy. It now seems

more likely that this effect may take place at the level of the blood vessels. With the discovery of the an AVP-like peptide (Hanley et al, 1984), it may be that this exerts post-ganglionic effects, and could show denervation supersensitivity as has been described for noradrenaline in PAF (Bannister et al, 1979). Indeed, a local receptor-receptor interaction on the blood vessels would be consistent with the observation that AVP in sub-pressor doses potentiates the pressor action of catecholamines (Bartelstone & Nasmyth, 1965) and that decreased sympathetic tone results in increased dependence of vascular tone on circulating vasopressin (Ribiero et al, 1986). As the specificity of V<sub>1</sub> and V<sub>2</sub> agonists and antagonists is increased, it may be possible to use them to more fully dissect out the roles of vasopressin in diabetic autonomic neuropathy.

This thesis has attempted to address aspects of diabetic autonomic neuropathy by comparing and contrasting the results of studies presented within it with those in other forms of autonomic dysfunction. As the recognition has grown that the autonomic nervous system has moved from the perspective of anatomical simplicity, to one in which neuropeptide transmission, endocrine influences and other factors play an increasingly complex part, the concepts of denervation have changed. Complete denervation is now becoming an untenable concept in man (Burnstock, Dhital &

Alafaci, in press). Even when 'complete' cardiac denervation occurs by surgical section of the nerves, complex intracardiac reflexes are maintained (Kaye, 1984). As yet undiscovered neuropeptidergic mechanisms may persist in man, even when there is evidence that severe autonomic damage is present. The present state of knowledge in this field is at present limited.

From the evidence I have presented, and with the comparisons with other groups of patients affected by autonomic dysfunction, it is clear that diabetics do display evidence of 'denervation' but that this may not manifest itself as floridly as in, for example, patients with progressive autonomic failure. Certainly, chapter three provides evidence that, unlike the vagotomised and catechol-depleted rat, the direct effects of partial agonism activity-containing beta adrenoreceptor blocking drugs cannot be shown in diabetic autonomic neuropathy, whereas in progressive autonomic failure they can. Chapter four shows for the first time that the hypothalamic-neurohypophyseal responses to osmotic stimulation are unaffected by autonomic neuropathy. Chapter five demonstrated an afferent defect in cardiovascular release of AVP in autonomic neuropathy, as is seen in other types of autonomic failure, and chapter six, suggests that vascular sensitivity to vasopressin is heightened in diabetics with autonomic failure, but as in

chapter three, the responses are not so clear-cut as in other disorders of autonomic failure. Perhaps the concepts of denervation, mooted by Cannon in his posthumous monograph (Cannon & Rosenblueth, 1949), do still hold in most circumstances, but may have to be modified to take account of the complexities of neuropeptidergic and other influences on the autonomic nervous system.

## REFERENCES

- Aellig, W.H. (1976) B-adrenoceptor blocking activity and duration of action in pindolol and propranolol in healthy volunteers. *British Journal of Clinical Pharmacology* 3, 251-257.
- Aellig, W.H. (1982) Pindolol - a B-adrenoreceptor blocking drug with partial agonist activity. Clinical pharmacological considerations. *British Journal of Clinical Pharmacology* 13, 187S-192S.
- Ahlquist, R.P. (1948) Study of adrenotropic receptors. *American Journal of Physiology* 153, 586-600.
- Altura, B.M. & Altura, B.T. (1984) Actions of vasopressin, oxytocin and synthetic analogs on vascular smooth muscle. *Federation Proceedings* 43, 80-86.
- Arnold, J.M.O. & McDevitt, D.G. (1983) An assessment of physiological finger tremor as an indicator of B-adrenoreceptor function. *British Journal of Clinical Pharmacology* 16, 167-174.
- Arnold, J.M.O. & McDevitt, D.G. (1984) Enhancement of physiological finger tremor by intravenous isoprenaline infusions in man: evaluation of its role in the assessment of B-adrenoreceptor antagonists. *British Journal of Clinical Pharmacology* 18, 145-152.
- Arnold, J.M.O., O'Connor, P.C., Riddell, J.G., Harron, D.W.G., Shanks, R.G. & McDevitt, D.G. (1985) Effects of the B<sub>2</sub>-adrenoreceptor antagonist ICI 118,551 on exercise tachycardia and isoprenaline-induced B-adrenoreceptor responses in man. *British Journal of Clinical Pharmacology* 19, 619-630.

Aylward, P.E., Floras, J.S., Leimbach, W.N. & Abboud, F.M. (1986) Effects of vasopressin on the circulation and its baroreflex control in healthy men. *Circulation* 73, 1145-1154.

Bannister, R., Ardill, L. & Fentem, P. (1969) An assessment of various methods of treatment of idiopathic orthostatic hypotension. *Quarterly Journal of Medicine* 38, 337-395.

Bannister, R., Davies, I.B., Holly, E., Rosenthal, T. & Sever, P. (1979) Defective cardiovascular reflexes and supersensitivity to sympathomimetic drugs in autonomic failure. *Brain* 102, 163-176.

Bannister, R. (1983) Introduction and classification. In: *Autonomic failure: a textbook of clinical disorders of the autonomic nervous system.* Ed, Bannister R. Oxford, Oxford University Press. p 1-13.

Bargen, J.A., Bollman, J.L. & Kepler, E.J. (1936) The "diarrhea of diabetes" and steatorrhea of pancreatic insufficiency. *Proceedings of the Staff Meetings of the Mayo Clinic* 11, 737-742.

Barrett, A.M. & Carter, J. (1970) Comparative chronotropic activity of B-adrenoreceptor antagonists. *British Journal of Pharmacology* 40, 373-381.

Baylis, P.H. & Robertson, G.L. (1980) Plasma vasopressin response to hypertonic saline infusion to assess posterior pituitary function. *Journal of the Royal Society of Medicine* 73, 255-280.

Baylis, P.H. (1987) Osmoregulation and control of vasopressin secretion in healthy humans. *American Journal of Physiology* 253, R671-678.

X Bell, G.M., Reid, W., Ewing, D.J., Cumming, A.D., Watson, M.L., Doig, A. & Clarke, B.F. (1987) Abnormal diurnal sodium and water excretion in diabetic autonomic neuropathy. *Clinical Science* 73, 259-265.

Bennett, T. (1983) Physiological investigation of diabetic autonomic failure. In: *Autonomic Failure: A Textbook of Clinical Disorders of the Autonomic Nervous System.* pp 406-436. Ed. Bannister, R. Oxford, Oxford University Press.

Bennett, T. & Gardiner, S.M. (1985) Involvement of vasopressin in cardiovascular regulation. *Cardiovascular Research* 19, 57-68.

Bennett, T. & Gardiner, S.M. (1986) Influence of exogenous vasopressin on baroreflex mechanisms. *Clinical Science* 70, 307-315.

Bie, P. & Warberg, J. (1983) Effects on intravascular pressures of vasopressin and angiotensin II in dogs. *American Journal of Physiology* 245, R906-914.

Bilski, A., Robertson, H.H. & Wale, J.L. (1979) A study of the relationship between cardiac B-adrenoreceptor blockade and intrinsic sympathomimetic activity in rats depleted of catecholamines. *Clinical and Experimental Pharmacology and Physiology (Oxford)* 6, 1-19.

Blum, I., Barkan, A., Kuritzky, A., Doron, M. & Karp, M. (1980) Cardiac denervation and other multisystem manifestations caused by isolated autonomic neuropathy in a young diabetic patient. *American Journal of the Medical Sciences* 280 87-93.

Boyd, G.W., Adamson, A.R., Fitz, A.E. & Peart, W.S. (1969) Radioimmunoassay determination of plasma renin activity. *Lancet* 1, 213-218.

Bradbury, S. & Eggleston, C. (1925) Postural hypotension. A report of three cases. *American Heart Journal* 1, 73-86.

Bradley, R.F. & Schonfield, A. (1962) Diminished pain in diabetic patients with acute myocardial infarction. *Geriatrics* 17, 322-326.

Brodde, O.E., Karad, K., Zarkowski, H.R., Rohm, W. & Reidemeister, J. Coexistence of B<sub>1</sub>- and B<sub>2</sub>-adrenoreceptors in human heart and lung. *British Journal of Pharmacology* 78, 72P.

Brown, A.M. (1979) Cardiac reflexes. In: Handbook of Physiology. Section 2: The Cardiovascular System. pp 677-689. Ed. Berne, R.M. Bethesda, American Physiological Society.

Brown, J.E., Macleod, A.A. & Shand, D.G. (1983) Evidence of cardiac B<sub>2</sub>-adrenoreceptors in man. *Clinical and Experimental Therapeutics* 33, 424-427.

Brunt, P.W. & McKusick, V.A. (1970) Familial dysautonomia. *Medicine (Baltimore)* 49, 343-374.

Burnstock, G., Dhital, K.K. & Alafaci, C. (in press, 1989) Structural and chemical organisation of the autonomic neuroeffector system. *New Issues in Neuroscience*.

Buzzard, T. (1890) Illustrations of some less known forms of peripheral neuritis - especially alcoholic monoplegia and diabetic neuritis. *British Medical Journal* 1, 1419-1422.

Campbell, A. & Conway, H. (1960) Gastric retention and hypoglycaemia in diabetes. *Scottish Medical Journal* 5, 167-168.

Campbell, I.W., Ewing, D.J., Anderton, J.L., Thompson, J.H., Horn, D.B. & Clarke, B.F. (1976) Plasma renin activity in diabetic autonomic neuropathy. *European Journal of Clinical Investigation* 6, 381-385.

Campbell, I.W., Ewing, D.J. & Clarke, B.F. (1978) Painful myocardial infarction in severe diabetic autonomic neuropathy. *Acta Diabetologia Latina* 15, 201-204.

Canal, N., Comi, G., Saibene, V., Musch, B. & Pozza, G. (1978) The relationship between peripheral and autonomic neuropathy in insulin dependent diabetes: a clinical and instrumental evaluation. In: *Peripheral Neuropathies*. Eds. Canal, N. & Pozza, G. pp 247-255. Amsterdam, Elsevier Biomedical Press.

Cannon, W.B. & Rosenblueth, A. (1949) A law of denervation. In: *The supersensitibility of denervated structures. A law of denervation.* pp 185-186. New York, The Macmillan Company.

Cantau, B., Keppens, S. De Wulf, H. & Jard, S. (1980) (<sup>3</sup>H)-vasopressin binding to isolated rat hepatocytes and liver membranes: regulation by GTP and relation to glycogen phosphorylase activation. *Journal of Receptor Research* 1, 137-168.

Christlieb, A.R., Munichoodappa, C. & Braaten, J.T. (1974) Decreased response of plasma renin activity to orthostasis in diabetic patients with orthostatic hypotension. *Diabetes* 23, 835-840.

Cignarelli, M., de Pergola, G., Paternostro, A., Corso, M., Cospite, M.R., Centaro, G.M. & Giorgino, R. (1986) Arginine-vasopressin response to supine-erect posture change: an index for evaluation of the integrity of the afferent component of baroregulatory system in diabetic neuropathy. *Diabete & Metabolisme (Paris)* 12, 28-33.

Clark, B.J., Menninger, K. & Bertholet, A. (1982) Pindolol - the pharmacology of a partial agonist. *British Journal of Clinical Pharmacology* 13, 149S-158S.

Clarke, B.F., Ewing, D.J. & Campbell, I.W. (1979) Diabetic autonomic neuropathy. *Diabetologia* 17, 195-212.

Clarke, B.F. & Ewing, D.J. (1982) Cardiovascular reflex tests in the natural history of diabetic autonomic neuropathy. *New York State Journal of Medicine* 82, 903-908.

Courtice, G.P., Kwong, T.E., Lumbers, E.R. & Potter, E.K. (1984) Excitation of the cardiac vagus by vasopressin in mammals. *Journal of Physiology (London)* 354, 547-556.

Cowley, A.W. & Barber, B.J. (1983) Vasopressin vascular and reflex effects - a theoretical analysis. In: *The Neurohypophysis - Structure, Function and Control*, Progress in Brain Research. 60, 415-424. Eds. Cross, B.A. & Leng, G. Elsevier Science Publishers, Amsterdam.

Cowley, A.W., Merrill, D., Osborn, J. & Barber, B.J. (1984) Influence of vasopressin and angiotensin on baroreflexes in the dog. *Circulation Research* 54, 163-172.

Creba, J.A., Downes, C.P., Hawkins, P.T., Brewster, G., Michell, R.H. & Kirk, C.J. (1983) Rapid breakdown of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-biphosphate in rat hepatocytes stimulated by vasopressin and other  $Ca^{++}$ -mobilising hormones. *Biochemical Journal* 212, 733-747.

Cryer, P.E., Silverberg, A.B., Santiago, J.V. & Shah, S.D. (1978) Plasma catecholamines in diabetes. The syndromes of hypoadrenergic and hyperadrenergic postural hypotension. *American Journal of Medicine* 64, 407-416.

Da Prada, M. & Zurcher, G. (1976) Simultaneous radioenzymatic determination of plasma and tissue adrenaline, noradrenaline and dopamine within the femtomole range. *Life Sciences* 19, 1161-1173.

Davies, R., Slater, J.D.H., Forsling, M.L. & Payne, N. (1976) The response of arginine vasopressin and plasma renin to postural change in normal man, with observations on syncope. *Clinical Science* 51, 267-274.

De Calvi, M. (1864) *Recherches sur les Accidents Diabetiques et essai d'une théorie générale du diabète*. Paris, P Asselin.

Dejgård, A., Hilsted, J. & Christensen, N.J. (1986) Noradrenaline and isoproterenol kinetics in diabetic patients with and without autonomic neuropathy. *Diabetologia* 291, 773-777.

Di Pette, D., Gavras, I., North, W., Di Pette, P. & Gavras, H. (1984) Vasopressin response to hyperosmotic stimulus: blood pressure effect in normal subjects and patients with impaired sympathetic system. *Clinical and Experimental Hypertension - Theory and Practice* A6(4), 851-861.

Dousa, T.P. (1985) Renal actions of vasopressin. In: *The Posterior Pituitary - hormone secretion in health and disease.* Ch5, pp 141-159. Eds. Baylis, P.H. & Padfield, P.L. New York: Marcel Dekker.

Drieu, L., Rainfray, M., Cabrol, C. & Ardaillou, R. (1986) Vasopressin, aldosterone and renin responses to volume depletion in heart-transplant recipients. *Clinical Science* 70, 233-241.

Du Vigneaud, V., Lawler, H.C. & Popenone, E.A. (1953) Enzymatic cleavage of glycinamide from vasopressin and a proposed structure for this pressor antidiuretic hormone of the posterior pituitary. *Journal of the American Chemical Society* 75, 4880-4881.

Ebert, T.J., Cowley, A.W. & Skelton, M. (1986) Vasopressin reduces cardiac function and augments cardiopulmonary baroreflex resistance increases in man. *Journal of Clinical Investigation* 77, 1136-1142.

Elisberg, E.I. (1963) Heart rate response to Valsalva manoeuvre as test of circulatory integrity. *Journal of the American Medical Association* 186, 200-205.

Ewing, D.J., Campbell, I.W., Burt, A.A. & Clarke, B.F. (1973) Vascular reflexes in diabetic autonomic neuropathy. *Lancet* 2, 1354-1356.

Ewing, D.J. (1978) Cardiovascular reflexes and autonomic neuropathy. *Clinical Science and Molecular Medicine* 55, 321-327.

Ewing, D.J., Campbell, I.W. & Clarke, B.F. (1980) The natural history of diabetic autonomic neuropathy. *Quarterly Journal of Medicine* 49, 95-108.

Ewing, D.J., Campbell, I.W. & Clarke, B.F. (1981) Heart rate changes in diabetes mellitus. *Lancet* 1, 183-186.

Ewing, D.J. & Clarke, B.F. (1982) Diagnosis and management of diabetic autonomic neuropathy. *British Medical Journal* 285, 916-918.

Ewing, D.J. (1983) Practical bedside investigation of diabetic autonomic failure. In: *Autonomic Failure: A textbook of clinical disorders of the autonomic nervous system.* pp 371-405. Ed. Bannister, R. Oxford, Oxford University Press.

Ewing, D.J. (1984) Cardiac autonomic neuropathy. In: *Diabetes and Heart Disease.* Ch 5, pp 99-132. Ed. Jarrett, R.J. Amsterdam, Elsevier Science Publishers BV.

Ewing, D.J., Neilson, J.M.M. & Travis, P. (1984) New method for assessing cardiac parasympathetic activity using 24 hour electrocardiograms. *British Heart Journal* 52, 396-402.

Ewing, D.J., Martyn, C.N., Young, R.J. & Clarke, B.F. (1985) The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 8, 491-498.

Ewing, D.J. & Clarke, B.F. (1986a) Diabetic autonomic neuropathy: present insights and future prospects. *Diabetes Care* 9, 648-665.

Ewing, D.J. & Clarke, B.F. (1986b) Autonomic neuropathy: its diagnosis and prognosis. *Clinics in Endocrinology and Metabolism* 15, 855-888.

Ewing, D.J., Bellavere, F., Espi, F., McGibben, B.M., Buchanan, K.D., Riemersma, R.A. & Clarke, B.F. (1986) Correlation of cardiovascular and neuroendocrine tests of autonomic function in diabetes. *Metabolism* 35, 349-353.

Fisher, B.M., Baylis, P.H. & Frier, B.M. (1987) Plasma oxytocin, arginine vasopressin and atrial natriuretic peptide response to insulin induced hypoglycaemia in man. *Clinical Endocrinology* 26, 179-185.

Fisher, B.M., Baylis, P.H., Thornton, S. & Frier, B.M. (1989) Arginine vasopressin and oxytocin responses to insulin-induced hypoglycaemia in Type I (insulin-dependent) diabetes. *Journal of Clinical Endocrinology and Metabolism* 68, 688-692.

Floras, J.S., Jones, J.V., Hassan, M.O. & Sleight, P. (1982) Ambulatory blood pressure during once-daily randomised double-blind administration of atenolol, metoprolol, pindolol and slow release propranolol. *British Medical Journal* **285**, 1387-1392.

Frier, B.M. (1986) Hypoglycaemia and diabetes. *Diabetic Medicine* **3**, 513-525.

Goldsmith, S.R. (1988) Baroreceptor-mediated suppression of osmotically stimulated vasopressin. *Journal of Applied Physiology* **65**, 1226-1231.

Goodman, J.I. (1966) Diabetic anhydrosis. *American Journal of Medicine* **41**, 831-835.

Graybiel, A. & Glendy, R.E. (1941) Circulatory effects following the intravenous administration of pitressin in normal persons and in patients with hypertension and angina pectoris. *American Heart Journal* **21**, 481-489.

Grimaldi, A., Pruszczyński, W., Thervet, F. & Ardaillou, R. (1985) Antidiuretic hormone response to volume depletion in diabetic patients with cardiac autonomic dysfunction. *Clinical Science* **68**, 545-552.

Grimaldi, A., Pruszczyński, W., Jacquin, V., Thervet, F. & Ardaillou, R. (1988) Vasopressin secretion during hyperglycaemia in insulin-dependent diabetics. *Diabete & Metabolisme (Paris)* **14**, 37-39.

Guillon, G., Butlen, D., Cantau, B., Barth, T. & Jard, S. (1982) Kinetic and pharmacological characterization of vasopressin membrane receptors from human kidney medulla: relation to adenylate cyclase activation. *European Journal of Pharmacology* **85**, 291-304.

Gundersen, H.J.G. & Christensen, N.J. (1977) Intravenous insulin causing loss of intravascular water and albumin and increased adrenergic nervous activity in diabetics. *Diabetes* **26**, 551-557.

Hanley, M.R., Benton, H.P., Lightman, S.L., Todd, K., Bone, E.A., Fretten, P., Palmer, S., Kirk, C.J. & Michell, R.H. (1984) A vasopressin-like peptide in the mammalian sympathetic nervous system. *Nature* **309**, 258-261.

Harry, J.D., Knapp, M.F. & Linden, R.J. (1974) The activities of a new B-adrenoreceptor blocking drug, ICI 66,082, in the rabbit papillary muscle and on the dog heart. *British Journal of Pharmacology* **51**, 169-177.

Harry, J.D. (1977) The demonstration of atenolol as a B-adrenoreceptor blocking drug in man. *Postgraduate Medical Journal* **53** (suppl 3), 65-69.

Hasser, E.M., Haywood, J.R., Johnson, A.K. & Bishop, V.S. (1984) The role of vasopressin and the sympathetic nervous system in the cardiovascular response to vagal cold block in the conscious dog. *Circulation Research* **55**, 454-462.

Hebden, R.A., Bennett, T. & Gardiner, S.M. (1987) Pressor sensitivities to vasopressin, angiotensin II, or methoxamine in diabetic rats. *American Journal of Physiology* **253**, R726-R734.

Helderman, J.H., Vestal, R.E., Rowe, J.W., Tobin, J.D., Andres, R. & Robertson, G.L. (1978) The response of arginine vasopressin to intravenous ethanol and hypertonic saline in man: the impact of aging. *Journal of Gerontology* **33**, 39-47.

Heller, H. (1974) History of neurohypophyseal research. In: Handbook of Physiology, section 7, Endocrinology, vol IV. The pituitary gland and its neuroendocrine control. pp 103-117. Eds. Sawyer, W.H. & Knobil, E. American Physiological Society, Washington DC.

Higgins, C.B., Vatner, S.F. & Braunwald, E. (1973) Parasympathetic control of the heart. *Pharmacological Reviews* 25, 119-155.

Hilsted, J., Parving, H-H., Christensen, N.J., Benn, J. & Galbo, H. (1981a) Haemodynamics in diabetic orthostatic hypotension. *Journal of Clinical Investigation* 68, 1427-1434.

Hilsted, J.H., Madsbad, S., Krarup, T., Sestoft, L., Christensen, N.J., Tronier, B. & Galbo, H. (1981b) Hormonal, metabolic, and cardiovascular responses to hypoglycaemia in diabetic autonomic neuropathy *Diabetes* 30, 626-633.

Hilsted, J., Richter, E., Madsbad, S., Tronier, B., Christensen, N.J., Hildebrandt, P., Damkjaer, M. & Galbo, H. (1987) Metabolic and cardiovascular responses to epinephrine in diabetic autonomic neuropathy. *New England Journal of Medicine* 317, 421-426.

Howell, W.H. (1898) The physiological effects of extracts of the hypophycerebri and infundibular body. *Journal of Experimental Medicine* 3, 245-258.

Howland, W.J. & Drinkard, R.V. (1963) Acute diabetic gastric atony. Gastroparesis diabeticorum. *Journal of the American Medical Association* 185, 214-216.

Jadzinsky, M., Faerman, I. & Fox, D. (1973) Visceral diabetic neuropathy. *Acta Diabetologia Latina* 10, 208-260.

Johnson, R.H. & Spalding, J.M.K. (1974) Arterial hypotension. In; Disorders of the Autonomic Nervous System. Ch 6, p 79-113. Eds. Johnson, R.H. & Spalding, J.M.K. Oxford: Blackwell Scientific Publications.

Jordan, W.R. (1936) Neuritic manifestations in diabetes mellitus. *Archives of Internal Medicine* 57, 307-366.

Jose, A.D. & Collson, D. (1970) The normal range and determinants of the intrinsic heart rate in man. *Cardiovascular Research* 4, 160-167.

Kaldor, A., Gachalyi, B., Szigeti, A., Juvancz, P & Szilagy, A. (1977) Intrinsic heart rate in diabetic neuropathy. *International Journal of Clinical Pharmacology* 15, 335-336.

Kassander, P. (1958) Asymptomatic gastric retention in diabetics (gastroparesis diabetorum). *Annals of Internal Medicine* 48, 797-812.

Kaye, M.P. (1984) Denervation and reinnervation of the heart. In: Nervous Control of Cardiovascular Function. pp 278-306. Ed. Randall, W.C. Oxford, Oxford University Press.

Keen, H. (1959) Autonomic neuropathy in diabetes mellitus. *Postgraduate Medical Journal* 35, 272-280.

Kent, K.M. & Cooper, T. (1974) The denervated heart - a model for studying autonomic control of the heart. *New England Journal of Medicine* 291, 1017-1021.

Kircheim, H.R. (1976) Systemic arterial baroreceptor reflexes. *Physiological Reviews* 56, 100-176.

Lands, A.M., Luduena, F.P. & Buzzo, H.H. (1967) Differentiation of receptors responsive to isoproterenol. *Life Sciences* 6, 2241-2249.

Langley, J.N. (1898) On the union of cranial autonomic (visceral) fibres with nerve cells of the superior cervical ganglion. *Journal of Physiology (London)* 23, 240-270.

Lefkowitz, R.J., Caron, M.G. & Stiles, G.L. (1984) Mechanisms of membrane-receptor regulation. *New England Journal of Medicine* 310, 1570-1579.

Leon, D.F., Shaver, J.A. & Leonard, J.J. (1970) Reflex heart rate control in man. *American Heart Journal* 80, 729-739.

Levy, M.N. (1971) Sympathetic-parasympathetic interactions in the heart. *Circulation Research* 29, 437-445.

Levy, M.N. & Martin, P. (1984) Parasympathetic control of the heart. In: *Nervous Control of Cardiovascular Function*. pp 68-94. Ed. Randall, W.C. Oxford, Oxford University Press.

Liard, J.F., Deriaz, O., Schelling, P. & Thibonnier, M. (1982) Cardiac output distribution during vasopressin infusions or dehydration in conscious dogs. *American Journal of Physiology* 243, H663-669.

Lightman, S.L., Todd, K. & Everitt, B.J. (1984) Ascending noradrenergic projections from the brainstem: evidence for a major role in the regulation of blood pressure and vasopressin secretion. *Experimental Brain Research* **55**, 145-151.

Lloyd-Mostyn, R.H. & Watkins. P.J. (1976) Total cardiac denervation in diabetic autonomic neuropathy. *Diabetes* **25**, 748-751.

Majzoub, J.A. (1985) Vasopressin biosynthesis. In: Vasopressin. pp 465-475. Ed. Schrier, R.W. New York, Raven Press.

Man in't Veld, A.J. & Schalekamp, M.A.D.H. (1981) Pindolol acts as beta-adrenoceptor agonist in orthostatic hypotension: therapeutic implications. *British Medical Journal* **282**, 929-931.

Man in't Veld, A.J., Boomsa, F. & Schalekamp, M.A.D.H. (1982) Effects of B-adrenoceptor agonists and antagonists in patients with peripheral autonomic neuropathy. *British Journal of Clinical Pharmacology* **13**, 367S-374S.

Manning. M. & Sawyer, W.H. (1985) Development of selective agonists and antagonists of vasopressin and oxytocin. In: Vasopressin. pp 131-144 Ed. Schrier, R.W. New York, Raven Press.

Marsden, C.D., Foley, T.H., Owen, D.A.L. & McAllister, R.G. (1967) Peripheral B-adrenergic receptors concerned with tremor. *Clinical Science* **33**, 53-65.

Martin, M.M. (1953) Diabetic neuropathy. A clinical study of 150 cases. *Brain* **76**, 594-624.

Martyn, C.N. & Ewing, D.J. (1986) Pupil cycle time: a simple way of measuring an autonomic reflex. *Journal of Neurology, Neurosurgery and Psychiatry* 49, 771-774.

Masaoka, S., Lev-Ran, A., Hill, R., Vatal, G. & Hon, E.H.G. (1985) Heart rate variability in diabetes: relationship to age and duration of the disease. *Diabetes Care* 8, 64-68.

Matthews, D.M., Martyn, C.N., Riemersma, R.A., Clarke, B.F. & Ewing, D.J. (1987) Noradrenaline response to edrophonium (tensilon) and its relation to other autonomic tests in diabetic subjects. *Diabetes Research* 6, 175-180.

Milles, J.J., Baylis, P.H. & Wright, A.D. (1981) Plasma vasopressin during insulin withdrawal in insulin dependent diabetes mellitus. *Diabetologia* 20, 607-611.

Mitchell, E.A., Wealthall, S.R. & Elliot, R.B. (1983) Diabetic autonomic neuropathy in children: immediate heart rate response to standing. *Australian Paediatric Journal* 19, 175-177.

Möhring, J., Glanzer, K., Maciel J.A. Jr., Dusing, R., Kramer, H.J., Arbogast, R. & Koch-Weser, J. (1980) Greatly enhanced pressor response to antidiuretic hormone in patients with impaired cardiovascular reflexes due to idiopathic orthostatic hypotension. *Journal of Cardiovascular Pharmacology* 2, 367-376.

Monos, E., Cox, R.H. & Peterson, L.H. (1978) Direct effects of physiological doses of arginine vasopressin on the arterial wall in vivo. *American Journal of Physiology* 234, H167-172.

Montani, J.P., Liard, J.F., Schoun J. & Möhring, J. (1980) Haemodynamic effects of exogenous and endogenous vasopressin at low plasma concentrations in conscious dogs. *Circulation Research* 47, 346-355.

McCaffrey, P.M., Riddell, J.G. & Shanks, R.G. (1986) The partial agonist activity of some B-adrenoceptor antagonists by their effect on the cardiovascular system and finger tremor. *British Journal of Clinical Pharmacology* 21, 582P-583P.

Mackay, J.D., Page, M.M., Cambridge, J. & Watkins, P.J. (1980) Diabetic autonomic neuropathy. The diagnostic value of heart rate monitoring. *Diabetologia* 18, 471-478.

Norris, S.C., Harry, J.D., Young, J. & Sharman, P. (1984) Demonstrating the cardioselectivity of the B-adrenoreceptor blocking agent ICI 141,292 in man. *British Journal of Clinical Pharmacology* 18, 271P-272P.

O'Connor, W.J. & Verney, E.B. (1942) The effect of removal of the posterior lobe of the pituitary on the inhibition of water-diuresis by emotional stress. *Quarterly Journal of Experimental Physiology* 31, 393-408.

Oliver, G. & Schafer, E.A. (1895) On the physiological action of extracts of the pituitary body and certain other glandular organs. *Journal of Physiology (London)* 18, 277-279.

Padfield, P.L., Lever, A.F., Brown, J.J., Morton, J.J. & Robertson, J.I.S. (1976) Changes of vasopressin in hypertension: cause or effect? *Lancet* i, 1255-1257.

Page, M.McB. & Watkins, P.J. (1976) Provocation of postural hypotension by insulin in diabetic autonomic neuropathy. *Diabetes* 25, 90-95.

Page, M. McB. & Watkins, P.J. (1978) Cardiorespiratory arrest and diabetic autonomic neuropathy. *Lancet* 1, 14-16.

Pavy, F.W. (1885) Introductory address to the discussion on the clinical aspects of glycosuria (in two parts). *Lancet* 2, 1033-1035; 1085-1087.

Pitres, M.A. (1902) Note sur l'état des réflexes cutanés et pupillaires et des sensibilités testiculaire et épigastrique profondez les diabétiques. *Comptes Rendus de la Societe de Biologie (Paris)*, 4, 1286-1287.

Poole, C.J.M., Williams, T.D.M., Lightman, S.L. & Frankel, H.L. (1987) Neuroendocrine control of vasopressin secretion and its effect on blood pressure in subjects with spinal cord transection. *Brain* 110, 727-735.

Pringle, T.H., O'Connor, P.C., McNeill, A.J., Finch, M.B., Riddell, J.G. & Shanks, R.G. (1986) Effects of ICI 141,292 on exercise tachycardia and isoprenaline-induced B-adrenoceptor responses in man. *British Journal of Clinical Pharmacology* 21, 249-258.

Pryce, T.D. (1893) On diabetic neuritis with a clinical and pathological description of three cases of diabetic pseudo-tabes. *Brain* 16, 416-424.

Pullan, P.T., Johnston, C.I., Anderson, W.P. & Korner, P.I. (1980) Plasma vasopressin in blood pressure homeostasis and in experimental renal hypertension. *American Journal of Physiology* 239, H81-H87.

Puritz, R., Lightman, S.L., Wilcox, C.S., Forsling, M. & Bannister, R. (1983) Blood pressure and vasopressin in progressive autonomic failure: response to postural stimulation, L DOPA and naloxone. *Brain* 106, 503-511.

Quail, A.W., Woods, R.L. & Korner, P.I. (1987) Cardiac and arterial baroreceptor influences in release of vasopressin and renin during hemorrhage. *American Journal of Physiology* 252, H1120-H1126.

Reid, J.L. & Rubin, P.C. (1987) Peptides and central neural regulation of the circulation. *Physiological Reviews* 67, 725-749.

Reske-Nielsen, E., Lundback, K. & Rafaelsen, O.J. (1965) Pathological changes in the central and peripheral nervous system of young long-term diabetics (diabetic encephalopathy). *Diabetologia* 1, 233-241.

Ribeiro, A., Mulinari, R., Gavras, I., Kohlmann, O., Ramos, O. & Gavras, H. (1986) Sequential elimination of pressor mechanisms in severe hypertension in man. *Hypertension* 8 (suppl 1), I169-I173.

Riley, C.M., Day, R.L., Greenly, D.M. & Langford, W.S. (1949) Central autonomic dysfunction with defective lacrimation: report of 5 cases. *Paediatrics* 3, 468-478.

Rizza, R.A., Cryer, P.E., Haymond, M.W. & Gerich, J.E. (1980) Adrenergic mechanisms for the effect of epinephrine on glucose production and clearance in man. *Journal of Clinical Investigation* 65, 682-689.

Robertson, G.L., Mahir, E.A., Athar, S. & Sinha, T. (1973) Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. *Journal of Clinical Investigation* 52, 2340-2352.

Robertson, G.L. & Athar, S. (1976) The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. *Journal of Clinical Endocrinology and Metabolism* 42, 613-620.

Robertson, G.L., Shelton, R.L. & Athar, S. (1976) The osmoregulation of vasopressin. *Kidney International* 10, 25-37.

Robertson, G.L., Aycinena, P. & Zerbe, R.L. (1982) Neurogenic disorders of osmoregulation. *American Journal of Medicine* 72, 339-353.

Roulston, J.E. & McGregor, G.A. (1978) Measurement of plasma renin activity by radioimmunoassay after prolonged cold storage. *Clinica Chimica Acta* 88, 45-48.

Rowe, J.W., Shelton, R.L., Heiderman, J.H., Vestal, R.E. & Robertson, G.L. (1979) Influence of the emetic reflex on vasopressin release in man. *Kidney International* 16, 729-735.

Rowe, J.W., Minaker, K.L., Sparrow, D. & Robertson, G.L. (1982) Age-related failure of volume-pressure-mediated vasopressin release. *Journal of Clinical Endocrinology and Metabolism* 54, 661-664.

Roy, C., Barth, T. & Jard, S. (1975) Vasopressin-sensitive kidney adenylate cyclase. *Journal of*

*Biological Chemistry* **250**, 3149-3156.

Rundles, R.W. (1945) Diabetic neuropathy. General review with report of 125 cases. *Medicine (Baltimore)* **24**, 111-160.

Saad, C.I., Ribeiro, A.B., Zanella, M.T., Mulinari, R.A., Gavras, I. & Gavras, H. (1988) The role of vasopressin in blood pressure maintenance in diabetic orthostatic hypotension. *Hypertension* **11** (suppl 1), I217-I221.

Sawchenko, P.E. & Swanson, L.W. (1981) Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. *Science* **214**, 685-687.

Sawchenko, P.E. & Swanson, L.W. (1982) The organisation of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. *Brain Research Review* **4**, 275-325.

Schiffrin, E.L. & Genest, J. (1983) <sup>3</sup>H-vasopressin in binding to the rat mesenteric artery. *Endocrinology* **113**, 409-411.

Schrier, R.W. & Berl, T. (1972) Mechanism of the antidiuretic effect associated with the interruption of parasympathetic pathways. *Journal of Clinical Investigation* **51**, 2613-2620.

Schrier, R.W., Berl, T., Harbottle, J.A. & McDonald, K.M. (1975) Catecholamines and renal water excretion. *Nephron* **15**, 186-196.

Schrier, R.W., Berl, T. & Anderson, R.J. (1979) Osmotic and nonosmotic control of vasopressin release. *American*

*Journal of Physiology* 236(4), F321-332.

Schwartz, J. & Reid, I.A. (1981) Effect of vasopressin blockade on blood pressure regulation during hemorrhage in conscious dogs. *Endocrinology* 109, 1178-1180.

Seckl, J.R., Williams, T.D.M. & Lightman, S.L. (1986) Oral hypertonic saline causes transient fall of vasopressin in humans. *American Journal of Physiology* 251, R214-R217.

Sharpey-Schafer, E.P. & Taylor, P.J. (1960) Absent circulatory reflexes in diabetic neuritis. *Lancet* 1, 559-562.

Sharpey-Schafer, E.P. (1965) Effect of respiratory acts on the circulation. In: *Handbook of Physiology*. Vol III, pp 1875-1886. Eds. Hamilton, W.E., Dow, P. Maryland, Waverley Press.

Shaver, J.A., Leon, D.F., Gray, S., Leonard, J.J. & Bahnson, H.T. (1969) Hemodynamic observations after cardiac transplantation. *New England Journal of Medicine* 281, 822-827.

Shimamoto, K. Murase, T. & Yamaji, T. (1976) A heterologous radioimmunoassay for arginine vasopressin. *Journal of Laboratory and Clinical Medicine* 87, 338-344.

Shy, G.M. & Drager, G.A. (1960) A neurological syndrome associated with orthostatic hypotension. *Archives of Neurology* 2, 511-527.

Silverman, A.J. & Zimmerman, E.A. (1982) Adrenalectomy increases sprouting in a peptidergic neurosecretory

system. *Neuroscience* 7, 2705-2714.

Simpson, H.C.R., Zubillaga, J.E., Collier, J.G., Bennett, E.D., Ang, V.T.Y., Mehta, N. & Jenkins, J.S. (1986) Haemodynamic effects of vasopressin in man are related to posture. *Clinical Science* 70, 177-184.

Sklar, A.H. & Schrier, R.W. (1983) Central nervous system mediators of vasopressin release. *Physiological Reviews* 63, 1243-1280.

Smith, H.J., Halliday, S.E., Earl, D.C.N. & Stribling, D. (1983) Effects of selective (B<sub>1</sub> and B<sub>2</sub>) and non-selective B-adrenoceptor antagonists on the cardiovascular and metabolic responses to isoproterenol: comparison with ICI 141,292. *Journal of Pharmacology and Experimental Therapeutics* 226, 211-216.

Smith, S.E., Smith, S.A., Brown, P.M., Fox, C. & Sonksen, P.H. (1978) Pupillary signs in diabetic autonomic neuropathy. *British Medical Journal* 2, 924-927.

Spokes, E.G., Bannister, R. & Oppenheimer, D.R. (1979) Multiple system atrophy with autonomic failure: clinical, histological and neurochemical observations on four cases. *Journal of Neurological Sciences* 43, 59-82.

Spruce, B.A., McCulloch, A.J., Burd, J., Ørskov, H., Heaton, A., Baylis, P.H. & Alberti, K.G.M.M. (1985) The effect of vasopressin on glucose metabolism in man. *Clinical Endocrinology* 22, 463-468.

Starling, E.H. & Verney, E.B. (1924) The secretion of urine as studied on the isolated kidney. *Proceedings of the Royal Society of London (Biology)* 97, 321-363.

Stiles, G.L., Caron, M.G. & Lefkowitz, R.J. (1984) B-adrenergic receptors: biochemical mechanisms of physiological regulation. *Physiological Reviews* 64, 661-743.

Sved, A.F., McDowell, F.H. & Blessing, W.W. (1985) Release of antidiuretic hormone in quadriplegic subjects in response to head-up tilt. *Neurology* 35, 78-82.

Swanson, L.W. & Sawchenko, P.E. (1980) The paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. *Neuroendocrinology* 31, 410-417.

Thompson, C.J., Burd, J.M. & Baylis, P.H. (1987) Acute suppression of plasma vasopressin and thirst after drinking in hypernatraemic humans. *American Journal of Physiology* 252, R1138-R1142.

Thompson, C.J., Davis, S.N., Butler, P.C., Charlton, J.A. & Baylis, P.H. (1988) Osmoregulation of thirst and vasopressin secretion in insulin-dependent diabetes mellitus. *Clinical Science* 74, 599-606.

Thompson, C.J., Davis, S.N. & Baylis, P.H. (1989) Effect of blood glucose concentration on osmoregulation in diabetes mellitus. *American Journal of Physiology* 256, R597-R604.

Thompson, C.J., Thow, J., Jones, I.R. & Baylis, P.H. (1989) Vasopressin secretion during insulin-induced hypoglycaemia. Exaggerated response in people with Type I diabetes. *Diabetic Medicine* 6, 158-163.

Thompson, D.A., Campbell, R.G., Lilavanthana, U. & Robertson, G.L. (1981) Increased thirst and plasma arginine vasopressin levels during 2-deoxy-D-glucose-induced glucoprivation in humans. *Journal of Clinical Investigation* 60, 1083-1093.

Verney, E.B. (1947) The antidiuretic hormone and the factors which determine its release. *Proceedings of the Royal Society of London (Biology)* 135, 27-106.

Vokes, T.P., Aycinena, P.R. & Robertson, G.L. (1987) Effect of insulin on osmoregulation of vasopressin. *American Journal of Physiology* 252, E538-548.

Vokes, T. & Robertson, G.L. (1985) Effects of insulin on the osmoregulation of thirst and vasopressin. In: *Vasopressin*. p 271-279. Ed. Schrier, R.W. New York, Raven Press.

Wagner, H.N. & Braunwald, E. (1956) The pressor effect of the antidiuretic principle of the posterior pituitary in orthostatic hypotension. *Journal of Clinical Investigation* 35, 1412-1418.

Walsh, C.H., Baylis, P.H. & Malins, J.M. (1979) Plasma arginine vasopressin in diabetic ketoacidosis. *Diabetologia* 16, 93-96.

Wang, B.C., Sundet, W.D., Hakumaki, M.O.K. & Goetz, K.L. (1983) Vasopressin and renin responses to hemorrhage in conscious, cardiac denervated dogs. *American Journal of Physiology* 245, H399-H405.

Wang, B.C., Flora-Ginter, G., Leadley, R.J. & Goetz, K.L. (1988) Ventricular receptors stimulate vasopressin release during hemorrhage. *American Journal of Physiology* 254, R204-R211.

Watkins, P.J. (1973) Facial sweating after food: a new sign of diabetic autonomic neuropathy. *British Medical Journal* 1, 583-587.

Watkins, P.J. & Mackay, J.D. (1980) Cardiac denervation in diabetic neuropathy. *Annals of Internal Medicine* 92, 304-307.

Watkins, P.J. & Edmonds, M.E. (1983) Clinical presentation of diabetic autonomic failure. In: *Autonomic Failure: a textbook of clinical disorders of the autonomic nervous system.* p 337-370. Ed. Bannister, R. Oxford, Oxford University Press.

Webber, R.H. (1978) Some aspects of the historical development of the autonomic nervous system. *Journal of Neural Transmitters* 42, 3-8.

Weiner, N. & Taylor, P. (1985) Neurohumoral transmission: the autonomic and somatic motor nervous system. In: *The pharmacological basis of therapeutics* (7th edition), p 66-99. Eds. Gilman, A.G., Goodman, L.S., Rall, T.N. & Murad, F. New York, Macmillan Publishing Company.

Wheeler, T. & Watkins, P.J. (1973) Cardiac denervation in diabetes mellitus. *British Medical Journal* 4, 584-586.

Williams, T.D.M., Carter, D.A. & Lightman, S.L. (1985) Sexual dimorphism in the posterior pituitary response to stress in the rat. *Endocrinology* 116, 738-740.

Williams, T.D.M., Lightman, S.L. & Bannister, R. (1985) Vasopressin secretion in progressive autonomic failure: evidence for defective afferent cardiovascular pathways. *Journal of Neurology, Neurosurgery and Psychiatry* 48, 225-228.

Williams, T.D.M., Lightman, S.L. & Leadbetter, M.J. (1986) Hormonal and cardiovascular responses to DDAVP in man. *Clinical Endocrinology* 24, 89-96.

Williams, T.D.M., Da Costa, D., Mathias, C.J., Bannister, R. & Lightman, S.L. (1986) Pressor effect of arginine vasopressin in progressive autonomic failure. *Clinical Science* 71, 173-178.

Winocour, P., Dhar, H. & Anderson, D.C. (1986) The relationship between autonomic neuropathy and urinary sodium and albumin excretion in insulin-treated diabetics. *Diabetic Medicine* 3, 436-440.

Wooten, R.L. & Meriwether, T.W. (1961) Diabetic gastric atony: a clinical study. *Journal of the American Medical Association* 176, 1082-1087.

Wurster, R.D. (1984) Central nervous system regulation of the heart: an overview. In: *Nervous Control of Cardiovascular Function*. Ch 13, pp 307-320. Ed. Randall, W.C. New York, Oxford University Press.

Zerbe, R.L., Vinicor, F. & Robertson, G.L. (1979) Plasma vasopressin in uncontrolled diabetes mellitus. *Diabetes* 28, 503-508.

Zerbe, R.L., Henry, D.P. & Robertson, G.L. (1983) Vasopressin response to orthostatic hypotension. Etiologic and clinical implications. *American Journal of Medicine* 74, 265-271.

Zerbe, R.L. & Robertson, G.L. (1983) Osmoregulation of thirst and vasopressin in human subjects: effects of various solutes. *American Journal of Physiology* 244, E607-E614.

Zerbe, R.L., Vinicor, F. & Robertson, G.L. (1985) Regulation of plasma vasopressin in insulin-dependent diabetes mellitus. *American Journal of Physiology* 249, E317-325.

Young, R.J., Ewing, D.J. & Clarke, B.F. (1983) Nerve function and metabolic control in teenage diabetics. *Diabetes* 32, 142-147.

## LIST OF ABBREVIATIONS

|   |   |
|---|---|
| Ach                                     | Acetylcholine   |
| AUC                                     | Area under the curve  |
| AVP                                     | Arginine vasopressin  |
| Beats min <sup>-1</sup>                 | Beats per minute  |
| CV                                      | Coefficient(s) of variation   |
| DAN +ve                                 | Diabetics with cardiovascular reflex test evidence of autonomic neuropathy    |
| DAN -ve                                 | Diabetics without cardiovascular reflex test evidence of autonomic neuropathy |
| HR                                      | Heart rate  |
| ISA                                     | Intrinsic sympathomimetic activity  |
| MAP/MBP                                 | Mean arterial pressure/mean blood pressure                                    |
| mmHg                                    | Millimetres of mercury  |
| mmol l <sup>-1</sup>                    | Millimoles per litre  |
| mosm kg <sup>-1</sup>                   | Milliosmoles per kilogram   |
| MSA                                     | Multiple system atrophy   |
| ng ml <sup>-1</sup> hr <sup>-1</sup>    | Nanograms per millilitre per hour   |
| nmol l <sup>-1</sup>                    | Nanomoles per litre   |
| NTS                                     | Nucleus of the tractus solitarius   |
| OT                                      | Oxytocin  |
| OVLTT                                   | Organum vasculosum of the lamina terminalis                                   |
| PAA                                     | Partial agonist activity  |
| PAF                                     | Progressive autonomic failure   |
| pg ml <sup>-1</sup>                     | Picograms per millilitre  |
| pmol l <sup>-1</sup>                    | Picomoles per litre   |
| pmol min <sup>-1</sup> kg <sup>-1</sup> | Picomoles per minute per kilogram   |
| PRA                                     | Plasma renin activity   |

PVN                    Paraventricular nucleus  
SON                    Supraoptic nucleus  
TPR                    Total peripheral resistance  
umol l<sup>-1</sup>                Micromoles per litre

