CARDIOVASCULAR AUTOREGULATION IN

INSULIN

DEPENDENT DIABETIC PATIENTS.

Submitted for the degree of M.D.

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'What is written without effort is in general read without pleasure.'

Samuel Johnson (1709-1784)

CHAPTER 1.

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INTRODUCTION

1.1 Background

Diabetes mellitus can be defined as an abnormality of glucose metabolism and is a common metabolic abnormality. The incidence of insulin dependent diabetes mellitus (IDDM) is often established from diabetes registries, many of which are coordinated within the Diabetes Epidemiology Research International Group (Diabetes Epidemiology Research International Group 1987). The work of this group has demonstrated an enormous range of incidence rates between populations and to a lesser extent within populations. Most studies of the incidence of IDDM have been conducted amongst children and young adults partly because older subjects presenting with diabetes mellitus are unlikely to have IDDM. The incidence of IDDM for the United Kingdom for the period 1980-83 was 21.7/100,000.

Diabetes mellitus would be nothing more than a annoying inconvenience for most patients were it not for the profound vascular abnormalities that develop as a consequence of the metabolic abnormalities. The excess premature mortality associated with IDDM can mostly be attributed to the increased incidence of cardiovascular disease as reported by the Framingham study (Kannel 1976). From this data, the average annual age-adjusted incidence of cardiovascular disease was increased 2.2 fold in diabetic men and 2.8 fold in diabetic women when compared with the non-diabetic population. Similarly, cardiovascular disease mortality was greater in diabetic men and women. When the categories of cardiovascular disease are further analysed, it is apparent that a wide spectrum of cardiovascular events including heart failure, coronary heart disease and peripheral vascular disease are all increased in IDDM. Even after correcting for

confounding factors such as cigarette smoking, hypercholesterolaemia and hypertension, the relative risk for this spectrum of cardiovascular morbidity and mortality remains significantly higher in IDDM patients (Kannel 1976). From this, it can be seen that diabetes is a major independent risk factor for the development of macrovascular disease. Diabetes mellitus is also complicated by microvascular disease due to damage and dysfunction of the capillary beds. The widespread vascular injury that results from microangiopathic damage is unique to diabetes and presents itself in many ways. It is clinically most apparent in the eye where it results in the characteristic diabetic retinopathy. Similar pathological changes develop in all other microvascular beds resulting in diabetic nephropathy, diabetic neuropathy (perhaps due to involvement of the vaso nervorum) and diabetic cardiomyopathy.

The pathogenesis of small vessel damage remains obscure. In 1949 light microscopic appearances of hyalinization (thickening) of the retinal capillaries was described (Ashton 1949). Subsequently, electron microscopic studies have revealed thickening of the capillary basement membrane and this phenomenon is now considered the ultrastructural hallmark of diabetic microvascular damage (Hidayat et al 1985). Whilst the concept of a single pathogenic mechanism for all diabetic complications is appealing the discordant evolution of different complications (e.g. retinopathy develops in almost all patients and nephropathy in less than half) is against this theory. In general, three categories of possible pathogenic mechanisms have been suggested: glucose-related, including abnormalities in polyol metabolism (Greene et al 1987) and excessive glycation of membrane bound proteins (Brownlee et al 1988); vascular mechanisms, including abnormalities of

endothelial function (Lorenzi et al 1991) and supporting cells such as mesangial cells in the glomerulus (Steffes et al 1989) as well as hyperfiltration and intra-renal hypertension in the kidney (Hostetter et al 1982); finally, rheological factors such as abnormalities of platelet function (Colwell et al 1983) and increases in coagulation factors such as Von Willebrand factor (Jensen et al 1989) may also play a role.

As the primary metabolic abnormality in IDDM is hyperglycaemia, it seems reasonable to assume that directly or indirectly the complications of IDDM can be attributed to hyperglycaemia. Although studies in animal models of diabetes (Engerman et al 1977) and epidemiological studies (Klein et al 1984) have long implicated hyperglycaemia in the pathogenesis of long-term diabetic complications, clinical trials had previously failed to show a consistent or convincing benefit of intensive blood glucose treatment in humans because of the small sample sizes, short duration of study and lack of tight glycaemic control through-out the study period (Kroc Collaborative Study Group 1984, Lauritzen T et al 1985, Brinchmann-Hansen et al 1988). In 1993 the Diabetes Control and Complications Trial (DCCT) finally and convincingly showed delay in the onset and slowing of progression of diabetic retinopathy, nephropathy and neuropathy (Diabetes Control and Complications Trial Research Group 1993). This was a multicentre, randomised clinical trial designed to compare intensive and conventional diabetes treatments with regard to their effects on the development and progression of the early vascular and neurologic complications of IDDM. The intensive therapy regimen, consisting of three or more insulin injections daily or treatment with a insulin pump, was compared with conventional therapy of once or twice-daily insulin injections. The two

cohorts were studied to see if intensive therapy prevented the development of diabetic retinopathy in patients with no retinopathy (i.e. primary prevention), and would intensive therapy affect the progression of early retinopathy (i.e. secondary prevention). Whilst retinopathy was the principal outcome of the study renal, neurologic and cardiovascular outcomes were also studied in the two groups. Over the six years of the study intensive therapy reduced the risk of developing retinopathy by 76% and slowed the progression of retinopathy by 54%. Subsequently the data for diabetic nephropathy (Diabetes Control and Complications Trial Research Group 1995a) and neuropathy (Diabetes Control and Complications Trial Research Group 1995b) have also been published. Intensive control of blood glucose reduced the risk of developing microalbuminuria (> 28μg/min) in the primary prevention group by 34%. In the secondary prevention group (those with retinopathy), in those patients with no microalbuminuria on entry into the study, intensive therapy delayed the development of microalbuminuria by 43%. Intensive therapy reduced the development of confirmed clinical neuropathy (defined as a history or physical examination consistent with clinical neuropathy confirmed by either abnormal nerve conduction or autonomic nervous system testing) by 64% in the combined cohorts after five years of follow up.

Further evidence for improved glycaemic control preventing or delaying the onset of diabetic nephropathy comes from the recent study by Krolewski and colleagues (Krolewski et al 1995). They looked prospectively at the risk of developing microalbuminuria for a given level of hyperglycaemia, as measured by the glycosylated haemoglobin (haemoglobin A_1) in a group of IDDM patients. The study showed that the

risk of microalbuminuria was strongly related to the degree of hyperglycaemia. Furthermore, if haemoglobin A_1 levels were less than 10% then the risk of developing microalbuminuria was independent of the level of hyperglycaemia and was possibly related to other components of the diabetic condition such as abnormalities of plasma insulin concentration (Nestler et al 1990, Abrass et al 1994). Thus, recent studies support the idea that the degree of hyperglycaemia is important in the development of diabetic complications and that tight glycaemic control can reduce the risk of developing these complications.

Strict glycaemic control is not without its difficulties. Firstly, the risk of hypoglycaemia was three times higher in the intensive treatment group of the DCCT and this was after those at high risk for hypoglycaemia were excluded from the study (Diabetes Control and Complications Trial Research Group 1993 and 1995c). The second concern was the greater cost incurred by the intensive therapy group, which has recently been estimated at three times the cost of conventional therapy (Diabetes Control and Complications Trial Research Group 1995d). This expense was not only due to the increased treatment but also due to the longer time spent by health care professionals in counseling and caring for patients; patients in the intensive treatment arm of the DCCT were often admitted to hospital to commence intensive therapy and were seen monthly in the clinic. In-between times, they were contacted by telephone to make daily adjustments to their therapeutic regimens. Clearly, in averting complications the hope is that the initial costs will be outweighed by the potential financial saving although this has not yet proven to be the case. Finally, there was an increase in the proportion of patients in the intensive treatment

group who became overweight (Diabetes Control and Complications Trial Research Group 1995c) although the consequences of this are as yet unclear. Thus, despite the clear long-term benefits in terms of complication rates to using intensive therapy in IDDM other factors have to be considered before using this as the norm for treating IDDM.

1.2 Diabetic neuropathy

1.2.1 Introduction

Epidemiology

Diabetic neuropathy encompasses a wide range of abnormalities involving both the somatic and autonomic nervous systems and has profound effects on the mortality and morbidity of affected diabetic patients (Pickup et al 1994). The prevalence and incidence of diabetic neuropathy are difficult to assess largely due to the inconsistencies of the definition: studies have estimated the prevalence of somatic neuropathy as low as 7% for symptomatic patients (Pirart 1974) to 100% when diagnosis is based on nerve conduction studies (Vinik et al 1992). In a large prospective study of diabetic out-patients the prevalence rose from 7.5% at the time of diagnosis of diabetes to 50% after 25 years. (Pirart 1974). In another prospective study, Palumbo and colleagues found the prevalence to be half this value although this study was confined to non-insulin dependent diabetic patients without neuropathy at the time of entry into the study (Palumbo et al 1978). In an attempt to standardize the diagnosis of diabetic neuropathy and allow staging of the evolution of the disease standard criteria have been defined (Dyck et al 1985).

1.2.2 Pathology

Most information on the pathological changes in diabetic neuropathy has derived from nerve biopsy specimens which obviously sample only a small portion of the peripheral nervous system. Patients with diabetic polyneuropathy show axonal loss, which increases in severity distally, plus moderate demyelination. In one biopsy study of untreated diabetic subjects the predominant change on sural nerve biopsy in asymptomatic patients was segmental demyelination and remyelination (Dyck et al 1980); patients with symptoms showed a combination of demyelination and remyelination plus axonal loss.

1.2.3 Neurophysiology

In asymptomatic diabetic patients, nerve conduction velocity of motor and sensory fibres is often slightly reduced and sensory nerve action potentials are frequently diminished in amplitude indicating subclinical neuropathy. In established diabetic polyneuropathy, the abnormalities are greater in the sensory than the motor fibres and more prominent in the lower than the upper limbs.

1.2.4 Pathogenesis of diabetic nerve damage

Woltman first reported nerve lesions associated with vascular changes in autopsies performed on diabetic patients (Woltman et al 1929). In 1959, it was reported that there was occlusion of the lumen together with thickening and hyalinization of the vessel walls of the epineural and endoneural vessels in diabetic patients with peripheral neuropathy

(Fagerberg 1959). It was suggested, therefore, that these vascular changes were the primary cause of diabetic neuropathy. Some time afterwards, however, other workers reported that the primary affected site was the peripheral nerve and the nerve root and, indeed, no vascular change could be found (Dolman 1963, Greenbaum et al 1964). Later still, as mentioned above, demyelination was found to be a consistent feature of diabetic neuropathy and thus the neuropathy was assumed to be a disease of the Schwann cell (Thomas et al 1965, Thomas et al 1966). Subsequently, axonal degeneration was demonstrated in myelinated and unmyelinated fibres and suggested that the main abnormality was found in the axon (Bischoff 1973). More recently, ultrastructural studies have disclosed the Schwann cell, axonal and vascular changes in more detail and they all appeared to develop independently (Behse 1977). However, the morphology of endoneurial vessels was not studied nor were the changes in the myelinated nerve fibres.

The vascular theory for the pathogenesis of diabetic neuropathy

The vascular theory for the pathogenesis of diabetic neuropathy received renewed support in the 1980s. It has been shown, as mentioned before, that the endoneurial vessels of diabetic patients with somatic neuropathy undergo characteristic changes with hyalin thickening and increased periodic acid-Schiff positive substances in their vessel wall (Fagerberg 1959). More recent work has shown a significant negative correlation between thickness of the basement membrane of the endoneurial vessels and myelinated fibre density and that the reduction in fibre density correlated with nerve conduction deficits (Yagihashi 1995). In 1986, a systematic exploration of diabetic patients with neurographic

studies found that focal myelinated fibre loss was the main pathology in diabetic polyneuropathy and that this suggested an ischaemic aetiology (Dyck 1986). At the same time oxygen tension in sural nerves of diabetics was found to be reduced thus reinforcing the ischaemic hypothesis (Newrick et al 1986). Furthermore, a close correlation was found between the severity of the neuropathy and the number of 'closed' capillaries, which was ascribed to the swelling and hyperplasia of endothelial cells with thickening of the basement membrane, seen on electron microscopy (Dyck et al 1985).

Focal fibre loss, however, has also been found to be a feature of neuropathies in which a vascular basis has been discounted such as Charcot-Marie-Tooth disease (Llewlyn et al 1988), thus focal segmental fibre loss is not always an indication of a vascular aetiology. Other workers found capillary closure in non-diabetic subjects and failed to show a specificity of capillary closure to diabetic neuropathy however the significance of the thickened endoneurial vessel wall was re-confirmed (Yasuda et al 1987, Bradley et al 1990). These findings led to a revision of the vascular theory of diabetic neuropathy and suggested that it was the overall functional and structural changes of endoneurial vessels which induced ischaemia and increased permeability of plasma components, rather than vascular closure (Dyck 1989).

The significance of microvascular changes in the pathogenesis of diabetic neuropathy has been reinforced by ultrastructural studies of peripheral nerves which have shown that nerve fibre alterations and microvascular changes proceed together (Britland 1990, Malik 1992, Malik 1993). Abnormalities of endoneurial vessels are universally demonstrated in human diabetic nerves and are correlated with the severity of the neuropathy. However,

conclusive evidence is still lacking demonstrating that nerve fibre pathology is the consequence of vascular lesions.

Owing to the inconclusive data for a vascular aetiology for diabetic neuropathy other mechanisms have been proposed as possible causes and will now be discussed.

Metabolic theories

Increasing levels of hyperglycaemia activate the polyol pathway in tissues such as nerve, retina, glomerulus and vessel wall. Glucose is converted to its polyol, sorbitol, and then sorbitol is converted to fructose. The first step is mediated by aldose reductase, the rate limiting enzyme, and the second by sorbitol dehydrogenase. This pathway is normally relatively inactive but under hyperglycaemic conditions the pathway is activated. Sorbitol then accumulates in the tissues which in turn results in a reciprocal depletion of another polyol, myoinositol, and taurine. These metabolic changes are, in turn, associated with severe nerve dysfunction (Stevens et al 1993). Reduced myoinositol concentration is associated with abnormal phosphoinositides and decreased membrane Na^+-K^+ ATPase activity, so impairing nerve function. This theory has been supported by studies in diabetic rats where improved nerve conduction velocity was observed when myoinositol was supplemented in the diet (Greene et al 1975, Greene et al 1983). Since myoinositol transport is dependent on Na^+- K^+ ATPase activity, a self-reinforcing cycle of reduced myoinositol uptake and reduced Na^+- K^+ ATPase activity results in an increased intra-axonal Na^+. It has been claimed that intra-axonal accumulation of Na^+ leads to axonal

swelling and axoglial dysjunction- the separation of the termination of the myelin lamellae from the axon- and further structural changes (Sima et al 1988, Sharma et al 1993).

Further evidence for the link between the polyol pathway and myoinositol depletion in the pathogenesis of diabetic neuropathy was demonstrated in animals by the corrective effects of aldose reductase inhibitors. Administration of aldose reductase inhibitors to rabbits with diabetic neuropathy was associated with improved nerve conduction velocities (Greene et al 1990). Long-term administration of fucose, a myoinositol antagonist, to rats resulted in a reduction of nerve conduction velocities and reduced myoinositol in peripheral nerves (Yorek et al 1992). Furthermore, treatment with myoinositol restored nerve conduction in these rats (Yorek et al 1993). However, evidence for the role of myoinositol in the pathogenesis of human diabetic neuropathy is lacking. Studies administering myoinositol to humans showed no benefit in peripheral nerve function (Gregersen et al 1978, 1983)

Whilst these changes in sorbitol metabolism have been demonstrated in animals and humans, a reduction in nerve myoinositol concentration has not been demonstrated in human studies (Hale et al 1987, Dyck et al 1988) and results of aldose reductase inhibitors in humans have been disappointing (Masson et al 1990). Furthermore, galactose-induced neuropathy, in which nerve myoinositol levels are also reduced, Na^+- K^+ ATPase is increased (Lambourne et al 1987) indicating a complex relationship between myoinositol metabolism and Na^+- K^+ ATPase activity.

The enhanced polyol pathway and glycation of structural proteins are claimed to be responsible for the basement membrane thickening seen in the retina and kidney of diabetic animals (Sima et al 1985, Kern et al 1987). It has been shown that increased cell fragility,

cell death and cell regeneration occur in response to the increased polyol pathway activity and to increased glycation. These effects may be responsible, in part, for the thickened and reduplicated basement membrane seen in diabetic capillaries (Vracko et al 1970,1973). Activation of the polyol pathway is accompanied by massive consumption of NAD when sorbitol is converted to fructose, resulting in increased NADH/NAD^+ ratios. This results in increased free radical production which has been linked to the alterations of cellular phosphoinositide metabolism and subsequent alterations in diacylglycerol and protein kinase C metabolism (Williamson et al 1992). These metabolic alterations resemble those seen in tissue hypoxia and have been termed 'diabetic pseudohypoxia' (Williamson et al 1993). Such metabolic changes have been associated with increased nerve blood flow in diabetic rats (Tilton et al 1989), although more recent studies have demonstrated a reduction in blood flow (Calcutt et al 1994). Comparisons between these studies are difficult because different methods of measuring nerve blood flow were used and therefore further work is required.

When hyperglycaemia activates the polyol pathway there is consumption of NADPH, which is used to convert glucose to sorbitol. NADPH is necessary for the production of nitric oxide (NO) by NO synthetase, using L-arginine as a substrate. Thus, consumption of NADPH inhibits the production of NO and reduces its release from endothelial cells. Cameron and colleagues have shown a reduction in nerve blood flow and slowed nerve conduction in rats administered an NO synthetase inhibitor (Cameron et al 1993). Thus, there appears to be a close link between the polyol pathway and NO production, and a reduction in NO production may be associated with abnormalities of nerve function.

As would be expected after the DCCT showed a benefit in tight blood sugar control in slowing the development of diabetic neuropathy, hyperglycaemia has been implicated in the pathogenesis of diabetic neuropathy. Increased glycation of nerve structure proteins including myelin proteins, tubulin and neurofilaments has been observed following experimentally induced hyperglycaemia (Williams et al 1982, Cullum et al 1991, Ryle et al 1994). Perineurial collagen and the basement membranes of vessel walls are also glycated during hyperglycaemia and, subsequently, this compromises the diffusion barrier. Furthermore, glycation of perineurial collagen may alter the permeability of the perineurium and lead to the compression of transperineurial arterioles and to endoneurial ischaemia (Myers et al 1989). Glycation of tubulin and neurofilaments may be related to disturbances of axonal transport which has been seen in experimental diabetic neuropathy (Sidenius et al 1979) and may contribute to the distal axonal atrophy seen in diabetic neuropathy. Glycation of myelin proteins may render the myelin membrane susceptible to external injuries or environmental insults and thus contribute to demyelination (Vlassara et al 1983). Advanced glycation end-products have been localised to the endoneurial vessels, perineurial basement membranes as well as the Schwann cell and axonal cytoplasm (Sugimoto et al 1995) showing excessive glycation of various neural proteins. Excessive glycation has been shown to promote the release of free radicals (Brownlee et al 1988) which, as mentioned earlier, interferes with endothelial function and alters endoneurial blood flow. Furthermore, advanced glycated end products activate macrophages to produce tumour necrosis factor-α and other cytokines (Brownlee et al 1994). A recent study administering N-acetylcysteine (a free radical scavenger and

inhibitor of tumour necrosis factor-α) has been shown to inhibit the development of functional and structural peripheral nerve abnormalities in diabetic rats (Sagara et al 1996). Finally, glycation of laminin has been suggested to inhibit nerve regeneration (Federoff et al 1993).

In summary, diabetic neuropathy encompasses diverse symptom complexes. The pathology of diabetic nerve lesions is heterogeneous suggesting that the aetiology is multifactorial. From animal models of diabetes, it has become clear that enhanced polyol pathway activity plays an important (but partial) role in the pathogenesis of diabetic neuropathy. Polyol pathway activation is associated with endoneurial vessel changes and altered blood flow which could contribute to the structural and functional changes seen in peripheral nerves. Glycation of neural structure proteins is involved in the pathogenesis of diabetic neuropathy, possibly by a combination oxidative stress and advanced glycation end-products.

1.3 Diabetic Autonomic Neuropathy

1.3.1 Introduction

Diabetes mellitus commonly affects the autonomic nervous system with up to 40% of diabetic patients demonstrating some abnormality on formal autonomic nervous system testing (Ewing et al 1985) although only relatively few have symptoms of autonomic neuropathy. As newer, more sensitive tests are used to study autonomic neuropathy the incidence rises. Symptoms from autonomic neuropathy occur relatively late in the natural history of the condition and are many and varied in nature.

Aetiology

As evidenced by several recent trials, hyperglycaemia is of paramount importance in the development of diabetic autonomic neuropathy. Young and colleagues (1986) in a large prospective study showed a clear relationship between deteriorating cardiovascular reflex tests and poor glycaemic control. Moreover, patients whose metabolic control was improved for two years by continuous subcutaneous insulin infusion have shown small but significant improvements in autonomic function tests (Jakobsen et al 1988). Finally, the DCCT showed a reduction of 44% in prevalence of abnormal autonomic function tests in the intensive treatment group compared with the conventionally treated arm of the study over the 5 years of follow up (The Diabetes Control and Complications Trial Research Group 1995b). The pathogenesis of autonomic neuropathy in diabetic patients thus appears to be the same for somatic and autonomic nerves. However it has also been suggested that diabetic autonomic neuropathy may have an immunological basis, as evidenced by the association between iritis and diabetic autonomic neuropathy (Guy et al 1984). This hypothesis has, however, been disputed (Martyn et al 1986) and additional studies are required to further explore these findings.

Relationship of diabetic autonomic neuropathy to other complications

Traditionally, it is supposed that diabetic autonomic neuropathy rarely occurs independently of peripheral neuropathy (Ellenberg 1976, Ewing et al 1981). However this view has recently been challenged by a large study by Flynn and colleagues (1995). They

looked at a large group of 546 insulin dependent diabetic patients and assessed them for evidence of peripheral neuropathy, as defined by absence of deep tendon reflexes and reduced vibration sensory threshold measured with a Biothesiometer, and autonomic neuropathy assessed by standard cardiovascular reflex tests. It was shown that half of the subjects with abnormal autonomic function had no clinical evidence of peripheral neuropathy. This disparity in trial findings can be explained partly by differences in definition of neuropathy and in a difference in the sensitivity of the techniques used to diagnose neuropathy. The earlier studies used nerve conduction velocities to diagnose peripheral neuropathy and slowing of nerve conduction has been demonstrated before the development of clinical signs or symptoms of neuropathy (Lawrence et al 1961).

A clinical association between neuropathy and nephropathy in diabetes has been suggested in some studies. It has been shown that there is a progressive increase in the prevalence of peripheral neuropathy in different stages of diabetic nephropathy from microalbuminuria to macroalbuminuria (Parving et al 1988). Other workers have shown a similar increasing prevalence and severity of autonomic neuropathy with progression of nephropathy. In particular, Zander and colleagues (1989) found an increase of 100% in abnormal cardiovascular reflex tests in patients with macroalbuminuria compared to those with normoalbuminuria. Moreover, chronic renal failure itself, regardless of the aetiology, can lead to an impairment of autonomic function (Malik et al 1986). Using spectral analysis methods to look at heart-rate variability, the link between diabetic autonomic neuropathy and diabetic nephropathy is further strengthened. A recent study showed that microalbuminuric patients (i.e. those patients with the earliest detectable clinical evidence

of diabetic nephropathy) had evidence of impaired vagal function and abnormal sympathovagal balance (Mølgaard 1994).

A pathogenetic relationship between autonomic neuropathy and nephropathy was suggested as early as 1982 when an increase in renal blood flow was found in IDDM patients with symptomatic diabetic autonomic neuropathy (Hilsted 1982). Animal studies have lead to further information in this area. In rats, renal nerve stimulation by direct micropuncture results in an increase in vascular resistance mediated through α_1 - adrenoreceptors which results in a reduction in renal blood flow and glomerular filtration rate (Pelayo et al 1989). Furthermore, renal sympathetic nerves regulate urinary sodium excretion by a direct effect on tubular reabsorption and also by changing renal haemodynamics and renin release. Thus, stimulation of renal sympathetic nerves reduces sodium excretion and increases sodium reabsorption, whilst decreased sympathetic activity has the opposite effect (Pelayo et al 1989). This action is thought to be mediated via the α_1 -adrenoreceptors on the tubular membranes activating the Na^+K^+ ATPase with resulting enhancement of the transepithelial sodium transport (DiBona 1994). Further studies in conscious animals have shown that changes in blood volume are sensed by atrial pressure receptors and these determine adjustments in renal sympathetic nerve activity and thus renal sodium reabsorption/excretion; head-up tilt, for example, results in a decrease in left atrial pressure, increased renal sympathetic activity and antinatriuresis (Miki et al 1993).

While the diuretic and natriuretic effects of denervation are easily demonstrable in experimental conditions, haemodynamic consequences of renal denervation are less clear.

The degree of renal sympathetic nerve stimulation needed to induce a change in blood flow is greater than that needed to induce a change in renin release or urinary sodium excretion (DiBona 1994). Furthermore, patients with autonomic failure are unable to reduce sodium excretion following sodium restriction (Wilcox et al 1977).

Whilst transposing these experimental results to the patient with diabetic autonomic neuropathy is difficult, the association between diabetic autonomic neuropathy and nephropathy could imply a pathogenetic significance rather than a chance association of two diabetic complications (Lilja et al 1985). Epidemiological studies have shown a striking increase in the incidence of nephropathy in a group of IDDM patients with symptomatic autonomic neuropathy (Sampson et al 1990, Sundkvist et al 1993). Renal perfusion, assessed using doppler techniques, has been found to be decreased in patients with familial autonomic failure while standing and during exercise (Axelrod et al 1993). It has been postulated that these changes in renal haemodynamics could be due to a global decreases in blood pressure associated with profound postural hypotension (Axelrod et al 1993). Furthermore, an excessive nocturnal diuresis and natriuresis and a consequent increase in morning postural hypotension has been demonstrated in patients with non-diabetic autonomic neuropathy, possibly due to defect in renal salt conservation as a consequence of renal denervation (Wilcox et al 1977).

Changes in the diurnal pattern of urine output and sodium excretion have also been described in diabetic patients with autonomic neuropathy (Wincour et al 1986, Bell et al 1987). Wincour and colleagues reported a higher nocturnal urinary albumin excretion and nocturnal sodium excretion rate in insulin treated diabetics with diabetic autonomic

neuropathy and suggested these abnormalities may be due to derangement of nocturnal renal haemodynamics as a result of impaired neural control. Other workers have observed a nightly rise in atrial naturetic peptide with an increased nocturnal diuresis and natriuresis in non-insulin dependent diabetic patients with autonomic neuropathy and a reversed circadian rhythm in systolic blood pressure. It was postulated to be due to water and sodium retention with postural pooling during the day followed by blood volume expansion with recumbency at night (Nakano et al 1994). Others have observed similar abnormalities in IDDM patients and have postulated that diabetic autonomic neuropathy decreases intraglomerular pressure during the day and raises it during the night, thus this relative nocturnal intraglomerular hypertension leads to a deterioration in glomerular filtration rate (Sundkvist et al 1993).

The hypothesis that diabetic autonomic neuropathy is involved in the development of diabetic nephropathy has received further support recently. Spallone and colleagues (1994) found that a group of IDDM patients with diabetic autonomic neuropathy displayed a lower nocturnal fall in systolic blood pressure than a group of IDDM patients with no autonomic dysfunction. Furthermore, urinary albumin excretion and urine volume also were reduced compared to the IDDM patients without diabetic autonomic neuropathy. This led them to suggest that the autonomic neuropathy resulted in a reduced nocturnal blood pressure fall and that this was directly responsible for the reduction in urinary albumin excretion and urine volume by increasing renal blood flow, glomerular filtration rate and impairing tubular water and sodium reabsorption. Furthermore, it was noted that for any given systolic blood pressure, neuropathic patients had a higher urinary

albumin excretion than those patients without neuropathy. Whilst neuropathic patients could simply have had more advanced nephropathy there was no significant difference in 24 hour urinary albumin excretion between those patients with diabetic autonomic neuropathy and those with normal autonomic function. Indeed, the relationship between urinary albumin excretion and systolic blood pressure in a group of IDDM patients was the same between those with microalbuminuria and those normoalbuminuric patients (Hansen et al 1992). It is thus possible that there is a pathogenetic relationship between diabetic autonomic neuropathy and nephropathy potentially due to the loss of compensatory mechanisms of glomerular haemodynamics, which are under control of the autonomic nervous system.

Similarly, it has been suggested that diabetic autonomic neuropathy may be a risk factor for early-onset proliferative retinopathy (Krolewski et al 1992). The proposed mechanism is unclear but may possibly be due to abnormalities of retinal blood flow.

In conclusion, it is possible to hypothesise a pathogenetic role for diabetic autonomic neuropathy in the development of diabetic neuropathy through the changes in nocturnal glomerular function and increased vulnerability of the kidney to haemodynamic influences.

Natural history of diabetic autonomic neuropathy

Whilst abnormal autonomic function can be detected in up to 40% of diabetic subjects only a small percentage of those will have symptoms attributable to the neuropathy (Ewing et al 1985). In a large study of 237 subjects, 26% showed a deterioration in cardiovascular autonomic function tests over a 5 year period (Ewing et al 1985). Over this

time 70% of patients showed no change in their autonomic function tests. Only 3% showed an 'improvement' in autonomic function over the study period and a similar lack of reversibility of autonomic dysfunction was seen in another large study of insulin dependent diabetics (Sampson et al 1990).

The epidemiological studies discussed above have also demonstrated an increased five-year mortality rate in diabetic patients with autonomic neuropathy but the incidence of this varies from 23% (Sampson et al 1990) to 56% (Ewing et al 1985). Furthermore, these studies have shown no excess mortality in those patients with asymptomatic autonomic defects. The wide variation in mortality rates can be explained in part by older age, longer duration of symptomatic neuropathy and recruitment of patients with renal impairment at the start of the study by Ewing and colleagues.

The causes of death in the different studies are, however, constant principally from renal failure followed by macrovascular complications such as myocardial infarction and cerebrovascular disease. However, a significant minority of the deaths were sudden and unexpected; reduced heart-rate variability (Bigger et al 1988, Liniger et al 1991), relative predominance of the sympathetic nervous system at night (Bernardi et al 1992) and loss of the night-time fall in systolic blood pressure (Verdecchia et al 1994) are all associated with an increase in cardiovascular morbidity and diabetic autonomic neuropathy. Thus, part of the increased cardiovascular mortality in patients with diabetic autonomic neuropathy may be due to altered neural control of the cardiovascular system just as the increase in nephropathy in IDDM patients with autonomic neuropathy may be due to impaired autonomic control of the renal microcirculation.

1.3.2 Diagnosis of cardiac autonomic dysfunction

The diagnosis of diabetic autonomic neuropathy was first based on symptoms but now depends on various objective reflex tests. The cardiovascular tests have been best described and the assumption has been made that cardiovascular reflex abnormalities reflect damage throughout the autonomic nervous system. A consensus statement has recently been published for the diagnosis and classification of autonomic neuropathy (Consensus Statement 1988); it was recommended that i) symptoms should not be considered by themselves as a marker for the presence of diabetic neuropathy; ii) an abnormality of more than one test of autonomic function is desirable to establish the diagnosis; iii) a battery of quantitative measures should be used to monitor improvement or deterioration of autonomic function.

1.3.3 Cardiovascular reflex tests

For the past 25 years, these tests have been the gold standard for the detection of diabetic autonomic neuropathy. They examine the response to various stimuli of either heart-rate or blood pressure and have been fully examined in non-diabetic patients of all age and non-diabetic age dependent values are available (O'Brien et al 1986).

Valsalva manoeuvre

The Valsalva manoeuvre was named after Antonio Valsalva (1666-1723) and was first shown to be abnormal in diabetic patients in 1960 (Sharpey-Schafer 1960). Subjects are

asked to perform a forced expiration at 40 mmHg for 15 seconds into a mouthpiece connected to a pressure measuring device. The mean ratio of the longest R-R interval shortly after the manoeuvre to the shortest R-R interval during the manoeuvre for three consecutive Valsalva manoeuvres is defined as the Valsalva ratio.

There are four phases of the Valsalva manoeuvre: Phase 1 is a short-lasting increase in heart-rate and systolic blood pressure associated with the taking of a deep breath prior to blowing. Phase 2: as intrathoracic pressure rises during the forced expiration, so cardiac output falls due to decreased venous return. Blood pressure, therefore, falls and heart-rate reflexly increases. Phase 3: immediately after release of the strain there is a further reduction in blood pressure. Phase 4: after release of the strain intrathoracic pressure plummets, there is an increase in venous return and a resultant increase in cardiac output. The increased cardiac output is forced into a vascular system that is still constricted with a resultant increase in blood pressure and a reflex bradycardia.

Thought of in the simplest terms, the Valsalva manoeuvre assesses the parasympathetic (phase 4) and sympathetic (phase 2) arms of the autonomic nervous system as well as the whole of the baroreflex mechanism. The fall of systolic blood pressure during phase 2 and 3 results in a baroreflex-mediated withdrawal of fast reacting parasympathetic function and later increasing in the slower responding sympathetic nerve activity which is responsible for the continued tachycardia and vasoconstriction of splanchnic and muscular vessels (McLeod et al 1987). Direct microneurographic studies of sympathetic nerve activity during the phases of the Valsalva manoeuvre have shown increased sympathetic

nerve activity during the whole of the hypotensive period of the Valsalva manoeuvre (Delius et al 1972).

The increase in heart-rate during the strain is mediated not only by this increase in sympathetic outflow to the sinoatrial node but also by withdrawal of vagal tone. During phase 4 baroreflex stimulation by the blood pressure overshoot after release of the strain results in a vagally mediated slowing of the heart-rate to below basal levels and a reduction in sympathetic nerve activity (Delius et al 1972).

Heart-rate response to standing

Standing from a supine position normally results in a predominantly parasympathetic mediated response in heart-rate. Usually, there is an immediate increase in heart-rate toward a primary peak at around 3 seconds and increases further to a secondary peak, maximal about the 15th beat after standing. This is followed by a relative bradycardia which is maximal around the 30th beat (Ewing et al 1982, Ten Harkel et al 1990, Imholz et al 1991). The primary rise in heart-rate is almost completely due to withdrawal of vagal tone but it is often impossible to identify this peak separately. The secondary heart-rate peak is also mainly vagally mediated although enhanced sympathetic outflow (which as mentioned before is much slower to respond) to the sinus node also contributes to this response; the stimulus is the initial fall in blood pressure on standing although this is not detectable by syphgmomanometry but only when using beat-to-beat blood pressure recording systems. The relative bradycardia is due to a vagal reflex which depends on the

recovery and overshoot of blood pressure mediated by sympathetically induced vasoconstriction (Sprangers et al 1991).

To perform this test, the patient is asked to stand from the supine position, with the electrocardiogram recording continuously. The ratio of the longest R-R interval (around the 30th beat after standing) to the shortest R-R interval is expressed as the 30:15 ratio. The period of resting supine before standing has profound effects on the value of the 30:15 ratio with longer periods of rest being associated with greater ratios (Ten Harkel et al 1990) thus standardization of the test protocol is imperative.

Heart-rate response to deep breathing

In normal subjects, heart-rate continually varies, mostly with respiration and this is described as respiratory sinus arrhythmia. The heart-rate increases during inspiration and decreases during expiration and these changes are under cardiac parasympathetic control- both vagal inhibition and activation are tested. To perform this test, the subject is asked to sit quietly and breathe deeply and evenly at six breaths per minute for one minute. The maximum and minimum heart-rate during the cycles is calculated and the differences during three successive cycles are averaged. Alternatively, the changes can be expressed as a ratio of the slowest heart-rate, during expiration, to its fastest during inspiration (the inspiration/expiration ratio). To observe vagally-mediated changes in heart-rate, some vagal tone should be present and therefore the test cannot be interpreted when the resting heart-rate is high.

Blood pressure response to standing

On standing, blood pools in the legs resulting in a fall in blood pressure which is normally rapidly corrected by a reflex combination of tachycardia and peripheral and splanchnic vasoconstriction- thus both sympathetic and parasympathetic pathways are involved. To perform the test, blood pressure is measured with the patient supine and again one minute after standing. The difference in systolic pressure is noted.

Blood pressure response to sustained handgrip

Isometric exercise raises blood pressure by stimulating sympathetic efferent pathways and is assessed by sustaining a handgrip of a third of maximum voluntary contraction for 3-4 minutes. Blood pressure is measured each minute and the difference between diastolic blood pressure before starting and just before release of the handgrip is taken as a measure of response.

1.3.4 Assessment of cardiovascular autonomic damage

Large numbers of non-diabetic subjects and diabetic patients have been assessed using the battery of cardiovascular tests mentioned above (Ewing et al 1985) and age-adjusted normal ranges are available (O'Brien et al 1986). In addition, these tests have proved to be reproducible despite the potential for intra and inter-individual variation (Smith 1982, Pfeifer et al 1982, Smith 1984, Lawrence et al 1992). A scoring system has been developed to help classification of autonomic neuropathy with a borderline abnormal test

scoring one point and an abnormal test scoring two points (Ewing et al 1985, Bellavere et al 1983). An overall 'autonomic test score' of 0-10 can then be obtained.

Early in the development of diabetic autonomic function it was assumed that the tests of heart-rate variability were tests of vagal (parasympathetic) function whereas the blood pressure responses were said to test sympathetic function (Ewing et al 1982). As discussed above, the autonomic pathways involved with these cardiovascular reflexes are complex for all of the tests, especially the Valsalva manoeuvre, which involves the sympathetic and parasympathetic systems to varying degrees. Abnormalities of autonomic function are not an all-or-nothing phenomenon, so with the use of a scoring system for autonomic neuropathy the labeling of abnormalities as sympathetic or parasympathetic is no longer valid. Furthermore, the scoring of autonomic function tests allows the progression of autonomic abnormalities to be followed. Autonomic dysfunction is the term applied to abnormalities of cardiovascular reflex tests whilst autonomic neuropathy should be reserved for those patients with autonomic symptoms as well as abnormal cardiovascular reflex tests.

1.3.5 Sequence of abnormalities

Autonomic damage is therefore not simply present or absent but can be anywhere on the spectrum of minimal to severe. Moreover it appears that once cardiovascular reflex tests are abnormal then the natural history is to progress to more severe neuropathy, but the patient does not necessarily become symptomatic (Sampson et al 1990). Conventionally, it is said that parasympathetic dysfunction precedes sympathetic abnormalities but this is

based on the standard battery of bedside cardiovascular reflex tests where the assumption was made that the heart-rate tests are purely testing parasympathetic function. Heart-rate tests are much more sensitive than blood pressure responses and thus more liable to be abnormal. Recent attempts to assess sympathetic nerve activity in more detail have involved looking at muscle sympathetic nerve activity using microneurographic techniques and radioactive isotopes of noradrenaline analogues to evaluate cardiac sympathetic innervation.

Muscle sympathetic nerve activity using microneurography was first described in 1968 (Haagbarth et al 1968). A fine-gauge tungsten microelectrode is used to directly record postganglionic muscle sympathetic nerve activity, often from the peroneal nerve at the fibular head (Valbo et al 1979). Using these microneurographic techniques, a reduction in muscle sympathetic nerve activity has been detected in a small number of diabetic patients with no clinical symptoms of autonomic dysfunction (Fagius 1982). No assessment of parasympathetic function was made and, furthermore, no information about the type of diabetes or the metabolic 'state' of the patients studied was provided. This is important information because insulin increases sympathetic activity in non-diabetic subjects (Rowe et al 1981, Anderson et al 1991), whilst in animal studies fasting and hyperglycaemia with insulin deficiency inhibit sympathetic activity (Daly et al 1990). More recently, others have found reduced muscle sympathetic nerve activity before abnormalities were present in the heart-rate variability tests which were used as a means of assessing cardiac parasympathetic function (Hoffman et al 1993). It has been assumed from these studies that the abnormalities of muscle sympathetic nerve activity are a direct result of autonomic

neuropathy with evidence of early sympathetic nerve damage and no parasympathetic dysfunction. It is, however, unreliable to extrapolate data from muscle autonomic function on to cardiac autonomic function. Whilst there is evidence that muscle sympathetic nerve activity is abnormal before any evidence of clinical autonomic dysfunction, current studies show that cardiac sympathetic dysfunction appears to develop after parasympathetic abnormalities possibly because the cardiac parasympathetic fibres are longer and more prone to damage (Ewing et al 1983). One concern with this assumption is that assessment of cardiac sympathetic function has been difficult. Direct assessment of cardiac sympathetic autonomic function has recently been investigated using radioisotope methods.

The myocardial uptake of meta-iodobenzylguanidine (MIBG) is a recently developed technique which allows evaluation of cardiac sympathetic neuropathy (Wieland et al 1980) by measuring post-ganglionic pre-synaptic noradrenergic uptake. Radioiodinated MIBG serves as an analogue of noradrenaline and shares the same uptake and storage mechanisms without being metabolized (Nakajo et al 1986, Sisson et al 1987). Therefore, it can be used for the imaging of organs with a rich sympathetic innervation such as the heart. Initial studies in IDDM patients with abnormal cardiovascular reflex tests (including the conventional sympathetic tests of blood pressure response to sustained handgrip and blood pressure response to changes in posture) showed a significant reduction of uptake of MIBG (Mantysaari et al 1992). Other studies addressing the interdependence between cardiac autonomic neuropathy (determined by bedside cardiovascular reflex tests) and MIBG scanning have given conflicting results probably due to the differing severity of the

autonomic dysfunction in the populations studied. Thus, in patients with symptomatic autonomic neuropathy, or with abnormal blood pressure reflex tests (Mantysaari et al 1992) then MIBG scanning appears to be abnormal; in patients with normal bedside tests of autonomic function there is increasing evidence that MIBG scanning can be abnormal although it is unclear if these abnormalities are of clinical significance (Schnell et al 1995, Murata et al 1996). The lack of sensitive bedside tests of sympathetic autonomic function makes comparisons difficult and a significant number of the patients included in these trials had abnormalities of heart-rate variability tests- traditionally described as parasympathetic tests. Furthermore, abnormalities in MIBG scans have been related to functional ventricular alterations as documented by echocardiography and ventriculography, suggesting a link between sympathetic neuropathy and ventricular dysfunction in diabetes (Mantysaaria et al 1993, Kreiner et al 1995.) Whilst of interest in research fields the use of MIBG scans has limited clinical application due to the presence of radioactive isotopes and the lack of information on the clinical significance of an 'abnormal' test.

In summary, on the basis of the current data cardiac parasymapthetic activity, as detected by heart-rate variability tests, is often abnormal despite normal bedside tests of blood pressure response. The assessment of cardiac sympathetic innervation using radioisotopes can detect abnormalities before the blood pressure response to sustained handgrip or changes in posture are abnormal although heart-rate tests are often already abnormal. Muscle sympathetic nerve activity is often impaired before any evidence of cardiac autonomic dysfunction although the clinical significance of this is unclear and data on cardiac innervation can only tentatively be extrapolated from this.

1.4 Prolongation of the QT interval

1.4.1 Introduction

Since cardiac autonomic dysfunction can have profound effects on the patients morbidity and mortality so further methods of assessing cardiac autonomic function have been sought, including abnormalities of cardiac depolarisation/repolarisation. These have the advantage of being non-invasive and easy to perform.

QT Interval

The QT interval on the surface ECG represents the sum of uncancelled potential differences during ventricular depolarisation and reploarisation. Abnormal repolarisation of the heart, manifest as a prolongation of rate adjusted QT interval (QTc) has been associated with an increased risk of sudden death in such diverse clinical settings as familial long QT syndrome (Schwartz 1982), sudden infant death syndrome (Schwartz 1987) and ischaemic heart disease (Barber et al 1985). This ECG abnormality has been interpreted as the consequence of the imbalance between left and right sympathetic innervation of the heart and a marker of cardiac electrical instability which could possibly lead to life-threatening ventricular arrhythmias (Abildskov 1991). Several population-based studies have examined the risks of sudden death associated with prolonged QT interval. In the Rotterdam QT project (Algra et al 1991) 6,693 consecutive patients who underwent 24 hour ambulatory electrocardiography were studied. These were a

heterogeneous population comprising patients with a variety of conditions, from transient ischaemic attacks to post-myocardial infarction subjects, undergoing 24 hour ECG recording for risk stratification. This study showed that those subjects with a QTc interval of greater than 0.44 s had a 2.3 times greater risk of sudden death compared with patients with QTc interval of less than 0.44 s. In patients with evidence of cardiac dysfunction (presence of symptoms or an ejection fraction of less than 40%) the risk of sudden death was 4.5 times higher. Similarly, apparently healthy individuals appear to have an increased risk of sudden death associated with longer QT intervals (Shouten et al 1991).

Information on the incidence of prolonged QTc in the diabetic population is available from a similar large cohort study of IDDM patients (Sivieri et al 1993). The prevalence of a QTc of > 0.44 s (the upper limit of normal) was 31% in those patients with autonomic neuropathy and 24% in those without autonomic neuropathy. Neuropathy in this study was diagnosed on the five standard tests of cardiovascular function as previously described. Diabetic autonomic neuropathy has also been associated with increased risk of sudden death (Page et al 1978, Ewing et al 1990), however, the relation between prolonged QTc and sudden death in patients with diabetic autonomic neuropathy is far from clear. There is still some debate as to whether prolonged QT interval in diabetic patients is associated with a poor prognosis (Kahn et al 1987, Bellavere et al 1988, Chambers et al 1990, Gonin et al 1990) although there is evidence that the association between prolonged QT and an increase in mortality parallels that in the non-diabetic population. Thus, a small study (n = 13) by Ewing and colleagues (1991) found that in diabetic patients with abnormal cardiovascular reflexes QT and QTc were significantly

longer in those patients who subsequently died. Furthermore, they found a close linear relationship between worsening autonomic function and increasing prolongation of QT interval. The association between worsening autonomic function and increasing prolongation of QT interval is also open to debate. Bravenboer and colleagues (1993) failed to observe an association between autonomic neuropathy score and the length of the QT interval concluding that QT interval was not a reliable indicator of the severity of diabetic autonomic neuropathy. Similarly Lo and colleagues (1993) found no association between severity of autonomic dysfunction and QT interval length. However, a more recent study supports Ewing's finding in an investigation of a large group of consecutively recruited diabetic patients, only a third of whom were insulin dependent(Veglio et al 1995). It was shown that patients with definite diabetic autonomic neuropathy, as shown by an autonomic neuropathy score of 4 or more, had a significantly longer QTc than patients with borderline or no diabetic autonomic neuropathy. Furthermore, they observed a significant correlation between QTc and total score of cardiovascular tests. The reasons for the discrepancies between the studies include: 1) patient selection (studies with only IDDM patients show a weaker correlation between QTc and autonomic neuropathy score). 2)Bravenboer and colleagues did not use age adjusted normal ranges to score the autonomic tests. 3) Small numbers of patients can influence study findings- Lo's study included only 3 patients with 2 or more abnormal autonomic function tests.

In recent work by Heller and colleagues (1995), QTc was shown to be prolonged during hypoglycemia, despite normal QT intervals when euglycaemic. In this study there was no

information about the presence or absence of autonomic system abnormalities but this change in QTc could clearly contribute to the cases of sudden unexplained deaths seen in young patients.

Finally, a recent study looking at IDDM patients with overt diabetic nephropathy found a prolonged QTc interval was associated with an increased risk of death and that prolongation of QTc was independent of the presence of autonomic neuropathy (Sawicki et al 1996).

Several criticisms have been leveled at the use of QTc prolongation as a marker for autonomic neuropathy. The calculation of QTc involves correcting for heart-rate usually using Bazett's formula ($QT/\sqrt[2]{RR}$) which has been shown to over-correct at heart-rates above 90 beats per minute (Kawataki et al 1984). This is especially relevant to diabetic subjects with autonomic dysfunction since they tend to have a higher resting heart-rate (Ewing et al 1983, Rubler et al 1985). Indeed, the only study not to use Bazett's formula to correct for heart-rate but an exponential correction formula has reported a circadian periodicity of QT interval and a *shortening* of QT interval in diabetic patients with autonomic neuropathy (Ong et al 1993). Finally, the sample sizes in the studies mentioned are small with variations in the 'normal' values of autonomic function tests and the 'type' of diabetics studied.

In summary, prolongation of the QT interval is a marker of abnormal ventricular repolarization and prolongation of QTc in non-diabetic subjects is associated with an increased risk of sudden death. There is some evidence to suggest that prolonged QT

interval is associated with diabetic autonomic neuropathy and this may be a marker of increased risk of sudden death amongst diabetic patients. Especially amongst non-insulin dependent diabetic patients, QTc prolongation appears to be correlated to the severity of autonomic dysfunction although evidence for this association in IDDM patients is weaker. Furthermore, other diabetic complications especially overt nephropathy can influence QT interval.

1.4.2 QT Dispersion

Historically, QT intervals across the surface ECG have been viewed as being of uniform length. Mirvis (Mirvis 1985) and others (Sylven et al 1984) measured QT in multiple torso leads and showed that there was variation in QT intervals amongst these leads. With some modifications Cowan developed the idea of QT dispersion measured from the standard 12 lead surface ECG (Cowan et al 1988). QT intervals are measured from all 12 leads and QT dispersion is calculated as the maximum minus the minimum QT interval; data are usually presented as QT dispersion and rate-adjusted QTc dispersion. QT dispersion may reflect dispersion of ventricular repolarisation (Day et al 1992) and any disparity in repolarisation of adjacent areas of myocardium could represent an area of potential re-entry tachycardias. QT dispersion thus has been suggested to be a predictor of sudden death due to ventricular arrhythmias in certain clinical settings (Buja et al 1993, Barr et al 1994) although there are an equal number of papers refuting this hypothesis (Davey et al 1994, Leitch et al 1995).

There is little data available about the role of the autonomic nervous system in the measurement of QT dispersion. One study reported no difference between QT dispersion in patients with primary autonomic failure and control subjects but found both QT and QTc intervals to be prolonged in the patients with primary autonomic failure (Lo et al 1993).

Similarly, information about QT dispersion in the diabetic population are lacking. In one small study looking at 13 diabetic patients (we are not told what type of diabetes) with diabetic nephropathy QTc dispersion was significantly greater in the diabetic population compared to a control group of uraemic patients with renal failure due to non-diabetic causes (Kirvellä et al 1994). The diabetic patients studied had high scores for autonomic dysfunction based on the five standard tests of autonomic function and it was suggested that the abnormal autonomic function could contribute to the increased QT dispersion. This study is further flawed because of the high incidence of coronary artery disease in the diabetic group which in itself is associated with increased QT dispersion (Mirvis 1985, Day et al 1991, Cox et al 1994).

In summary, QT dispersion, which possibly reflects ventricular repolarisation, may be a marker for arrhythmogenic potential and this appears to be independent of the autonomic nervous system. Data on QT dispersion in diabetic subjects are sparse, although one imperfect study reported that QT dispersion was increased in diabetic patients with diabetic renal disease and autonomic neuropathy.

1.5 Circadian Rhythms

In keeping with many other biological systems both blood pressure and heart-rate are subject to circadian variations and abnormalities in the circadian rhythm can have profound consequences on the patient's morbidity and mortality.

1.5.1 Circadian blood pressure variation

Since the first automated intra-arterial blood pressure recording in unrestricted man (Bevin et al 1969), circadian fluctuation of blood pressure, with higher levels during the day and lower levels during the night, has been well described in both normal and hypertensive subjects. In a detailed study of twenty-four hour blood pressure profiles in hypertensive subjects, Miller-Craig et al (1978) reported that blood pressure was highest at 10 am then progressively fell through the day reaching its lowest levels during sleep at 3 am. Blood pressure then rose and from 6 am it increased rapidly, even more so after waking at 7 am. Sleep is the primary determinant of the nocturnal fall in blood pressure. Non-REM sleep is associated with a fall in blood pressure and a reduction in blood pressure variability whilst REM sleep increases blood pressure variability and is associated with a slight rise in average blood pressure readings (Mancia et al 1980). More recent data using non-invasive blood pressure measurement and microneurographic recordings of muscle sympathetic nerve activity have shown a reduction in heart-rate, blood pressure and sympathetic nerve activity during non-REM sleep (Okada et al 1991, Somers et al 1993) whilst REM sleep is

associated with a marked increase in sympathetic activity and a rise in heart-rate and blood pressure often to levels seen whilst awake.

Further evidence for the role of the autonomic nervous system in the circadian modulation of blood pressure comes from a variety of clinical settings. Thus, in patients with primary autonomic failure there is a reduction in the circadian variation of blood pressure (Mann et al 1988) and the normal circadian pattern may well be reversed, with blood pressure increasing at night and falling on waking. Similarly in tetraplegic patients, who have impaired autonomic reflexes, and in patients following cardiac transplant, circadian fluctuations of blood pressure is inhibited (Reeves et al 1986). Richards and colleagues (1986) related fluctuation in blood pressure to sympathetic activity, as assessed by plasma noradrenaline levels, over a 24-hour period and found a close coupling giving further support for a major role of the sympathetic nervous system in controlling blood pressure levels. The simplest method of assessing circadian changes in blood pressure is with the difference between mean day-time and mean night-time pressures. A decrease of more than 10% from day to night in mean blood pressure is the norm and patients are classified as being 'dippers' whilst a fall of less than 10% is seen in 'non-dippers' (O'Brien et al 1988, Verdecchia et al 1990). Approximately 17% of hypertensive patients can be classified as non-dippers (O'Brien et al 1988) and other pathophysiological conditions, such as endogenous glucocorticoid excess and hyperthyroidism as well as some causes of secondary hypertension are associated with an absent fall of blood pressure at night (Littler et al 1979, Imai et al 1988). Reduced day-night variation of blood pressure has also been reported in accelerated hypertension, eclampsia and congestive cardiac failure

(Imai et al 1990). Imai and colleagues suggested the lack of diurnal variation of blood pressure under these conditions could be due to cerebral ischaemia which activates a compensatory mechanism preventing a fall in nocturnal blood pressure and thus a further reduction in cerebral blood flow at night (Imai et al 1990). Whilst controversial this hypothesis received some support from the observation that an excessive nocturnal fall in blood pressure critically lowers the perfusion to organs such as the heart and brain (Stanton et al 1993).

1.5.2 Blood pressure profile in diabetic subjects

A blunted or reversed circadian pattern of blood pressure has been described in diabetic patients. The earliest studies of circadian blood pressure variation in diabetic subjects stressed the importance of autonomic dysfunction in the loss of the nocturnal dip in blood pressure. This relationship was first observed by invasive direct intraarterial blood pressure measurement (Hornung et al 1989), and confirmed using a non-invasive ambulatory blood pressure monitoring system (Wiegmann et al 1990, Felici et al 1991). In the study by Wiegmann and associates no measure of autonomic function was made,however, they showed that in IDDM subjects ambulatory blood pressure significantly exceeded control values at all time-points measured, despite patients being identified as 'normotensive' on clinic sphygmomanometer readings. The concept of blood pressure load or burden was also introduced at this time and using an arbitrary value of 135/85 mmHg, 50% of readings in the diabetic population were found to be higher than this value. Furthermore, compared to day-time readings, the mean night-time blood pressure values were not

significantly lower in the IDDM population unlike in the control group. The albumin excretion rate was 18.2 μmol/min/m^2 for the diabetic group and although no assessment of albumin excretion was made in the control population the changes in circadian blood pressure variation were said to be independent of kidney function.

Felici and colleagues found that there was a loss of the normal nocturnal decline in blood pressure in a population of NIDDM and IDDM patients with clinical evidence of autonomic dysfunction. Their population of diabetic subjects was, however, mixed with over half being hypertensive on clinic blood pressure readings. Furthermore, no detailed assessment of diabetic nephropathy was made, with patients included in the study if their creatinine clearance was greater than 20 ml/min/m^2.

Liniger and colleagues (1991) assessed the relationship between twenty four-hour ambulatory blood pressure profiles and autonomic function as assessed by the cardiovascular reflex tests. They found that three-quarters of insulin-dependent diabetic patients with abnormal cardiovascular reflex tests had an impairment of the normal circadian pattern of blood pressure. Moreover, in half of this group, the systolic blood pressure rose at night whilst in the others nighttime blood pressure did not differ from daytime levels. They postulated that as autonomic damage became more severe (as assessed by an increasing autonomic neuropathy 'score') so the circadian changes in blood pressure became more abnormal. The patients in whom systolic blood pressure rose at night all had a systolic blood pressure fall on standing. On closer examination of the small number of patients in each group, there was a significantly higher 24 hour protein excretion in the IDDM group with abnormal circadian blood pressure profiles compared

to those with a normal nighttime dip in blood pressure, as well as a higher serum creatinine although this was not statistically significant.

In a more recent study the relation between 24 hour blood pressure and autonomic function has become a little clearer. Phong Chau and colleagues (1993) found that a mixed group of IDDM and NIDDM patients with abnormal cardiovascular reflex tests had a significant reduction in night-time blood pressure change compared with a control population matched for age and clinic blood pressure. Furthermore, they found an increased variability of systolic blood pressure during the day in those patients with autonomic dysfunction although this did not reach statistical significance. The association between diabetic nephropathy and circadian changes in blood pressure was also investigated revealing that those patients with autonomic dysfunction who had microalbuminuria had a further reduction in the change of systolic blood pressure at night. A similar loss of the diurnal variation of blood pressure has been observed in a non-diabetic population with renal disease (Baumgart et al 1989) leading to the suggestion that the loss of circadian change in blood pressure in the diabetic population is secondary to diabetic renal disease. In particular, an attenuated fall of nocturnal blood pressure in IDDM patients with microalbuminuria has been reported. For example, in a study looking at IDDM patients who were normotensive on clinic blood pressure readings (< 140/90 mmHg) Benhamou and colleagues (1992) showed an increased ambulatory blood pressure at night in IDDM patients with microalbuminuria as compared with a control population without microalbuminuria. No formal assessment of autonomic function was made, but reviewing there data shows that nocturnal heart-rate was significantly higher in the

microalbuminuric group- possibly an early marker of autonomic dysfunction. Other workers have reported a similar loss of circadian variation of blood pressure (Hansen et al 1992, Lurbe et al 1993) and have postulated that the early renal damage results in abnormal circadian blood pressure changes.

Consequently, there has been some uncertainty whether the abnormal circadian blood pressure pattern is actually dependent on the presence of neuropathy or nephropathy. In one study in young IDDM patients microalbuminuria was associated with slightly increased blood pressure levels compared to normoalbuminuric subjects but there were no differences in circadian blood pressure variation unless autonomic neuropathy was present (Spallone et al 1993). Furthermore, the same group found that the loss of the nocturnal fall in blood pressure was related to an abnormal sympathetic predominance at night, suggesting a possible pathogenetic link between autonomic dysfunction and impaired circadian fluctuations of blood pressure. The interrelationship between autonomic neuropathy and 24-hour blood pressure profiles has been further explored by Torffvit and colleagues (1993) who showed that all patients with clinical diabetic nephropathy (defined as a positive urine dipstix for albumin) had evidence of autonomic neuropathy. Moreover, those patients with diabetic nephropathy showed an impairment of circadian blood pressure change.

Finally, recent work has suggested that latent overhydration may play a role in the nocturnal hypertension seen in diabetic patients (Hansen et al 1992, Mulec et al 1995). When extracellular volume was measured in a population of insulin dependent diabetic patients with diabetic nephropathy (GFR 40 ml/min/m^2) who had an impaired reduction of

night time blood pressure it was found to be significantly higher than in a group of 'dipping' IDDM patients with nephropathy. No assessment of autonomic function was made since it was assumed that all patients would have some degree of neuropathy. It was stated that there was no correlation between day or night heart-rate and the night/day blood pressure ratio. However, significant numbers of patients in the group were treated with beta-blockers. The assumption that fluid overload contributes to the lack of a nocturnal dip in systolic blood pressure does not exclude a role of the autonomic nervous system. As mentioned previously, the autonomic nervous system has an integral role in fluid-balance homeostasis, through the renin-angiotensin system.

In summary, impaired circadian changes of blood pressure are well documented in diabetic subjects. There appears to be a close association between reduced night-time fall in blood pressure and autonomic dysfunction and this may be an inverse linear relationship; the worse the autonomic dysfunction the lower the decline in night-time blood pressure. In those patients with severe autonomic dysfunction blood pressure may even rise at night (cycle inversion). Patients with microalbuminuria also have impaired circadian changes of blood pressure but it appears that autonomic dysfunction may be the pivotal factor in this It may, therefore, be possible to assume that diabetic patients with a blunted nocturnal fall in blood pressure all have some degree of autonomic dysfunction.

1.5.3 Significance of the abnormal circadian pattern of blood pressure

Cross sectional and retrospective studies indicate that damage to target organs is more closely linked to twenty-four hour blood pressure profiles than random clinic blood

pressures (Mancia et al 1988). Hypertensive patients who are non-dippers have an increased prevalence of strokes (O'Brien et al 1988), atherosclerosis (Shimada et al 1992), and left ventricular hypertrophy (Verdecchia et al 1990). The situation in diabetic subjects is less clear. Liniger and colleagues (1991) found a significantly higher incidence of fatal and severe non-fatal cardiovascular events in the small number of patients who had a nocturnal rise in blood pressure (i.e. those with severe autonomic dysfunction). It should be remembered, however, that there is a higher risk of cardiovascular events in diabetic subjects with autonomic dysfunction (Ewing et al 1982, Sampson et al 1990). A more recent study of 23 microalbuminuric, IDDM patients showed no significant increase in left ventricular mass index or cardiovascular disease (Hansen et al 1995) although only small numbers of non-dippers were studied and no assessment of autonomic function was made in this group. Another study looking at left ventricular mass in normotensive patients with diabetic autonomic neuropathy demonstrated an increase in left ventricular mass index despite normal renal function (Gambardella et al 1993).

1.5.4 Does abnormal blood pressure load result in or result from diabetic nephropathy?

It has been documented that if albumin excretion is normal in diabetic patients then the prevalence of hypertension is the same as in the control population (Nørgaard et al 1990). However, if microalbuminuria is present there is a dramatic increase in the prevalence of

hypertension and this increase is even more pronounced in overt diabetic nephropathy (Mogensen et al 1992). Initially, these results were taken to suggest that elevated blood pressure was secondary to renal abnormalities and some support for this was received from longitudinal studies (Rudberg et al 1992, Mathiesen et al 1990). However, these studies did not utilize twenty four hour ambulatory blood pressure recordings but relied on repeated clinic blood pressures. By contrast, other workers have recently reported that raised blood pressure precedes the development of microalbuminuria, although blood pressure was again measured by conventional syphgmomanometry (Microalbuminuria Collaborative Study Group 1993). Ambulatory blood pressure recording clearly has advantages over single clinic readings allowing assessment of the circadian change in blood pressure, as well as better reflecting the risk of developing end-organ damage in non-diabetic patients (O'Brien et al 1988, Verdecchia et al 1990). However, a debate continues as to whether increases in 24 hour blood pressure (particularly at night) predate the development of microalbuminuria or are a consequence of early renal disease. Poulsen and colleagues (1994) reported that clinic and 24 hour ambulatory blood pressure was normal until after the development of microalbuminuria in a longitudinal study of IDDM patients without microalbuminuria. Interestingly, those patients who eventually progressed onto microalbuminuria had a diminished reduction in nocturnal diastolic blood pressure, although this could be related to the duration of diabetes which was greater in this group. In another study, IDDM patients with microalbuminuria were noted to have a blunted nocturnal fall in blood pressure although the mean 24 hour blood pressure was normal (Lurbe et al 1993). A sub-population of patients without microalbuminuria in this study

showed a reduced decline in nocturnal blood pressure, and the authors suggested that the blood pressure dysregulation and microalbuminuria were independent of each other. Further studies (Moore et al 1992, Page et al 1994) again confirmed the finding that patients with microalbuminuria had a higher mean 24 hour blood pressure than those without microalbuminuria. However, in these studies, patients without microalbuminuria had raised ambulatory blood pressure readings intermediate between non-diabetic subjects and those patients with microalbuminuria. There was an increase in left ventricular mass index in both diabetic groups and it was suggested that arterial pressure rises before the development of overt microalbuminuria. In the study by Page and colleagues (1994), the 'non-microalbuminuric' group had a significantly higher mean urinary albumin excretion of 19.5 mg/24h- higher than the 'non-microalbuminuric' in the other studies; such 'incipient microalbuminuria' may influence blood pressure profiles.

In summary, as microalbuminuria develops in IDDM patients there is an increase in blood pressure. The current studies do not answer the question as to whether an initial rise in blood pressure triggers or is triggered by an increase in urinary albumin excretion but there is little doubt that the two phenomena are closely linked. Abnormalities of the circadian blood pressure change, and thus an increase in the blood pressure load may be important in determining the development of diabetic nephropathy.

1.6 Autonomic control of cardiovascular activity

1.6.1 Introduction

The regulation of blood pressure and heart-rate is traditionally described in terms of homeostasis- although the blood pressure is constantly perturbed by external stimulation there is always a tendency for it to return back to a reference set point (Guyton 1994). In recent years it has become apparent that it is not only the average blood pressure (the reference set point) that is important but the fluctuations around the average blood pressure can provide considerable insight into the mechanisms of cardiovascular control (Akselrod et al 1981, Mancia et al 1983, Malliani et al 1991). Cardiovascular fluctuations can be studied through beat-to-beat blood pressure and heart-rate monitoring and calculation of the variance (standard deviation) of their average values (Littler et al 1978, Mancia et al 1983) i.e. in the time domain. Recently, frequency domain analysis has also been used to study the variability of blood pressure and heart-rate, dividing this variability into different frequency components and quantifying the variance or power at each frequency (Kay et al 1981, Akselrod et al 1981, 1985, Malliani et al 1991, Parati et al 1995). Variability of cardiovascular parameters can therefore be evaluated by a variety of methods of which the simplest are the time-domain measures.

1.6.2 Time-domain analysis of heart-rate and blood pressure variations

Data recorded from 24-hour blood pressure monitors or 24-hour Holter ECG recordings allow the assessment of the variability of these two parameters. The simplest time domain measures include the mean difference between consecutive values, the mean heart-rate or

blood pressure value, the day-night difference etc. Thus, variability can be expressed in terms of the standard deviation, the coefficient of variation (calculated from the standard deviation divided by the mean multiplied by 100 to give a percentage) and the range. It should be mentioned that total variance of heart-rate variability increases with the length of the sequence analysed. The drawback with all of these methods is that they ignore the sequence of the data, so other workers have used the root of the mean squared successive differences (RMSSD) as a parameter for blood pressure and R-R interval variability which allows identification of linear trends and circadian rhythms. It has been shown for blood pressure that using RMSSD has the advantage of being insensitive to changes in blood pressure levels so is not influenced by the circadian change in blood pressure level (Schächinger et al 1989). Conversely, the circadian change in blood pressure is important as an independent cardiovascular risk factor so the analysis of blood pressure variability should not be based solely on RMSSD but also consider standard deviation and the coefficient of variation.

Ewing and colleagues (1994) have adopted another method of assessing heart-rate variability based on 24-hour ECG recordings. They identified beat-to-beat changes in R-R interval that were superimposed on the sinus arrhythmia. They applied a threshold of fifty milliseconds as a cut off and they identified several hundred such changes each hour. Furthermore, they found that in patients with diabetic autonomic neuropathy and in subjects post-cardiac transplantation there was a marked reduction in the number of these sequences and concluded from this that these variations in R-R interval were parasympathetic in origin.

1.6.3 Power Spectral Analysis

Introduction

Rhythmic changes in blood pressure and heart-rate in response to respiration were first described by Hales (Hales 1733). Some years later Mayer reported that blood pressure also oscillated at frequencies slower than the respiratory rhythm suggesting these oscillations were related to vasomotor activity (Mayer 1876, Penaz 1978). With the development of faster data processing techniques, more sophisticated approaches to the study of these rhythmic circulatory phenomena have been applied, especially in the frequency domain using power spectral analysis.

Power spectral analysis

Any waveform may be visualised in an ideal form as a sinusoidal curve with the wavelength as the distance between similar points on the curve (e.g. from crest to crest). When the horizontal axis is time, the words 'period' or 'cycle' replace wavelength. The frequency is the number of cycles in a specified time period where the units are labeled as Hz (one Hertz equals one cycle per second). Thus, the spectrum of any phenomenon may be considered as the breakdown of the phenomenon in space or time.

The calculation of a spectrum involves the fitting, by least-squares analysis, of sinusoidal curves of different frequencies to a set of data (Rayner 1971). Thus, in this respect the method is equivalent to multiple regression with trigonometric transformations of the independent variable. However, the fitting turns out to be reasonably simple because the

parameters (coefficients of regression) are written in terms of simple sums of products. The number of coefficients obtained is equal to the number of data points and the process is known as a Fourier transformation. The power of the frequency bands is measured by the area under the curve and are therefore expressed in squared units (ms^2 or $mmHg^2$), but may also be expressed in normalised units (nu) which represent the relative value of each power in proportion to the total power minus the very low frequency component (Malliani et al 1991, Pagani et al 1986) (see below).

Spectral analysis therefore refers to the process, calculating and interpreting a spectrum. Depending upon whether the data are assumed to be periodic or not can result in different forms of spectra. Periodic data, where the data repeat themselves indefinitely both forwards from the beginning and backwards from the end, provides a spectrum of discrete distribution made up of a finite number of frequencies (i.e. coefficients from regression). With non-periodic data, the frequencies are assumed to be continuous, with each coefficient representing a band of frequencies which combine to produce a continuous density spectrum.

Fourier analysis

Harmonic or Fourier analysis is the process of fitting Fourier series to data and calculating the amplitude of the combined sine and cosine waves and the phase angle (the distance from the origin to the first crest). Thus, a complicated function is reduced to a series of simple functions, sinusoidal waves.

If the spectral results are to be interpreted some assumptions have to be made. It is especially important that the data repeats itself completely i.e. the data are completely periodic and demonstrates 'stationarity'. Clearly, there are few truly periodic phenomena in nature, although for simplicity this assumption is often made on biological data. This is probably a safe assumption for short periods of data but becomes more difficult with longer recordings especially those over 24 hours.

The spectrum resulting from a fast Fourier transformation of the data is derived from all the data present so it includes the entire signal variance regardless of whether its frequency components appear as specific spectral peaks or as non-peaked broadband data. Other methods of spectral analysis of data include autoregressive modelling (Kay 1981, Pagani et al 1986). Here the raw data are used to identify a best fitting model from which the final spectrum is derived. The components of the signal not fitting the model are treated as noise and partially or totally removed. It is clear that if the Fourier transform-derived spectra are used with a smoothing function then they will approach the spectra close to that derived from an autoregressive method. Similarly, when a high model order is applied to the data then the autoregressive spectra will approach the fast Fourier transformation spectra.

Strict periodicity of the data is essential for fast Fourier transformation and is frequently used with pre-selection of the number and frequency range of oscillatory components (Rayner 1971). However, autoregressive models automatically furnish the number, amplitude and center frequency of the oscillatory components.

Heart-rate and blood pressure spectra in man

Using the analytical techniques described above on heart-rate and blood-pressure data reveals three rhythmic oscillations, all with a period of less than 1 minute (Sayers 1973, Akselrod et al 1985, Saul et al 1991). Oscillations with a frequency around 0.2 to 0.4 Hz, a frequency similar to that of normal respiratory activity, are defined as high frequency. Oscillations with a frequency of approximately 0.1 Hz are defined as low-frequency and correspond in the case of blood pressure to the classic Mayer waves described before. Finally, oscillations with a frequency between 0.02 and 0.07 Hz are defined as very low frequency and are probably related to a variety of cardiorespiratory, thermoregulatory and vasomotor mechanisms. To study this low frequency oscillation, longer periods of data collection are required such as 24-hour blood pressure and heart-rate recordings. Other studies have shown that the amplitude and frequency of these oscillations are not constant but vary as a function of different behavioral conditions. This is especially so for the slower, very low frequency oscillations and, because of this, these fluctuations are often disregarded, analysis concentrating on the low and high frequency bands (Pagani et al 1986, Malliani et al 1991).

Recent workers have suggested that the non-rhythmic fluctuations of blood pressure and heart-rate variability are also important to the cardiovascular control mechanisms. Rather than appearing in the spectrum as defined peaks these non-rhythmic fluctuations appear as powers spread over a broad frequency region. Sinoaortic denervation in unanaesthetised cats (which removes the baroreceptor mediated restraint of sympathetic activity) is accompanied by systematic changes in non-peaked blood pressure and heart-rate across

several frequency regions (Di Rienzo et al 1991). In normotensive and hypertensive subjects non-peaked blood pressure and heart-rate powers are modified in a systematic fashion if sympathetic activity is reduced and vagal activity increased such as occurs during sleep (Parati et al 1990). Thus, consideration of broadband powers rather than peaks alone may offer a broader description of cardiovascular regulation.

Interpretation of spectral data

Most workers have focused on the variability components of the power spectra with frequencies between 0.025 Hz and 0.5 Hz based on the evidence that heart-rate and blood pressure spectra in this frequency region are modulated by autonomic neural mechanisms (Akselrod et al 1981, Malliani et al 1991), although the precise nature of these neural influences are open to debate. Owing to the slow periodicity of oscillation in the very low and ultra low frequency region these areas are often ignored but recent work has shown that a reduction in core body temperature is associated with an increase in the ultra low-frequency region (0.0039-0.04 Hz). (Fleisher et al 1996).

Heart-rate spectra

It is stated that vagal cardiac control acts as a low pass filter with a relatively high cut-off frequency, modulating heart-rate up to 1 Hz. Sympathetic cardiac control operates as a low pass filter with a much lower cut-off, modulating frequencies below 0.15 Hz. This is supported by several pieces of evidence. In dogs, stimulating the vagus nerve with broadband electrical stimulations results in heart-rate changes with minimal dampening up

to at least 0.7 Hz, whereas stimulating the right stellate ganglion in a similar way is followed by heart-rate changes with a delay of 2 seconds and a dampening that leads to a minimal heart-rate response above 0.15 Hz (Pagani et al 1986, Saul et al 1991). Total parasympathetic blockade in dogs and humans by atropine eliminates most heart-rate fluctuations above 0.15 Hz whilst those below this are relatively unaffected (Fouad et al 1984, Saul et al 1990). Cardiac sympathetic blockade with propranolol reduces heart-rate fluctuations below 0.15 Hz whilst leaving those above 0.15 Hz relatively unaffected (Saul 1989, Saul 1991). Thus heart-rate changes at frequencies above 0.15 Hz seem to be primarily caused by modulation of the cardiac vagal efferent activity. Similarly, since respiration usually occurs at frequencies greater than 9 breaths per minute (0.15 Hz) respiratory fluctuations in heart-rate, so-called sinus arrhythmia, are likely to be mediated primarily by parasympathetic efferent pathways. Clearly if respiratory rate was to fall below 9 breaths per minute then sympathetic modulation of heart-rate changes would occur.

The issue is not, however, quite as straightforward as it may appear. Despite combined pharmacological blockade of parasympathetic and sympathetic systems there is still a small respiratory sinus arrhythmia, postulated to be due to mechanical modulation of sinus rate by stretch of the vessel wall (Saul et al 1991). A similar phenomenon is seen after cardiac transplantation where there is total denervation of the transplanted heart. These oscillations have again been attributed to intrinsic cardiac mechanisms such myocardial wall stretch with the respiratory cycle, and indeed may be independent of cardiac innervation (Bernardi et al 1989, Bernardi et al 1990). Therefore, it can be said that heart-

rate power in the high frequency band is a satisfactory but incomplete measure of cardiac vagal control.

The situation is less clear for the specificity of the very low and low frequency powers for a single control mechanism. In animal studies heart-rate fluctuations at frequencies below 0.15 Hz are affected by stimulation of the vagal and sympathetic cardiac nerve fibres (Saul et al 1991). In humans, heart-rate powers between 0.03 and 0.15 Hz are reduced by either parasympathetic or sympathetic pharmacological blockade (Saul et al 1991, Saul et al 1989, Pomeranz et al 1985). Furthermore, heart-rate fluctuations in this frequency range have been associated with a wide variety of stimuli such as thermoregulation, periodic breathing and haemodynamic instability. Thus, heart-rate spectra in the low and very low frequency regions are not invariably a specific marker of sympathetic activity as previously suggested (Pagani et al 1986, Malliani et al 1990) but can also be dependent on vagal and other influences. The reliability of these spectral indices to reflect cardiac sympathetic modulation can, however, be enhanced by selectively activating the sympathetic nervous system such as during head up tilting (Saul et al 1990, Pagani et al 1986) and with selective pharmacological blockade (Pomeranz et al 1985).

Interpretation of blood pressure spectra

A similar spectrum is seen for blood pressure as for heart-rate with three main peaks identified. In patients with denervated donor hearts there is a persistent high frequency power and it has been suggested that this is due to the mechanical effects of respiration on the heart and large thoracic vessels (Saul et al 1991, Macov et al 1994). Similarly,

sinoaortic denervation results in minor reductions in high frequency blood pressure power in conscious cats (Di Rienzo et al 1991).

Combined pharmacological blockade of the autonomic nervous system with atropine and propranolol eliminates only a fraction of the blood pressure variability below 0.15 Hz, implying that the very low and low frequency powers for the blood pressure spectrum are caused predominantly by fluctuations in vasomotor tone and systemic vascular resistance (Saul et al 1991, Parati et al 1995). Indeed, at the very low frequency powers, vascular modulation has been regarded as being due to the renin-angiotensin system, endothelial factors and local thermoregulation (Akselrod et al 1985, Dutrey-Dupagne et al 1991). Increasing sympathetic drive by head-up tilt or mental stress has been associated with an increase in the low frequency band of the blood pressure spectrum; decreased sympathetic drive during sleep and α adrenergic blockade results in a decrease in the power around this frequency band (Malliani et al 1991, Parati et al 1990, Furlan et al 1990). Thus, as with the heart-rate spectrum there is a suggestion that the low frequency band (0.05-0.15 Hz) is a marker of sympathetic vasomotor tone although this is still open to some debate.

Heart-rate and blood pressure spectral powers; are they an index of autonomic modulation?

From the current evidence there seems little doubt that the high frequency band of the heart-rate variability spectrum is a reasonable marker of cardiac vagal tone. This is supported by pharmacological evidence that administration of atropine abolishes the respiratory component of the heart-rate spectrum (Pomeranz et al 1985).

The reliability of the blood pressure or heart-rate powers around 0.1 Hz as a marker of sympathetic tone as was initially described is, however, now open to question (See discussion of baroreceptor-cardiac reflex sensitivity derived from spectral analysis for further details). In the study by Pomeranz and colleagues (Pomeranz et al 1985), atropine administered to patients who were supine and breathing in a controlled manner, reduced the low frequency component of the power spectrum. This implies that under these conditions the low frequency band is predominately mediated by the parasympathetic system. (Metronome breathing, where the subject is asked to breath in time with a ticking metronome, has been shown to increase vagal drive (Pagani et al 1986) so in normal conditions this situation does not necessarily hold true). Cohen and colleagues have shown that despite increases in direct measures of sympathetic nerve activity (as recorded by microneurographic techniques) and in vascular resistance (measured with forearm venous occlusion plethysmography) caused by lower body negative pressure there was no increase in the 0.1 Hz heart-rate power. (Cohen et al 1991). Further conflicting results have come from studies using phenylephrine which raises blood pressure without directly affecting heart-rate. This results in activation of the baroreflex and thus a reduction in sympathetic activity but this was not seen as an increase of the 0.1 Hz power of the heart-rate spectrum (Saul et al 1990). In heart failure patients, where muscle sympathetic nerve activity is increased, there is not only a reduction in the high frequency power of heart-rate variability but also in the low frequency (0.1 Hz) region (Kienzle et al 1992). Similarly, Adamopoulis and colleagues (1992) showed that spectral measures of autonomic activity correlated poorly with other measures of autonomic function.

As mentioned before, there is some evidence to show that reducing the large number of input variables on heart-rate and blood pressure to narrow frequency regions is too simplistic. Broad-band spectral analysis, considering rhythmic and non-rhythmic fluctuations may be more relevant to neural cardiovascular regulation but there is little work in this area at present.

Thus, in summary, spectral analysis can be used to examine the complexities of the neural regulation of the cardiovascular system but it is probably incorrect to artificially isolate the influences of either the sympathetic or parasympathetic outflow in the individual spectral peaks but to see the spectrum as a dynamic assessment of the neural pathways of the heart. It should be remembered for example that atropine increases the heart-rate and thus reduces the overall variance of the heart-rate so reducing both low and high frequency spectral powers (both of which are fraction of the overall variability). It is usually the case that sympathetic and vagal influences are normally altered in opposite directions- an increase in vagal activity, such as during sleep, is accompanied by a reduction in sympathetic activity and an increase in sympathetic activity, such as head up tilt or standing, is accompanied by a reduction in vagal activity. One can therefore increase the limited sensitivity of the low frequency and high frequency as respective markers of sympathetic and vagal activity by using their ratio as a measure of sympathovagal balance (Pagani et al 1986). Another useful tool is to look at the relationship between blood pressure and heart-rate variables and use these data to evaluate the overall gain of the baroreceptor-heart rate mechanisms (see below).

Spectral analysis of heart-rate and blood pressure variability in diabetic patients.

Power spectral analysis of heart-rate variability has been applied to the study of diabetic autonomic neuropathy. This has shown that there is a reduction of both low frequency and high frequency spectral powers in diabetic patients with abnormal cardiovascular reflex tests and that this becomes more apparent if sympathetic activation is induced by tilting (Pagani et al 1988, Comi et al 1990, Bellavere et al 1992). Some caution needs to be applied to patients with severe, advanced neuropathy who show a marked reduction in heart-rate variability. In these patients there is occasionally a paradoxical presence of high frequency oscillations. This is synchronous with respiration and is probably due to mechanical factors similar to those seen in transplanted hearts (Bernardi et al 1989, Bernardi 1990).

Sympathovagal activity is not constant throughout the day but undergoes a similar circadian rhythm to blood pressure: there is a prevalence of sympathetic tone during the day and the first hours after waking and a marked relative increase in parasympathetic tone during the night (Furlan et al 1990, Bernardi et al 1992). There is a significant reduction in the nocturnal rise of parasympathetic activity in diabetic patients with autonomic neuropathy, as shown by a reduction in the power of the high frequency band of the spectra of heart-rate variability (Bernardi et al 1990, Spallone et al 1993). Thus, there is a relative sympathetic predominance throughout the night and this abnormal circadian pattern of sympathovagal balance is thought to be related to the similar abnormality in circadian blood pressure variation (Spallone et al 1993) although it is not clear whether the relative sympathetic activity at night is responsible for the blunted fall in blood

pressure directly or if an altered common neural regulatory mechanism, such as abnormal sleep control, underlies both abnormalities.

A circadian pattern also exists in the general population in the incidence of cardiovascular and cerebrovascular events. The morning peak of cardiovascular events has been attributed to the morning surge of a triggering event such as the morning rise in sympathetic activity (Muller et al 1989, Furlan et al 1990, Quyyumi et al 1990). There is a well recognised reversal of the normal circadian pattern of cardiovascular events in the diabetic population (Hjalmarson et 1989, Fava et al 1995); this could be associated with the reversal of the normal pattern of sympathovagal activity, although this is speculative at present.

1.7 Baroreceptor-cardiac reflexes

1.7.1 Introduction

It follows from the previous section that cardiovascular control is complex and dependent on a variety of mechanisms. The arterial baroreceptors have a major role to play in cardiovascular regulation and it is becoming increasingly apparent that abnormalities of baroreceptor-cardiac reflex sensitivity can have important diagnostic implications.

Arterial baroreceptor reflex

Arterial baroreceptors are located in the wall of the major vessels of the neck and thorax but are concentrated in the aortic arch (aortic baroreceptors) and the carotid sinus (carotid baroreceptors). These specialised nerve endings are 'stretch' receptors which are

stimulated by distention of the blood vessel wall caused by an increase in blood pressure (Kirchheim 1976). The activation threshold is different for different receptors, but is well below the prevailing arterial blood pressure, so that a reduction in arterial pressure deactivates the receptors and an increase in arterial pressure activates them. Stimulation of the arterial baroreceptors reflexly reduces sympathetic activity and augments vagal drive causing arteriolar dilatation, venodilatation, bradycardia and reduced myocardial contraction. Conversely, deactivation of the arterial baroreceptors results in a reflex increase in sympathetic activity and a reduction in vagal drive thus causing excitatory cardiac and vascular responses. It is clear, therefore, that the baroreceptor's role is to oppose blood pressure falls and rises i.e. to reduce blood pressure variability (Cowley et al 1973). This view is supported by the observation that, denervation of the arterial baroreceptors in unrestrained cats by division of the carotid sinuses and the aortic nerves, results in a greatly increased standard deviation of systolic blood pressure values recorded over a prolonged period of time (Ramirez et al 1985) showing that blood pressure variability has increased. The reflex change in heart-rate in response to blood pressure changes are mediated through the vagal and sympathetic outflows to the sinus node. The fast vagal response permits a beat-to-beat regulation of heart-rate whilst the slower sympathetic outflow to the sinus node contributes to the cardioacceleration during sustained decreases in carotid sinus pressure.

The effect of the baroreceptors on vascular and cardiac responses are made more complex by additional mechanisms which amplify or blunt the receptors responsiveness to physiological stimuli and they make the arterial baroreceptor reflex highly interactive with

other reflexes controlling the circulation, as well as central circulatory influences that participate in cardiovascular modulation (Grassi et al 1995).

1.7.2 Assessment of the baroreceptor-cardiac reflex

Baroreceptor control of heart-rate

Whilst animal studies have helped to clarify the physiology and pathophysiology of the arterial baroreceptors investigation of the baroreceptor-cardiac reflex in humans is more difficult. Since the 1960s baroreceptor-cardiac reflex has been assessed using pharmacological agents. Initially angiotensin (Smyth et al 1969), then subsequently phenylephrine and methoxamine have all been used to stimulate an increase in blood pressure to activate the baroreceptors. Angiotensin was used initially (Smyth et al 1969) but this was shown to have direct cardiac affects and produce central stimulation of sympathetic pathways (Koch-Weser 1965, Scroop et al 1966). The most commonly used agent is phenylephrine, an α-adrenergic agent that has no direct effect on heart-rate but which causes rise in blood pressure of up to 30 mmHg. The pharmacologically induced rise in blood pressure stimulates the arterial baroreceptors and produces a reflex slowing of the heart-rate. Drugs can be given as a bolus to produce a short lasting rise in blood pressure or as an infusion to stimulate the baroreceptors in a more sustained fashion. Similarly, the infusion of a vasodepressor agent such as nitroglycerine or nitroprusside can be used to decrease blood pressure, deactivating the baroreceptors and thus reflexly increasing heart-rate. Combining the two pharmacological agents allows a large portion of the reflex stimulus-response curve to be explored. Baroreceptor-cardiac reflex sensitivity

can be expressed as the slope of the linear relationship between systolic blood pressure change and the resulting change in pulse interval. The units for baroreceptor-cardiac reflex sensitivity are therefore expressed as ms/mmHg.

The Oxford group of Sleight and Pickering (Bristow et al 1969) were the first centre to study baroreceptor-cardiac reflex sensitivity systematically. They used the phenylephrine method, and demonstrated that baroreceptor cardiac reflex sensitivity was impaired in a group of patients with essential hypertension i.e. for the same drug-induced change in blood pressure the reflex heart-rate responses were much less pronounced in the patients with essential hypertension. Furthermore, not only transient heart-rate responses to boluses of vasoactive agents but sustained bradycardic-tachycardic effects of steady state blood pressure alterations as produced by a continuous stepwise infusion of the vasoactive drugs (the Oxford ramp technique) were found to be abnormal in patients with essential hypertension.

A further technique used in the early days to study baroreceptor cardiac reflex sensitivity was the neck suction technique. This vacuum device is applied to the neck and the baroreceptors are directly stimulated by lowering the air pressure within the cuff. This results in an increase carotid transmural pressure stimulating the carotid baroreceptors to produce a reflex bradycardia (Eckberg et al 1975, 1979). Similarly, increasing the pressure across the cuff can reduce carotid transmural pressure and deactivate the carotid baroreceptors resulting in a reflex tachycardia (Mancia et al 1977).

Baroreceptor control of blood pressure

The changes in arterial blood pressure with activation or deactivation of the carotid baroreceptors result from the balance of changes in cardiac output and total peripheral resistance. Total pharmacological autonomic blockade does not impair the buffering action of the carotid sinus baroreceptors, implying that adjustments in cardiac output are not essential and that beat-by-beat control of blood pressure by the arterial baroreceptors depends primarily on changes in total systemic vascular resistance through sympathetically mediated adjustments to vasoconstrictor tone (Shepherd et al 1986).

Animal studies provide some evidence that baroreceptor modulation of the sinus node may not always reflect baroreceptor modulation of the peripheral circulation and blood pressure (Guo et al 1981). Similarly, in normotensive human subjects there is some evidence to suggest differential modulation of the heart-rate and peripheral circulation by carotid and aortic baroreceptors (Mancia et al 1977, 1978).

Again, using the neck suction technique, in hypertensive subjects Mancia and colleagues found a reduction in the pressor baroreflex induced by decreasing carotid transmural pressure but the depressor response (induced by increasing carotid transmural pressure) was increased so the overall blood pressure change was not different in the hypertensive and normotensive groups. They postulated that essential hypertension impairs baroreceptor control of peripheral circulation much less than the baroreceptor control of heart-rate. It was suggested, therefore, that essential hypertension affects baroreceptor control of vagal function rather more than baroreceptor control of sympathetic activity

which predominates in modulation of vasomotor tone. A similar scenario has been postulated in diabetic patients (Watkins et al 1980).

1.7.3 Cardiopulmonary receptor reflex

Cardiopulmonary receptors are located in the cardiac walls, pulmonary arteries and the lungs. They are classified as stretch receptors and are innervated by myelinated and unmyelinated afferent fibres which travel with the vagus nerve to the brainstem. As with the arterial baroreceptors they are responsive to deformation of the structure in which they are located. In the case of cardiac receptors, deformation can originate from myocardial contraction but for the majority of stretch receptors the major stimulus is distention of the cardiac walls which in this low pressure system is volume dependent (i.e. results from cardiac filling) rather than dependent on changes in pressure. The cardiopulmonary volume receptors exert a tonic restraint on sympathetic activity so acting like arterial baroreceptors. The sympathetic restraint induced by the cardiopulmonary baroreceptors is especially marked in the kidney and to the sympathetic modulation of renin release. Vasopressin release, from the hypothalamus, is also reflexly inhibited by stimulation of the cardiopulmonary receptors which are therefore involved not only in blood pressure control but blood volume homeostasis as well (Grassi et al 1988, Robertson et al 1976).

The cardiopulmonary mechanoreceptors can be studied by increasing or decreasing venous return with passive leg raising or lower negative body pressure. The application of lower body negative pressure has been used to study cardiopulmonary baroreceptors. This is achieved by 'pulling' blood from the central circulation to the periphery thereby reducing

cardiac filling pressure and unloading the baroreceptors. When only small negative pressures are used this reduces central pressure without changing arterial pressure and ensures that the arterial baroreceptors are not unloaded (Shepherd et al 1986). It has been shown using these techniques that the cardiopulmonary receptors exert a major tonic inhibition of sympathetic vasoconstrictor tone to the skeletal muscle (Shepherd et al 1986). The sympathetic supply to the splanchnic vascular bed is also inhibited by stimulation of the cardiopulmonary receptors (Grassi et al 1988, Robertson et al 1976). There is a marked reduction or disappearance of the vascular (as assessed by reflex constriction of forearm resistance vessels- Mohanty et al 1987) and humoral responses (Gianattasio et al 1993) to alterations in central blood volume in heart transplant patients, implying that the cardiac baroreceptors are predominant over the pulmonary baroreceptors.

1.7.4 Other methods of assessing baroreceptor-cardiac reflex sensitivity

There are some concerns that the supra-physiological changes in blood pressure induced by the pharmacological agents used to assess baroreceptor-cardiac reflex sensitivity do not give a representative picture of the sensitivity 'in vivo'. Furthermore, it has beeen suggested that phenylephrine can alter the viscoelastic properties of the arterial wall thus affecting baroreceptor-cardiac reflex sensitivity by altering the stretch in the vessel wall induced by a change in blood pressure (Kircheim 1976). As a result of these concerns, other workers have sought non-pharmacological, non-invasive methods of assessing baroreceptor-cardiac reflex sensitivity.

Sequence analysis

This method was initially described in unanaesthetised, unrestrained cats who had blood pressure and pulse interval data recorded intra-arterially for 3 hours (Bertinieri et al 1985). These data were then processed by a computer programmed to identify spontaneous sequences of three or more consecutive beats in which systolic blood pressure increased and pulse interval progressively lengthened (type 1 sequence). Similarly, sequences of three or more consecutive beats where systolic blood pressure progressively fell and pulse interval progressively shortened were identified (type 2 sequences). The regression between systolic blood pressure values and pulse interval values of the following cycle was calculated for each sequence. It was found that there were a large number of sequences with three or more beats and that these all had a high correlation coefficient. The cats then underwent sinoaortic denervation, to denervate the arterial baroreceptors, and the studies repeated. After denervation, there was a 90% reduction in the number of type 1 and type 2 sequences indicating that the sequences were baroreflex in nature.

Parati and colleagues (1988) used this technique to look at normotensive and hypertensive humans. Using intra-arterial monitoring over 24 hours to record systolic blood pressure they identified three or more consecutive beats where blood pressure increased or decreased by at least 1 mmHg and pulse interval changed in the correct direction by at least 6ms. Baroreceptor-cardiac reflex was calculated for each individual sequence from the regression of the relationship between systolic blood pressure and pulse interval and

slope values were averaged to give an overall estimate of baroreceptor-cardiac reflex sensitivity for each subject. They suggested that baroreflex induced changes in pulse interval lag behind changes in systolic blood pressure by one beat. Once again, they found the relationship between systolic blood pressure and subsequent pulse interval to be linear with a correlation coefficient of at least 0.85. There were several hundred 'baroreflex sequences' throughout the 24 hours of the recording providing the first direct evidence that the baroreceptors constantly limit the extent of blood pressure oscillations in humans. Furthermore, they showed that the number of sequences per hour was not constant but followed a circadian pattern- there were fewer sequences during the night than the daytime. By contrast, however, the mean hourly slope (i.e. the baroreceptor-cardiac reflex sensitivity) increased during the night in the normotensive subjects. A similar reduction in the number of sequences and an increase in the slope was seen in normotensive subjects during a daytime siesta. Thus, baroreflex sensitivity was seen to show intra-individual variation dependent on sleep and this confirmed the same findings obtained with pharmacological methods (Smyth et al 1969, Conway et al 1983). Finally, hypertensive subjects were found to have fewer type 1 and type 2 sequences and the overall baroreceptor-cardiac reflex sensitivity, assessed from the slope of the relationship between systolic blood pressure and pulse interval, was reduced compared to the normotensive subjects. Moreover, the hypertensive subjects failed to show the increase in slope during sleep. This study also looked at the question of lag between systolic blood pressure and pulse interval response and found that there was a stronger influence of arterial pressure on pulse interval of the subsequent rather than the current cycle.

Several other interesting findings stem from this study. Firstly, the baroreceptor-cardiac reflex sensitivity decreased progressively as the time over which the baroreceptors modulated the sinus node lengthened- implying that the reflex was most effective during brief increases/decreases in systolic blood pressure. Secondly, where baseline heart-rate values were low the baroreceptor-cardiac reflex sensitivity was higher and similarly when the heart-rate was high the baroreceptor-cardiac reflex sensitivity was lower. This confirmed the previous findings from pharmacological studies of baroreceptor-cardiac reflex sensitivity (Smyth et al 1969) but whether the increased baroreceptor-cardiac reflex sensitivity results in a bradycardia or vice versa remains unclear.

Finally, sequence analysis has been performed on blood pressure data recorded non-invasively using the Finapres beat-to-beat blood pressure recording device (see Section 1.8). In a group of normal healthy volunteers Steptoe and Vögele (1990) recorded 5-minute sequences of resting heart-rate and blood pressure data and calculated baroreceptor-cardiac reflex sensitivity for the type 1 and type 2 sequences. They renamed the type 1 sequences as 'up baroreflex sequences' and the type 2 sequences as 'down baroreflex sequences'. Despite the short recording period they found a large number of linear sequences with a correlation coefficient of 0.8 or greater. When subjects moved from sitting to standing there was a significant reduction in baroreceptor-cardiac reflex sensitivity; again confirming the postural variation in baroreceptor-cardiac reflex sensitivity noted with the phenylephrine technique (Pickering et al 1971).

There are some potential difficulties with the technique of sequence analysis in the assessment of baroreceptor-cardiac reflex sensitivity. Firstly, the studies involving

sequence analysis have used a correlation coefficient of 0.8 or greater as an indicator of a relationship between systolic blood pressure and pulse interval. No mention was made of the significance of these relations unlike the original pharmacological method where a p value of < 0.05 was used to denote a significant relationship between systolic blood pressure and pulse interval (Bristow et al 1969, Smyth et al 1969). However, Bristow and colleagues found that a significance value of 0.05 for the association between systolic blood pressure and pulse interval was associated with a correlation of 0.65 or greater. Moreover, the spontaneously occurring blood pressure and heart-rate sequences are shorter than those obtained by the pharmacological methods, where there are often ten or more beats producing potentially inaccurate regression coefficients in the spontaneous sequences. However, this clearly shows that drug induced changes in blood pressure and pulse interval are not commonly seen under physiological conditions. On the other hand it is reassuring that the relations between sleep, posture and systolic blood pressure are the same for the sequence derived baroreceptor-cardiac reflex sensitivity and the pharmacologically derived results.

Another concern about sequence analysis is the assumption of a latency, or lag in the response of pulse interval to systolic blood pressure. Animal studies suggested that there was a latency in the reflex of 1 or 2 beats (Jewett et al 1964) and this lag of 1 beat was used in the first pharmacological studies of the baroreceptor-cardiac reflex (Smyth et al 1969). However, one study (Pickering et al 1973) has shown that length of the R-R interval changed with the same beat (i.e. lag of 0). In a study by Blaber and colleagues (1995) they found the latency of the baroreflex to be approximately 775 ms. Thus if the

mean R-R interval of subject is less than 775 ms then a lag of 1 or more beats is appropriate and Pickering et al (1973) showed that if pulse interval was 400 ms or less then a lag of 2 was more appropriate. Indeed, the population studied by Blaber and colleagues had a pulse interval of greater than 900 ms and they found that spontaneous baroreflex responses were not influenced by changing lag, whilst other workers (Steptoe et al 1990, Parati et al 1988) studied populations with a mean pulse interval of less than this so a lag of 1 or more beats was more appropriate.

Due to the technical problem of identifying significant baroreflex sequences it is assumed that sequences of change in three or more systolic blood pressure readings with associated pulse interval changes are baroreflex in nature. It can not be assumed that the baroreceptor-cardiac reflex is active only under these conditions but there may well be beat by beat influences on pulse interval that are considered as 'noise' using the current method of sequence analysis.

Another limitation of sequence analysis, and indeed with the pharmacological methods of assessing baroreceptor-cardiac reflex sensitivity lies in the inability to assess baroreceptor control of blood pressure and the peripheral circulation.

The advantages of the sequence technique are that it studies physiological changes in systolic blood pressure and examines the stimulus/response relationship of the baroreceptors across the physiological range of blood pressure. There are theoretical concerns that pharmacological agents may affect the baroreceptor reflex arc or alter the viscoelastic properties of the arterial wall thus influencing baroreceptor-cardiac reflex sensitivity. When the Finapres device is utilised (Section 1.8.3) the technique becomes

totally non-invasive. Furthermore, sequence analysis allows a large number of observations to be collected.

In summary, sequence analysis of resting heart-rate and blood pressure data obtained by invasive or non-invasive recording provides an estimation of baroreceptor-cardiac reflex sensitivity during activation and deactivation of the arterial baroreceptors that is comparable to pharmacological methods of assessing baroreceptor-cardiac reflex sensitivity. Moreover, there are some theoretical reasons why non-invasive methods of assessing baroreceptor-cardiac reflex sensitivity are preferable to pharmacological methods.

Assessment of baroreceptor-cardiac reflex by spectral analysis methods

As previously stated, baroreflex sensitivity can be expressed as the slope of the regression between pharmacologically induced changes in systolic blood pressure and reflex changes in pulse interval. This method of assessing baroreceptor-cardiac reflex sensitivity is often described as an open-loop system with the stimulus (in this case, an increase in blood pressure) leading onto a response (the increase in pulse interval). Several concerns about the open-loop model have been raised: one is that a change in pulse interval could produce a change in the following systolic blood pressure. For example, if pulse interval lengthens the filling period of the heart during diastole will also increase, producing a greater stroke volume and hence a higher systolic blood pressure. Similarly, changes in systolic blood pressure could affect pulse interval via positive and negative feedback mechanisms (Pagani et al 1988). These concerns, plus the invasive nature of the pharmacological techniques

and the moment by moment change in baroreceptor control of heart-rate due to other factors modifying the gain of the baroreflex have led to the use of closed loop models to provide a quantitative index of the overall gain of the baroreflex mechanisms. Akselrod and colleagues (1985) proposed a closed-loop feedback system with two transfer functions: one concerned with the effect of blood pressure on heart-rate and the other the effect of heart-rate on blood pressure. Noise is introduced into the model from mechanical disturbances (i.e. respiration) and from central mechanisms which can cause variation of heart-rate or systolic blood pressure independently of blood pressure or heart-rate. Clearly, the influence of noise on the closed loop is impossible to assess and so an assumption is made that all disturbances enter the system at the systolic blood pressure level (Akselrod et al 1985, Pagani et al 1988). Making these assumption allows the gain of the transfer function between variations in systolic blood pressure and pulse interval to be assessed from the frequency domain analysis of simultaneous beat-to-beat recordings of pulse interval and systolic blood pressure variability. The overall gain, usually expressed as the α-coefficient, is calculated from the square root of the ratio of the spectral components of pulse interval and the systolic blood pressure variabilities.

Mulder and colleague (1985) were among the first to utilize this closed loop model for assessing baroreceptor-cardiac reflex sensitivity. They assumed that the low-frequency band (0.07-0.14 Hz) was the 'site' of the baroreflex activity and calculated the modulus (gain) of the transfer function between variations in blood pressure and heart-rate in this band as a quantification of baroreceptor-cardiac reflex sensitivity. Robbe and colleagues (1987) further developed this to assess baroreceptor-cardiac reflex sensitivity with

phenylephrine and compare this to the modulus values of the mid-frequency region. It was shown that there was a high correlation between the gain of the transfer function between heart-rate and systolic blood pressure and the phenylephrine derived baroreceptor-cardiac reflex sensitivity.

Pagani and colleagues (1988) used spectral analysis methods and phenylephrine injection to look at baroreceptor-cardiac reflex sensitivity in 'mild' hypertensive subjects and compared baroreceptor-cardiac reflex sensitivity before and after a structured exercise programme. They assessed the α coefficient in both the low frequency (α_{LF}) and the high frequency (α_{HF}) regions. They found that the gain was the same for both frequency regions suggesting that the assumption about noise in the closed loop model apply to both low and high-frequency regions. Once again, there was good correlation between the baroreceptor-cardiac reflex sensitivity derived from the phenylephrine method and that derived from the assessment of α coefficient. Furthermore, it was demonstrated that improved physical fitness increased the gain of the relationship between pulse interval and systolic blood pressure when assessed by either method and that hypertension reduced the α coefficient, mirroring the changes in pharmacologically assessed baroreceptor-cardiac reflex sensitivity in hypertensives (Bristow et al 1969).

Other simple, closed loop models for assessing baroreceptor-cardiac reflex sensitivity have been postulated, notably by De Boer and colleagues (1987). They proposed a mathematical model of the circulation which examines the interactions of the fast vagal response to baroreceptor stimulation and the slower response of the sympathetic efferents controlling arterial smooth muscle. It is suggested that the oscillations around 0.1 Hz of

the pulse interval and blood pressure spectra are due to the phase lag in the baroreceptor loop: it is implied that respiration determines changes in venous return to the right, and then the left heart and accordingly in stroke volume which in turn determines the respiration induced changes in blood pressure. These rises in blood pressure stimulate the baroreceptors, which causes a fast vagal response to the heart and a slow sympathetic withdrawal to the blood vessels. Since the sympathetic response is slower than the parasympathetic response the sympathetic response is out of phase with the initial change in blood pressure and so instead of buffering it produces a new oscillation which is sensed by the baroreceptors and the cycle continues. The new oscillation is more dependent on the delay of the overall vascular response to the baroreceptors than the initial stimulus. As a consequence, variation in sympathetic discharge at a frequency of about 0.1 Hz (i.e. the low frequency region of heart-rate and blood pressure spectra) produces resonance between the fast vagal and slow sympathetic response to baroreceptor stimulation. This is dependent on the sympathetic efferents being intact, that there is a functional baroreflex and there is a reactive vascular system. It was stated earlier that atropine abolishes the high frequency and reduces the mid-frequency heart-rate fluctuations (Pomeranz et al 1985) and De Boer postulated this was due to removal of the fast vagal response which accentuates the resonance caused by the slow sympathetic response to baroreceptor stimulation. Other workers have shown that conditions where there is reduced gain in arterial baroreflex control, such as heart failure and exercise (Eckberg et al 1971, Ellenbogen et al 1989) are also associated with a *reduction* in the low-frequency peak despite evidence of increased sympathetic tone from direct measures of sympathetic

function (Cohn et al 1984, Leimbach et al 1986). Furthermore, after sinoaortic denervation the power in the region of 0.1 Hz is significantly reduced for blood pressure and pulse interval variability, compared to the situation with an intact baroreflex (Pagani et al 1986, Cerutti et al 1994).

This model has been tested by Bernardi and colleagues (1994) using neck suction to stimulate the arterial baroreceptors with a Finapres to record beat-to-beat blood pressure changes and peripheral vascular tone assessed using skin blood flow recorded using an infrared photoplethysmograph. To remove the complicating influence of changes in venous return during respiration, neck suction was applied during apnoea and they showed that stimulation of the baroreceptors generated a 0.1 Hz oscillation in the cardiovascular system. From this finding it was implied that the low frequency oscillations in power spectra of cardiovascular parameters were due to baroreceptor sensed blood pressure fluctuations. Sleight and colleagues (1995) further examined the De Boer hypothesis in a small study (n = 3) where arterial baroreceptors were again stimulated using the neck suction technique in a sinusoidal fashion and beat-to-beat blood pressure was recorded with the Finapres device. The arterial baroreceptors were stimulated with sinusoidal neck suction at two frequencies 0.1 Hz and 0.2 Hz. They assessed baroreflex sensitivity using the traditional phenylephrine method and studied a normal control subject plus two patients with congestive cardiac failure, one of whom had impaired baroreflex sensitivity. They showed that in the two heart failure patients (in whom sympathetic drive was assumed to be increased) the low frequency peak was increased only if the baroreflex was intact and if the rate of stimulating the arterial baroreceptors was slow enough for the

sympathetic efferents to be able to respond i.e. at 0.1 Hz. On the basis of this, they suggested that the low frequency power is determined by the baroreflex and as such is an index of baroreflex sensitivity. Furthermore, they postulated that the power spectra of heart-rate and blood pressure variability may be a sensitive descriptor of circulatory reflexes and that these reflexes set the absolute level of parasympathetic and sympathetic tone. In conclusion, it is suggested that power spectral analysis of circulatory variables may be an index of this sensitivity (gain) of the control by the baroreflex rather than specific sympathetic or parasympathetic tone.

Clearly, there are problems with the assumptions made from this study. Firstly, the small number of patients makes any statistical assessment of the data meaningless. Secondly, mental stress and short term physical exercise which reduces baroreceptor-cardiac reflex sensitivity is associated with an increase in the power around 0.1 Hz region (Malliani et al 1991). Finally, the low frequency components of blood pressure and pulse interval spectra decrease at night despite a well documented increase in baroreflex gain (Pagani et al 1988).

There are several problems in using the frequency domain to study baroreceptor-cardiac reflex sensitivity. Firstly, there is the question of coherence of the pulse interval and blood pressure data. The coherence function is the amount of linear coupling between the two signals and as such is equivalent to the regression coefficient in regression analysis in the time domain, except that it is computed for each frequency region (Robbe et al 1987). It is therefore apparent that for the α coefficient to be valid the coherence for the mid and high-frequency regions should be high (>0.5) although in many spectral studies it is not

mentioned. Another concern is that there are several techniques in use to estimate pulse interval and blood pressure spectra such as a fast Fourier (Robbe et al 1987) and autoregressive models (Pagani et al 1988, Lucini et al 1994) and until recently it was unclear whether the application of these different analytical techniques could influence α. Clayton and colleagues (1995) addressed this problem and analysed heart-rate and blood pressure data using five most commonly used methods of spectral analysis, including fast Fourier transformation and autoregressive models, to calculate α coefficient. They found that differences in transfer function gain between the different methods used were small provided the coherence between the data was acceptable. Finally, debate continues about the significance of the oscillations around 0.1 Hz and whether this is a pure baroreflex phenomenon due to resonance owing to delay of the sympathetic control loop of the baroreflex. It seems that exclusively attributing a cardiovascular rhythm to any single neural circuit is an oversimplification of a complex interaction of mechanisms and whilst this hypothesis may explain the apparent paradox of reduced power around 0.1 Hz in conditions where sympathetic drive is increased it is also apparent that low and high frequency components are present in both sympathetic and vagal efferent activities.

In summary, frequency domain analysis of simultaneously recorded heart-rate and blood pressure variability allows assessment of the baroreceptor-cardiac reflex sensitivity without the need for intravenous agents. Whilst there is some debate as to the best method to use and the interpretation of the spectral results this is a useful and expanding field of research.

Assessment of baroreceptor-cardiac reflex sensitivity from the Valsalva manoeuvre.

There has been concern voiced that vasoactive drugs can influence the baroreceptor-cardiac reflex by altering the viscoelastic properties of the arterial wall and also by having direct central effects on the reflex and may activate other reflex systems, especially the cardiopulmonary receptors. Workers have therefore turned to the Valsalva manoeuvre to search for a more 'pure' and physiological stimulation of the baroreceptor-cardiac reflex. The Valsalva manoeuvre is a complex reflex which can be divided into four phases as described in Section 1.3.3. The reflex tachycardia of phase 2 is a baroreceptor mediated response to a fall in systolic blood pressure during the forced expiration. After release of the strain, as blood is forced into the still constricted peripheral circulation, leading to a rebound hypertension and a baroreflex mediated bradycardia (phase 4). Thus, the relationship between systolic blood pressure and pulse interval during phase 2 or 4 of the Valsalva manoeuvre can potentially be used to assess baroreceptor-cardiac reflex sensitivity. Palmero and colleagues (1981) compared baroreceptor-cardiac reflex sensitivity assessed by the phenylephrine method with baroreceptor-cardiac reflex sensitivity assessed from phase 4 of the Valsalva manoeuvre. Included in the study were nine healthy volunteers, twenty five patients with Chagas disease (which is known to affect the autonomic nervous system) and ten hypertensive patients. Beat-to-beat blood pressure was recorded using intra-arterial monitoring and baroreceptor-cardiac reflex sensitivity was assessed during phase 4 of the Valsalva manoeuvre and then after a bolus of phenylephrine. The baroreceptor-cardiac reflex sensitivity was estimated from the linear relationship between pulse interval and systolic blood pressure. They found a good

relationship between baroreceptor-cardiac reflex sensitivity assessed by the two methods (r= 0.91). This study also recognised that there are two periods of phase 4; the first, immediately after release of the strain, where increases in blood pressure were not associated with changes in pulse interval and a second where progressive slowing of the heart-rate was associated with increasing pressure. When all beats in phase 4 were included in the analysis, baroreceptor-cardiac reflex sensitivity was half that obtained from the phenylephrine method whereas when the second period of phase 4 was used the correlation between the phenylephrine and the Valsalva manoeuvre method was 0.9. Other workers have previously demonstrated that there is inhibition of the baroreceptor-cardiac reflex during the initial seconds of the Valsalva manoeuvre (Sharpey-Schaffer et al 1955, Delius et al 1972) and it is postulated that this is a consequence of persistent activation of the adrenergic system that normally occurs during phase 2 of the Valsalva manoeuvre. However, the impulse-response curve between pulse interval and systolic blood pressure during phase 4 of the Valsalva manoeuvre is similar to that observed during the infusion of phenylephrine i.e. a sigmoid shaped curve (Palmero et al 1981, Pagani et al 1995).
Other workers have used the Valsalva manoeuvre to assess baroreceptor-cardiac reflex sensitivity but the studies vary in the stage of phase 4 used. Pickering and Sleight (1969) used the blood pressure overshoot (equivalent to Palmero's second period of phase 4) and used a lag of one beat in pulse interval to assess the baroreflex. Shimada and colleagues (1986) excluded the first part of phase 4, although they did not explain how this was done, and again used a lag of one beat. Goldstein et al (1982), however, used the whole of phase 4 and found Valsalva derived baroreceptor-cardiac reflex sensitivity to be

significantly lower than that derived using the phenylephrine method. Finally, Smith and colleagues (1987) examined the blood pressure overshoot and found a close correlation between this and the phenylephrine method of assessing baroreceptor-cardiac reflex sensitivity.

Thus, baroreceptor-cardiac reflex can be derived from phase 4 of the Valsalva manoeuvre and the values obtained are comparable to those from the 'gold standard' phenylephrine method. However, it is also clear that the different periods of phase 4 can give differing values for baroreceptor-cardiac reflex sensitivity so it is imperative that the method used is clearly stated. Moreover, it should be remembered that changes in venous return to the heart due to the raised intrathoracic pressure during the forced expiration can stimulate the low pressure baroreceptors, and this could modify the blood pressure rise seen in phase 4. Likewise, a 'hangover' effect of sympathetic activation from phase 2 of the Valsalva manoeuvre could also influence blood pressure changes, and hence baroreceptor-cardiac reflex sensitivity during phase 4 of the Valsalva manoeuvre.

1.8 The Finapres Device

One limitation in studying cardiovascular regulation is the need to record beat-to-beat changes in blood pressure. Until recently the only available method was intra-arterial monitoring which, whilst accurate and allowing 24-hour recordings to be made, is invasive and thus not suitable for the study of large numbers of patients or for repeatability studies. With the development of non-invasive beat-to-beat blood pressure monitoring systems,

such as the Finapres device (Ohmeda Monitoring Systems. Englewood, Colorado, USA) the study of cardiovascular rhythms should become more accessible.

1.8.1 Finapres

This device, a FINger Arterial PRESsure monitor, consists of a finger cuff that has a built in infra-red source and sensor. The cuff is wrapped around the middle phalanx of the middle finger and the cuff is attached to a front end box which in turn is attached to the main unit containing an air supply, the electronics and a recorder. The device measures blood volume under the cuff via the infra-red sensors and the cuff pressure via a pressure transducer.

The device measures blood pressure by utilizing a modified version of the volume clamp technique first described by the Czech physiologist, Peñáz (1973). The volume of blood under the cuff is monitored by the infra-red sensor and clamped to constant volume (two thirds of the maximum arterial blood volume) by varying the cuff pressure in parallel with intra-arterial pressure, using a fast servo system. If the intra-arterial pressure were to rise then the volume of blood under the cuff would increase; this is detected by the infra-red sensor and the servo counteracts immediately by increasing cuff pressure by the same extent as finger artery pressure. Clearly, one must calibrate for the correct level of actual finger arterial pressure. This is achieved by clamping the arterial blood volume at a level corresponding to the unloaded state of the arterial wall; in this situation cuff pressure is equal to finger arterial pressure. The procedure used in the Finapres to establish the correct volume clamp level is the Physiocal (physiological calibration). Regularly (every

ten beats to start and then every seventy beats once the measurement is stabilised) cuff pressure is not varied to properly clamp blood volume, but kept constant while the now pulsatile infra-red sensor output is evaluated. This plethysmogram is judged by the machine for its size and shape and if necessary the volume clamp is adjusted to make cuff pressure nearly identical to finger arterial pressure.

Finger arterial recordings are similar to but not identical to intra-arterial measurements. Propagation of the pressure wave towards the periphery results in mean and diastolic pressures that are lower than intra-arterial measurements and amplification of the pulse wave may result in higher systolic pressure values (Parati et al 1989, Imholz et al 1990). Parati and colleagues (1989) evaluated the Finapres against intra-arterial measurements in twenty four normotensive or essential hypertensive subjects at rest and during a variety of physiological manoeuvres, including the Valsalva manoeuvre, as well as during the injection of vasoactive drugs. They found that the finger blood pressures recorded by the Finapres were comparable to the direct radial artery readings albeit with an off-set that remained fairly constant throughout the various blood pressure manipulations; differences between systolic and diastolic pressure usually deviated by less than five mmHg. These differences are within the requirements for new blood pressure monitoring devices as stated by the Association for the Advancement of Medical Instrumentation, who consider a deviation of eight mmHg or less as acceptable for new non-invasive blood pressure measuring devices when compared with intra-arterial measures (AAMI 1980). Furthermore, the correspondence between finger and intra-arterial blood pressure readings was satisfactory even when blood pressure was undergoing fast and dramatic changes

such as those seen during the Valsalva manoeuvre or after bolus injection of vasoactive drugs. Phenylephrine is a potent vasoconstrictor and there was still a good correlation between finger blood pressures and intra-arterial readings remained after bolus injection of this agent so systemic vasoconstriction did not appear to impair the accuracy of the Finapres. It should be stressed, however, that the blood pressure values derived from the two methods were similar but not superimposable.

Other workers have confirmed the reliability of the Finapres for the measurement of blood pressure changes during the Valsalva manoeuvre (Imholz et al 1988) and during forms of orthostatic stress (Imholz et al 1990, Freidman et al 1990).

Imholz and colleagues infused progressively increasing doses of phenylephrine whilst recording intra-arterial blood pressure (via a brachial artery catheter) and non-invasive blood pressure using the Finapres. At rest the non-invasive systolic blood pressures recorded by the Finapres were higher than the invasive pressures. It was postulated that this could be due to pressure wave amplification between the aorta and the peripheries; the amplitude of the pressure wave increases as it travels toward the peripheries and the wave front becomes steeper, thus the peripheral pressure is greater than the central pressure (Latham et al 1985, Berger et al 1993). Imholz and colleagues also found that with increasing doses of phenylephrine there was a significant underestimation of the increase in systolic blood pressure using the Finapres compared with the intra-arterial recordings whereas diastolic blood pressure and mean arterial pressure were nearly identical with the two methods. It was suggested that when prolonged infusions of phenylephrine are used mean blood pressure should be used for analysis.

1.8.2 Using the Finapres to assess blood pressure variability

Whilst it seems that the Finapres can record average blood pressure values accurately, if it could accurately assess blood pressure variability this would allow the non-invasive study of the mechanisms regulating blood pressure both in the time and frequency domain. Parati et al (1989) found the standard deviation of mean arterial pressure recorded over a thirty minute period to be identical whether calculated from the Finapres or intra-arterial data.

Omboni and colleagues (1993) studied fourteen essential hypertensive patients and recorded blood pressure non-invasively using the Finapres device for thirty minutes and compared these readings to simultaneously recorded intra-arterial values. To allow for continuous, undisturbed, recording the automatic calibration mechanism was inactivated once the recording was stabilised. Once again, they found that the there were no significant differences between intra-arterial and finger systolic, diastolic and mean blood pressures. Furthermore, the standard deviations of the average blood pressure values for the whole of the recording period were not significantly different between the two methods. Moreover, when analysing blood pressure data using spectral analysis, Omboni found similar results for the pulse interval spectra recorded with the two methods. Similarly, the spectral powers for diastolic blood pressure and mean arterial pressure were not different, however, systolic blood pressure powers in the low frequency region and, to a lesser degree, for the high frequency region of the spectrum were significantly overestimated by the Finapres. Very low frequency systolic blood pressure variations were

also overestimated by the Finapres. Whilst the simplest explanation for these findings is that the Finapres fails to precisely reproduce slow oscillations in systolic blood pressure, the converse could also be true: the intra-arterial method, which depends on a fluid filled connection between the artery and the transducer, underestimate low and very low frequency oscillations in systolic blood pressure. Clearly, systolic blood pressure variations between the two sites do not need to be similar and rhythmic changes in arteriolar tone that have been shown to occur in the low and very low frequency bands could influence the blood pressure variability powers.

Thus, the Finapres has been shown to be suitable for assessing average blood pressure values over prolonged monitoring periods. It allows the non-invasive estimation of blood pressure variability by a variety of methods but, however, some caution should be exercised in the interpretation of slow systolic pressure oscillations since these are magnified by the Finapres.

1.8.3 The non-invasive assessment of baroreceptor-cardiac reflex sensitivity

Since the Finapres appears to allow accurate, non-invasive, blood pressure variations to be recorded over long periods it is very well suited to the study of cardiovascular regulation, and thus assessment of baroreceptor-cardiac reflex sensitivity.

Parati et al (1989) examined baroreceptor-cardiac reflex sensitivity using bolus injections of vasoactive drugs and the baroreceptor control of blood pressure via the neck chamber technique and found Finapres derived values of baroreceptor-cardiac reflex sensitivity to be similar to intra-arterial values. The baroreceptor-blood pressure reflex assessed with the

neck suction technique showed a lower correlation between non-invasive and intra-arterial derived values of baroreceptor reflex sensitivity.

Conversely, Imholz and colleagues (1992) found that a stepwise, continuous, infusion of phenylephrine yielded baroreceptor-cardiac reflex sensitivity values that were significantly higher than those derived from intra-arterial measurements. However, they found that dynamic assessment of baroreceptor-cardiac reflex sensitivity (by bolus injection of phenylephrine) showed similar results with data recorded from the two methods and using mean arterial pressure change rather than changes in systolic blood pressure again gave good concordance between the two methods.

Omboni and colleagues (1993) assessed baroreceptor-cardiac reflex sensitivity in the time domain using sequence analysis from data recorded invasively and non-invasively with the Finapres device. They found that the number of sequences was the same for both methods and that the baroreceptor-cardiac reflex sensitivity for type 1 and type 2 sequences was the same for both recording techniques.

More recently, baroreceptor-cardiac reflex sensitivity was assessed in a population of patients with ischaemic heart disease, using the phenylephrine method and recording blood pressure with the Finapres. Intra-arterial blood pressure data was recorded from the aorta during cardiac catheterization (Hartikainen et al 1995). Non-invasive systolic and diastolic blood pressures were significantly higher than corresponding invasive values although mean arterial blood pressure was not significantly different. The change in systolic blood pressure after the bolus of phenylephrine was, unsurprisingly, 25% lower in non-invasive systolic blood pressure compared to the corresponding invasive change and a similar

underestimation of diastolic blood pressure change was also seen. Mean arterial blood pressure change, however, was again comparable between the two methods.

In this study they found a high linear congruence between baroreceptor-cardiac reflex sensitivity values determined with invasive and non-invasively recorded blood pressure data. Since the changes in systolic blood pressure were different, the estimations of baroreceptor-cardiac reflex sensitivity from the Finapres were significantly higher than those estimated from intra-arterial data. The reason for the higher systolic blood pressure values for this and many of the other studies is unclear. It has been postulated that pressure wave amplification could account for the difference (see above). Hartikainen and colleagues (1995) also showed that with increasing age and systolic blood pressure the difference between non-invasive and intra-arterial blood pressure reduced possibly due to a decrease in the elasticity of the peripheral arteries reducing the pressure wave amplification. It is possible that vasoconstriction by infusion of phenylephrine may be responsible for the blunted increase in non-invasively systolic blood pressure because the finger-brachial artery pressure has been shown to increase by cooling the hand (vasoconstriction) and reduce by vasodilatation (heating the hand) (Tanaka et al 1993).

1.8.4 Summary

Non-invasive estimation of beat-to-beat blood pressure values have been shown to be possible using the Finapres device. The resting systolic, diastolic and mean arterial blood pressures recorded with this device correlated well with intra-arterial blood pressure values albeit with an offset that remains reasonably constant. Furthermore, assessment of

blood pressure variability at rest and during a variety of physiological manoeuvres have shown the Finapres to be reliable during rapid changes of blood pressure. The baroreceptor-cardiac reflex can be assessed using the Finapres device although there does appear to be overestimation of the sensitivity of the reflex when assessed non-invasively compared to intra-arterial recordings. The Finapres therefore provides a reliable, non-invasive, means of recording beat-to-beat blood pressure changes.

1.9 Aims

This thesis explores the possibility of detecting diabetic autonomic neuropathy and the consequences of autonomic neuropathy on cardiovascular regulation in a population of healthy, insulin dependent diabetic patients. The timely detection of diabetic autonomic neuropathy may allow the use of effective means of improving autonomic function and slowing its progression.

The specific aims were:

1) To assess cardiac autonomic neuropathy using the five 'traditional' cardiovascular reflex tests.

2) To use the newer techniques of frequency domain analysis of heart-rate and systolic blood pressure data to detect cardiovascular autonomic dysfunction and to compare these newer methods with the traditional tests of autonomic function.

3) To assess the baroreceptor-cardiac reflex using non-invasive methods and attempt to develop these techniques as a screening test for cardiac dysregulation.

4) To explore the possible consequences of autonomic dysfunction with respect to cardiovascular autoregulation of heart-rate and systolic blood pressure and assess end-organ damage with assessment of ventricular repolarisation and left ventricular mass index.

5) To explore twenty four hour blood pressure regulation and compare any abnormalities with the assessment of cardiovascular control.

CHAPTER 2

THE STUDY GROUPS AND THE ASSESSMENT OF AUTONOMIC FUNCTION

2.1.1 Description of the Study Groups

All clinical studies were performed at the Leicester Royal Infirmary and the Glenfield General Hospital. All patients gave informed consent, the protocols having been approved by the Leicester District Health Authority ethical committee.

Patients and methods

Patients

A total of sixty five insulin dependent diabetic patients were recruited from the local diabetic clinic at the Leicester Royal Infirmary. Subjects were initially approached via a mail shot of names taken from the Diabetic Register of the desired age range (20-55 years old). The subjects who responded were then interviewed either over the telephone or in person, if they attended the Diabetic Clinic within one month of the mail shot, to confirm they were eligible for entry into the study.

Inclusion Criteria

Patients were between 20-55 years old. From patients' notes and interviewing the patient, the diagnosis of insulin dependent diabetes mellitus was confirmed from the initial history at presentation and by the presence of ketonuria on the urine samples provided at presentation to the diabetic clinic.

All patients were normotensive as diagnosed by three or more clinic syphgmomanometer blood pressure readings over the preceding year of 140/90 mmHg or less.

Volunteers were all taking subcutaneous insulin either twice daily or four times daily and were on no other medication, except for the oral contraceptive pill in the female volunteers. In addition, none of the study group smoked.

Exclusion criteria

Those patients with clinical evidence of diabetic complications at the initial interview were excluded from entering the study. Thus, patients with a history of cerebrovascular or cardiovascular disease were excluded. Those patients known to have diabetic retinopathy (including those with background diabetic retinopathy detected on routine screening at the diabetic clinic) were also excluded as were those patients with known diabetic renal disease including those with normal serum creatinine but urine positive for protein on routine dipstix testing in the clinic. Patients with a clinical history suggestive of autonomic dysfunction were excluded. To assess this, patients were asked about the symptoms of autonomic dysfunction, including impotence in the male population, and those with symptoms of autonomic dysfunction were excluded from the study. Furthermore, patients with clinical evidence of peripheral neuropathy, on history and/or on examination to detect loss of peripheral reflexes, were also excluded.

Patients recruited

Five hundred patients were initially approached to take part in the study. Of those, sixty five patients (forty females) agreed to take part and met the inclusion/exclusion criteria for the study. All of these subjects gave informed consent to take part in the study and it was approved by the Local Ethical Committee.

Control subjects

Control volunteers were recruited from the staff of the Leicester Royal Infirmary and their relations. They were all healthy volunteers who gave informed consent. None of the volunteers had any medical conditions and were taking no medication other than the oral contraceptive pill amongst the female volunteers. Only non-smokers were recruited as control subjects.

Baseline investigations

All subjects attended the test laboratory at least two hours post-prandial and at least two hours after their last insulin dose and they underwent anthropometric measurements of height and weight. From this data body mass index was calculated using the formula weight/height^2. Subjects had hip and waist measurements taken as described by Stuhldreher and colleagues (Stuhldreher et al 1992). The subjects were standing and three measurements of maximal hip circumference were taken and the mean of the three readings calculated. Similarly, three waist circumference measurements were taken from the mid-point between the iliac crest and lower costal margin and the mean calculated. The ratio of mean waist to mean hip measurement was calculated and expressed as the waist/hip ratio.

A blood sample was drawn to analyse for serum electrolytes, creatinine and plasma glucose. A sample for glycosylated haemoglobin (HbA_1) was also taken. In addition, a survey of the notes of the IDDM subjects was undertaken and HbA_1 for the duration of their disease were recorded and averaged (records were available dating back to 1978). Random serum total cholesterol, HDL and LDL cholesterol as well as serum triglycerides were also determined.

Subjects were asked to collect three overnight urine samples, according to the protocol of Mogensen and colleagues (1995). The subjects were asked to void urine normally on the day of the collection up to and including the last sample before retiring to bed. If the subject woke during the night to urinate, they were asked to collect these samples in the bottle provided for them as well as collecting the first sample of the day in the bottle. Three separate overnight urine collections over a period of one month were made for each patient using this method and analysed for microalbuminuria and creatinine. Because of the potential variability in urine volume which can significantly affect the values for albumin excretion the urinary albumin/creatinine ratio, which has been shown to decrease the variability of urinary albumin estimation (Price et al 1985), was calculated for each subject.

2.1.2 Results

The baseline characteristics of the IDDM patients and the control population are presented in Table 2.1. There were no significant differences in the anthropometric measurements between the two groups.

There was no significant difference between the groups for systolic or diastolic blood pressure recorded at the time of enrollment into the study using a standard syphgmomanometer.

Only the HbA_1 and random blood glucose were significantly higher for the IDDM patients compared with the control subjects.

None of the diabetic subjects had evidence of microalbuminuria with the mean albumin/creatinine ratio for the group being 2.6 mg/mmol/Cr (normal range = 0 - 3.5 mg/mmol/Cr). Furthermore, none of the three samples provided by each IDDM subject

showed evidence of microalbuminuria. Twenty control subjects provided urine samples for assessment of albumin/creatinine ratio as described for the IDDM population. None showed evidence of microalbuminuria and the mean value was not statistically significant from the IDDM group (Table 2.1).

2.1.3 Conclusion.

The two groups were well matched for age, sex and systolic blood pressure. There was no evidence for diabetic renal disease in the IDDM patients studied and the renal function was normal and similar to the control population.

2.2 Assessment of autonomic function by bedside cardiovascular reflex tests

2.2.1 Introduction

Autonomic dysfunction has been reported in 20-40% of unselected diabetic patients when assessed using standard bedside tests (Ewing et al 1985). This is of clinical significance because diabetic patients with severely impaired cardiac autonomic function have a poor prognosis and an increased risk of sudden death (Page et al 1978, Ewing et al 1985, Sampson et al 1990).

For the past 25 years evidence for autonomic dysfunction has been sought by using a battery of non-invasive cardiovascular reflex tests (Ewing et al 1982). These include the ratio of maximal and minimal heart rate variation during deep breathing (I/E ratio), the ratio of maximum and minimum R-R interval during the Valsalva manoeuvre, the heart rate response to standing (30:15 ratio) and the blood pressure responses to standing and sustained hand grip.

ble 2.1: **Characteristics of cases and controls. (Mean ± SD)**

	Controls (n = 65)	Diabetics (n = 65)
ge (years)	37 ± 8.6	37.8 ± 4.4
uration of diabetes (years)	N/A	14.2 ± 7.8
bA_1 (%)	5.8 ± 0.6†	10 ± 1.2*
eatinine (μmol/ml)	97 ± 10.1	97 ± 10.7
CR**(mg/μmol/Cr)	(n = 20) 2.0 ± 1.1	2.6 ± 1.3
BP (mmHg)	122 ± 13	126 ± 12
BP (mmHg)	74 ± 11	76 ± 13
MI (kg/m^2)	26.2 ± 0.8	25.2 ± 0.7
/H‡	0.86 ± 0.04	0.84 ± 0.03

**Average HbA_1 since diagnosis.*

‡ Waist/Hip ratio

*** Albumin:Creatinine ratio*

† $p<0.001$

These tests were used to assess cardiovascular autonomic function in the IDDM and control subjects described in the previous chapter and the development of an automated device for assessing cardiovascular autonomic function is also described.

2.2.2 Methods

The sixty five insulin dependent diabetic patients and control patients recruited into the study performed the five standard cardiovascular reflex tests as described by Ewing and Clarke (Ewing et al 1982, Ewing et al 1985). All subjects were at least two hours post-prandial and rested for up to fifteen minutes prior to performing the tests. Subjects were seated on a bed and three electrocardiogram electrodes were stuck onto the anterior chest wall and connected to a Fukuda Denshi FCP 4101 electrocardiogram recorder (Fukuda Denshi Co. Ltd, Tokyo, Japan).

Valsalva Manoeuvre

The Valsalva manoeuvre was explained to the subject and after five minutes rest they were asked to perform a forced expiration through a mouthpiece connected to pressure transducer with a digital display. The subjects were asked to hold the expiration at 40 mmHg for fifteen seconds, the effort was then released and the heart rate was continually recorded for a further minute. Four separate Valsalva manoeuvres were performed with the first one not recorded but used as a practice attempt to ensure an adequate technique and allow the equipment to be properly set up. The tracing was marked to show the period of the expiratory effort and the recording period after the release of the strain.

Inspiration/Expiration ratio

The subjects were sitting comfortably with the electrocardiogram recording the heart rate. The patients were asked to breath at six breaths per minute with audible prompts given by the investigator when to breath in and out. The subjects performed six inspiration/expiration cycles (i.e. controlled breathing for one minute) to familiarise themselves with the technique as well as to ensure an adequate technique and then repeated this with the recorder switched on. During the recording the trace was marked to show when inspiration and expiration commenced.

Heart rate response to standing

The subjects were asked to rest supine on a bed for up to five minutes and during this time the test was explained to them. They were asked to stand from the supine position as quickly and as smoothly as possible during which time the heart rate was continually recorded for up to one minute after standing. The recording was quickly assessed for movement artifacts and if it was thought to unusable the test was repeated.

Blood pressure response to sustained handgrip

The patients were supine on a bed with a mercury syphgmomanometer cuff of the correct size applied to the non-dominant upper arm and the average blood pressure of three consecutive recordings taken. Using a Smedley Hand Dynamometer (Smedley, Jackson, Michigan, USA) the subjects were asked to perform a maximum contraction using their

dominant hand. One third of this maximum voluntary contraction is then maintained for up to five minutes with the blood pressure being measured each minute.

Blood pressure response to standing

The subjects were again supine with the syphgmomanometer cuff applied to the non-dominant upper arm. After resting supine for one minute the subjects were asked to stand and the blood pressure repeated one minute after standing.

Analysis of data

Valsalva manoeuvre

The R-R intervals were measured for whole of the recording period and the maximum R-R interval (usually shortly after release of the strain) and the minimum R-R interval (usually during the forced expiration) were noted. The ratio of the longest to the shortest R-R interval was expressed as the Valsalva ratio. This procedure was repeated for the three Valsalva manoeuvres that were recorded and the mean of the three values given as the overall Valsalva ratio.

Inspiration/Expiration ratio

From the marked tracing the periods of inspiration and expiration were identified and the maximum and minimum heart rate was measured for each inspiration/expiration cycle. The difference between the maximum (during inspiration) and minimum (during expiration) heart rate was calculated for each cycle and the mean calculated for the duration of the

recording period. The mean of the ratio of the maximum to minimum heart rate was also calculated for the recording period.

Heart rate response to standing

From the recording the R-R intervals were measured and the ratio of the longest R-R interval to the shortest R-R interval was calculated. Ewing and Clarke have found the longest R-R interval to be around the thirtieth beat after standing and the shortest R-R interval to be around the fifteenth beat after standing, expressing their results as the 30:15 ratio (Ewing et al 1985). In the current study, all patients had the 30:15 ratio measured but, in addition, had the actual ratio of the longest R-R to the shortest R-R interval calculated regardless of where in the recording it occurred.

Blood pressure response to sustained handgrip

The difference in the diastolic blood pressure just before release of the handgrip and before starting was calculated as a measure of the response.

Blood pressure response to standing

The difference in systolic blood pressure before and after standing was calculated.

2.3 Results of the assessment of autonomic function in the study population

The whole population of IDDM study group (sixty five patients) and forty two of the control population undertook the standard measure of autonomic function as detailed above.

The values for the five bedside tests of cardiovascular autonomic function are presented in Table 2.2. There were no differences in the tests between the control group and the IDDM subjects studied. Furthermore, none of the subjects studied had abnormal tests when compared to the age adjusted values presented by O'Brien and colleagues (O'Brien et al 1986) thus all subjects had an 'autonomic neuropathy score' of zero (Bellavere et al 1983, Ewing et al 1985).

2.4 Conclusion

None of the subjects studied had evidence of defective cardiovascular regulation in response to the five standard bedside tests of autonomic function. There were no differences in these traditional measures of autonomic function between the IDDM and control groups. The IDDM group had no historical evidence for autonomic or peripheral nerve dysfunction (including impotence amongst the male patients). The two groups were very closely matched for other variables that may affect autonomic function, in particular systolic blood pressure and renal function were not different between the two groups.

Table 2.2- Standard clinical tests of autonomic function. (Mean ± SD)

	Controls (n= 42)	Diabetic subjects (n= 65)	p‡
Valsalva ratio	1.75 ± 0.4	1.70 ± 0.6	0.74
I/E* (beats/min)	21.5 ± 0.3	22.1 ± 0.12	0.66
30:15 ratio	1.47 ± 0.03	1.45 ± 0.2	0.8
BP Handgrip (mmHg)	35 ± 6.3	35 ± 5.7	0.72
BP L-S† (mmHg)	3 ± 2.2	3 ± 3.2	0.61

**Inspiration/Expiration ratio.*

†*Systolic BP standing minus systolic BP lying.*

‡ *P value refers to t-test values between controls and diabetic subjects.*

2.5 Automation of the tests of autonomic function- the development of the ANT

2.5.1 Introduction

It can be seen that the tests of heart rate variability rely on the recording of heart rate onto paper and then manually measuring the change in heart rate/R-R interval in response to the physiological manoeuvres. Manual measurements are time consuming and error prone so automation of the analysis to eliminate chart analysis time and reduce measurement errors is desirable and with this aim the development of an Autonomic Neuropathy Tester (ANT) is described.

2.5.2 Method

ECG signal processing

R-R intervals were recorded from 2 wrist electrodes. The ECG signal was conditioned to optimise detection of the R-wave by the following method. An instrumentation amplifier with a gain of 70dB amplifies the signal and the signal frequencies were then band-pass filtered (6 Hz to 28 Hz at -3 dB) to enhance the R-wave element of the ECG. A modified voltage doubler circuit generated a negative pulse from the R-wave regardless of the polarity of the ECG signal at the input.

Detection was then achieved using the comparator output of a peak detector circuit. This allows the detection threshold to change with the amplitude of the R-wave. The time constant of the peak detector storage capacitor was of the order of the R-R interval to allow the system to respond to rapid changes in R-wave amplitude. Conversely, the time constant was still sufficiently long to prevent the threshold from falling to the noise level

between R-waves. The pulse width of the detected R-wave was extended to 200ms by a monostable to avoid multiple counts in the timing circuit from one R-wave. Physiological limitations would prevent any further real QRS complexes from occurring during this period.

Using the machine

The small, portable device gave timed instructions to the subject using a series of LED lights on the back of the machine, thus dispensing with the inaccurate, audible prompts to the patient from the investigator. Input commands such as selection of the desired test, and the command to start acquisition of the data and output the results were selected via the front panel controls.

2.5.3 Testing the device

Accuracy of R-R interval measurements

To assess the accuracy of the ANT device fourteen electronically simulated heart rates using the Laederal Heartstim were fed into the machine whilst simultaneously using an ECG chart recorder to allow manual measurement of the heart rate. The simulated heart rates varied from 48-126 beats per minute and the deviation of the ANT measurements from the true values was analysed for a total of 155 beats.

The Valsalva ratio, inspiration/expiration ratio and heart rate response to standing were then assessed using the automated method and simultaneously by ECG chart recording in

25 subjects (10 control subjects and 15 IDDM patients). The ANT unit was connected to two wrist electrodes whilst the chart ECG was connected to three chest electrodes.

2.5.4 Results

For the R-R interval data collected by chart recordings from the simulated heart rates, the upper and lower 95% confidence was ± 19ms, equivalent to ± 0.5 division of the ECG chart paper with a chart speed of 25mm per second and 1mm divisions. The corresponding confidence limits for the ANT device were ± 2ms consistent with the temporal precision of the device.

For the twenty five subjects studied similar results for the accuracy of the ANT to detect variations in R-R interval were found for each of the three tests. The agreement for the ANT and chart recordings during the inspiration/expiration test was greater than for the Valsalva manoeuvre or the standing test. The coefficient of variation between the two sets of data for the inspiration/expiration test was 0.5% compared to 1.9% for the Valsalva manoeuvre and 1.4% for the standing test. The measurements were also compared using the method of Bland and Altman (Bland et al 1986) to describe the differences in the ratios derived from the two methods. For the inspiration/expiration test the 95% confidence intervals for the differences in ratio were ± 0.04, for the standing test ± 0.08 and for the Valsalva manoeuvre ± 0.09.

Re-assessment of the data for these tests showed the differences between the two methods of data collection were due to inappropriate selection of maximum and minimum R-R intervals from the chart recordings.

Reliability of R-wave detection

Reliability of R-wave detection by the ANT was assessed by inspecting the heart rate trend for outlying values using a personal computer to view the data. This was done for the three heart rate variability tests on 71 subjects. Each of the 213 data sets contained approximately 85 R-R intervals and of these 12 showed a single outlying data point. Of these 12 points, 3 were due to ectopic beats whilst the other 9 points were due to a low signal to noise ratio; the Valsalva manoeuvre had the most error points predominantly due to muscle noise and movement artifact resulting from the stress of the test but these were all identified by the ANT.

Immunity to noise, ectopic beats and cardiac arrhythmias

The ability of the ANT to detect or reject noise, ectopic beats and cardiac arrhythmia was assessed using an ECG stimulator (Laederal Heartstim). Each rhythm was combined with an electronically induced noise signal of 60 Hz and simulated muscle noise of -7 dB and -13 dB.

The addition of the 60 Hz noise signal to the simulated ECG produced no measurable effect on the ANT performance (this frequency would be rejected by the 6-28 Hz band pass filter). The detection of the R-wave was accurate for all rhythms with R-R intervals up to 1.5 s. At very slow heart rates simulated muscle noise caused an increasing number

of errors, however amongst the 71 subjects who performed the 3 heart rate variability tests, resting heart rates as low as 34 beats per minute were accurately analysed.

2.5.5 Conclusion

The development of this automated device for assessing heart rate variability has dispensed with the laborious interpretation of ECG charts and R-R interval measurement accuracy is improved. There was a good correlation between automated measurements and manual measurements for a wide range of simulated heart rates. There were no significant differences in Valsalva ratio, inspiration/expiration ratio or in the heart rate response to standing recorded with the ANT and from simultaneously recorded chart tracings. Furthermore, maximum and minimum heart rate were more reliably identified from a trend graph displayed on an associated portable computer. In summary, with the development of the Autonomic Neuropathy Tester it is possible to automate the standard autonomic function tests and this machine enables autonomic function to be assessed quickly and simply in a diabetic clinic setting.

CHAPTER 3

SPECTRAL ANALYSIS OF HEART RATE AND BLOOD PRESSURE VARIABILITY

3.1 Introduction

Autonomic neuropathy is a common complication of diabetes mellitus (Ewing et al 1982, Ewing et al 1985, Sampson et al 1990) and is associated with increased mortality (Page et al 1978, Ewing et al 1985, Sampson et al 1990). As has been discussed, evidence for autonomic dysfunction is usually sought by using a battery of non-invasive cardiovascular reflex tests (Ewing et al 1985). In the current study, none of the patients had clinical or historical evidence for cardiovascular autonomic neuropathy based on these 'standard' tests of cardiac autonomic function. It is well recognised that Leicestershire has a low incidence of diabetic complications, possibly due to intensive education/treatment programmes in place for IDDM patients since 1945 (McNally et al 1995). Whilst easy to perform, the standard tests of autonomic neuropathy offer only a crude assessment of autonomic function and among their limitations it should be remembered that 13% of the general population may have one abnormal test (O'Brien et al 1986) probably because they involve active participation from the subject. Moreover, discrimination between nerve damage in the sympathetic and parasympathetic arms of the autonomic nervous system is not always possible (Ewing et al 1985, Pagani et al 1988). Owing to these limitations assessment of heart rate variability using power spectral anlaysis is increasingly used to search for autonomic neuropathy. This technique does not require active participation on behalf of the subject and allows an evaluation of the interaction between parasympathetic and sympathetic arms of the autonomic nervous system.

With this in mind, a randomly selected subset of the study group under went tests of heart rate and systolic blood pressure variability. The aim of this study was to compare these

more sensitive measures of autonomic function with the standard bedside cardiovascular reflex tests.

3.2 Patients and methods

Subjects

Thirty five of the insulin dependent diabetic group aged between 20 to 54 (34.8 ± 1.6 years, mean ± SEM) and their age, sex and systolic blood pressure matched non-diabetic control subjects were studied. They were selected at random from the main study group.

Protocol

Subjects attended the test laboratory at least 2 hours post-prandial and the tests were performed in a quiet room with the temperature controlled between 20-24°C. Due to time these studies were performed separately from the initial assessment of autonomic function. After 10 minutes of supine rest the Finapres cuff was applied to the middle finger of the left hand and surface ECG electrodes fitted to record R-R interval. The arm was supported at the level of the right atrium and subjects were asked not to talk. The Finapres was calibrated and when stable, resting systolic BP and R-R interval were recorded for 15 minutes with the subjects supine. Patients were then asked to stand and the system recalibrated: once stable data were collected for a further 15 minutes. During the recording periods the self-servo mechanism of the Finapres was disabled. The respiratory rate was visually monitored throughout the recording periods and data discarded if the

respiratory rate was less the 15 breaths per minute. Syphmomanometer blood pressures were measured before and after the Finapres recording to compare with the Finapres readings and data discarded if wildly discordant. Data were converted from analogue to digital using an on-line personal computer sampling at 200 Hz. A QRS detection algorithm was employed to mark automatically the R-R intervals (from the surface ECG). Sequences of R-R interval and systolic BP data were interpolated with a third order polynomial and re-sampled at 2 Hz to produce signals with a uniform time base. Three segments of data with 512 samples were used to estimate the power spectra of R-R interval and systolic BP for each patient by means of a fast Fourier transform algorithm after removing linear trends and applying a cosine tapered window. The power spectra were smoothed with a 9-point triangular window producing estimates with 42 degrees of freedom per harmonic. The total powers of R-R interval and systolic blood pressure were computed for the low frequency (0.05-0.15 Hz) and high frequency (0.2-0.35 Hz) spectral bands. The ratio of low frequency power to high frequency power was calculated to give an approximation of sympathovagal balance (Malliani et al 1990, Bernardi et al 1992)

Statistics

In the study all measures were approximately normally distributed as assessed by the Shapiro-Wilk *W* test. Continuous data were expressed as mean ± standard error of the mean (SEM). Between-group comparisons were made using Student's two-tailed unpaired t-test. The determinants of spectral power were assessed using multiple regression analysis with age, systolic blood pressure, HbA_1 and duration of diabetes as the

independent predictor variables. A p value of < 0.05 was regarded as statistically significant.

The coherence function was computed from the cross spectra and used to automatically assess the relationship between systolic blood pressure and heart rate at each harmonic (Robbe et al 1987). Simulated sequences of Gaussian random variates were used to establish the 95% lower confidence limits for the squared coherence (γ^2) using the same procedure adopted for the spectral analysis. Only patients with γ^2 above the lower 95% confidence limit for both the low and high frequency bands were included in the study.

3.3 Results

Reproducibility of spectral parameters

Ten control subjects and two IDDM patients agreed to repeat the tests after a median interval of 3 weeks (range 2-12 weeks). The intra-individual coefficient of variation between the two study days was 21.1% for low frequency power, 19.4% for high frequency power and 13.6% for the low frequency/ high frequency ratio.

Results

The 35 control subjects were closely matched for age and systolic blood pressure with the 35 IDDM patients (Table 3.1) with only the HbA_1 significantly higher in the IDDM group. None of the recordings in either group had to be discarded because of a low respiratory rate. The resting heart rate for the diabetic population was higher than the control group but this difference was not statistically significant (Table 3.1). In keeping

with the main patient set, there were no differences in the standard tests of autonomic function between IDDM patients and control subjects (Table 3.2) nor did either group show a difference from age-specific reference values (O'Brien et al 1986). The 95% lower confidence limit of γ^2, as estimated by simulation, was 0.4. All patients and control subjects had values of γ^2 above 0.5 for both the low frequency and high frequency bands when standing and supine.

There was a significant reduction in total power, low frequency and high frequency powers of R-R interval in the IDDM group compared to the control group in both the supine and standing positions (Fig 3.1 and Table 3.3). In addition, there was a significant increase in low frequency spectral power for systolic blood pressure in the diabetic group (Table 3.3). High frequency systolic blood pressure power also was greater in the diabetic group but the difference did not reach statistical significance (Table 3.3). Differences were also detected between the two groups when data were expressed in normalised units. Before normalisation, the low frequency power of heart rate variability was greater in the control group. However, this difference was reversed when the data was normalised; the IDDM group showing a significant increase in low frequency power compared to the control group (Table 3.3). Furthermore, sympathovagal balance, as assessed from the ratio of low frequency power to high frequency power was increased in the IDDM group compared to controls (Table 3.3). This difference was seen supine and more so when standing. Finally, total power and high frequency power showed a significant negative correlation with duration of diabetes ($r = -0.44$ $p < 0.01$) and glycaemic control ($r = -0.49$ $p<0.005$). Control subjects showed a significant correlation between age and high

frequency power ($r = -0.59$ $p < 0.001$) but this was not seen in the IDDM group. No relations were seen with low frequency power.

3.4 Discussion

This study is unique in showing abnormalities of heart rate variability in a group of IDDM subjects with no clinical evidence of autonomic dysfunction. In addition, the abnormalities detected in blood pressure spectra derived from the spectral studies have not previously been reported in IDDM patients.

Using spectral analysis methods to assess heart rate variability significant abnormalities of cardiovascular neural control were detected in a group of asymptomatic IDDM patients that went undetected using traditional tests of autonomic function. There was a significant reduction in high frequency spectral power of heart rate variability in the IDDM patients which has been suggested to be a marker of parasympathetic dysfunction (Pomeranz et al 1985, Pagani et al 1986, Malliani et al 1991). The ratio of low frequency to high frequency power, which has been postulated as a measure of sympathovagal balance (Pagani et al 1986, Bernardi et 1992) was significantly higher in the IDDM group thus implying a relative sympathetic predominance in these patients. The differences between control and IDDM groups in the spectral measures were observed in both the supine and standing positions. The difference in sympathovagal balance between the groups was greater in the standing position which is in keeping with the well documented increase in sympathetic tone on standing (Malliani et al 1991).

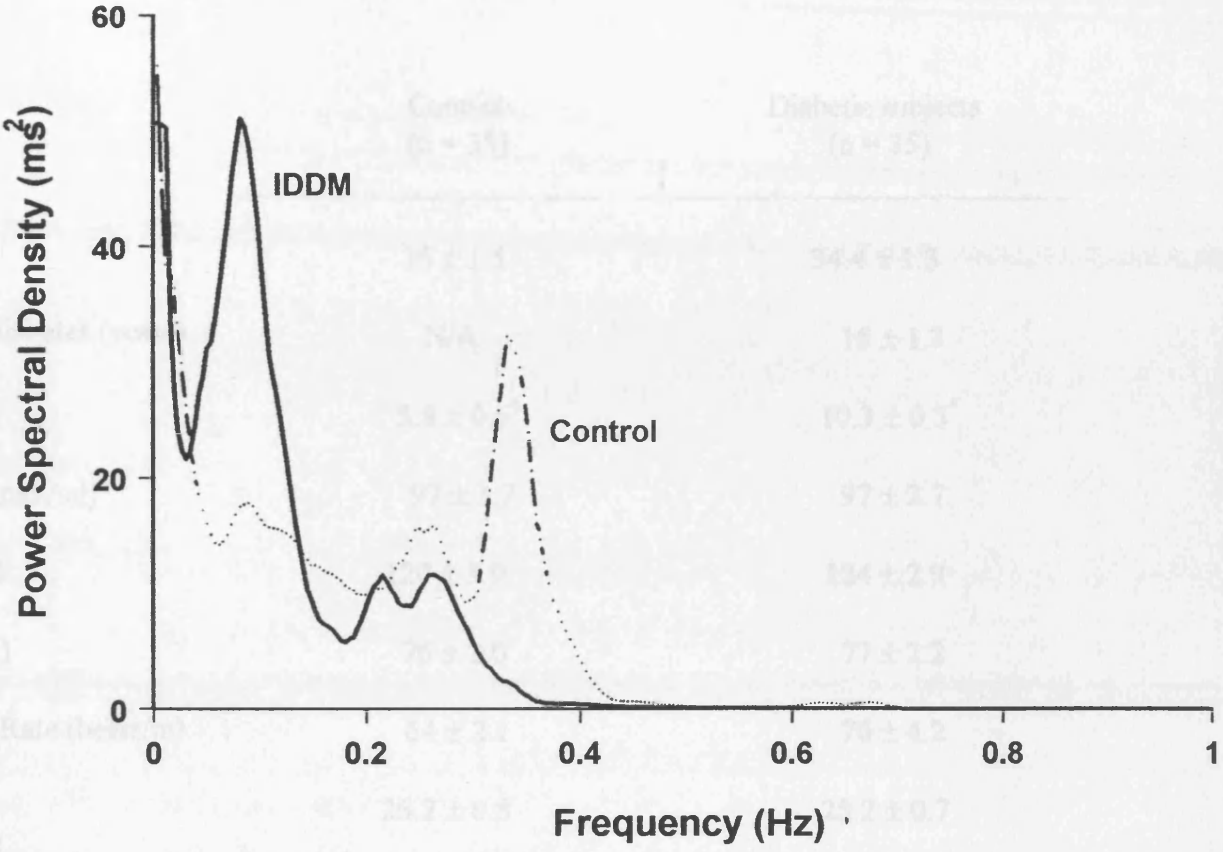

Fig 3.1: Combined power spectral density curves for heart rate variability in the supine position. The IDDM subjects, (black solid line), have a significantly reduced high frequency component (around 0.4 Hz) compared to the control subjects (interrupted blue line).

Table 3.1: Characteristics of diabetic subjects and controls. (Mean ± SEM).

	Controls (n = 35)	Diabetic subjects (n = 35)
Age (years)	35 ± 1.5	34.4 ± 1.3
Duration of diabetes (years)	N/A	15 ± 1.3
HbA_1 (%)	5.8 ± 0.6†	10.3 ± 0.3*
Creatinine (μmol/ml)	97 ± 3.7	97 ± 2.7
SBP (mmHg)	120 ± 3.9	124 ± 2.9
DBP (mmHg)	76 ± 2.0	77 ± 2.2
Resting Heart Rate (beats/m)	64 ± 2.1	70 ± 4.2
BMI (kg/m^2)	26.2 ± 0.8	25.2 ± 0.7
W/H‡	0.86 ± 0.04	0.84 ± 0.03

**Average HbA_1 since diagnosis.*

‡ Waist/Hip ratio

† $p<0.001$

Table 3.2: **Standard clinical tests of autonomic function. (Mean ± SEM)**

	Controls (n = 35)	Diabetic subjects (n = 35)	p
Valsalva ratio	1.75 ± 0.4	1.70 ± 0.09	0.74
E/I* (beats/min)	20.5 ± 0.13	21.1 ± 0.03	0.66
30:15 ratio	1.37 ± 0.03	1.36 ± 0.02	0.8
BP Handgrip (mmHg)	32 ± 0.3	32 ± 0.2	0.72
BP L-S† (mmHg)	5 ± 1.0	4 ± 0.5	0.61

* *Expiration/ Inspiration ratio.*

† *Systolic BP standing minus systolic BP lying.*

P-value refers to the t-test values between control and IDDM subjects.

Table 3.3: Comparison of spectral analysis results of resting, supine and standing pulse interval and systolic blood pressure for the control and IDDM populations.(Mean ± SEM).

SUPINE	Control	Diabetic	p
Power LF (ms^2)*	746.6 ± 77.6 (62 ± 2.3)	473.3 ± 62.8 (79 ± 1.7)	0.002 (0.002)
Power HF (ms^2)†	515.1 ± 67.3 (41 ± 8.1)	126.5 ± 12.3 (20 ± 4.1)	<0.0001 (0.002)
LF/HF‡	2.05 ± 0.76 (2.2 ± 0.53)	4.86 ± 0.67 (5.1 ± 0.55)	0.002 (0.002)
Power LF ($mmHg^2$)**	6.6 ± 0.7	9.3 ± 1.2	< 0.05
Power HF ($mmHg^2$)††	1.1 ± 0.3	1.4 ± 0.22	0.2
STANDING			
Power LF (ms^2)*	600 ± 52.3 (78 ± 2.3)	582.9 ± 36.1 (85.8 ± 1.7)	0.002 (0.002)
Power HF (ms^2)†	159.3 ± 48.5 (19.9 ± 3.1)	77.9 ± 20.2 (15 ± 1.1)	<0.001 (0.004)
LF/HF‡	3.97 ± 0.72 (3.8 ± 0.49)	8.8 ± 2.74 (6.6 ± 0.55)	<0.001 (<0.001)
Power LF ($mmHg^2$)**	9.6 ± 3.91	12.3 ± 2.91	<0.05
Power HF ($mmHg^2$)††	1.22 ± 0.8	1.9 ± 1.1	0.3

Key for Table 3.3.

* Total power of low frequency band (0.05-0.15 Hz). Normalised units given in parentheses.

† Total power of high frequency band (0.2-0.35 Hz). Normalised units given in parentheses.

‡ Ratio of low frequency to high frequency power. Normalised units given in parentheses.

** Power of low frequency band of systolic BP spectra.

†† Power of high frequency band of systolic BP spectra.

P values in parentheses relate to normalised data.

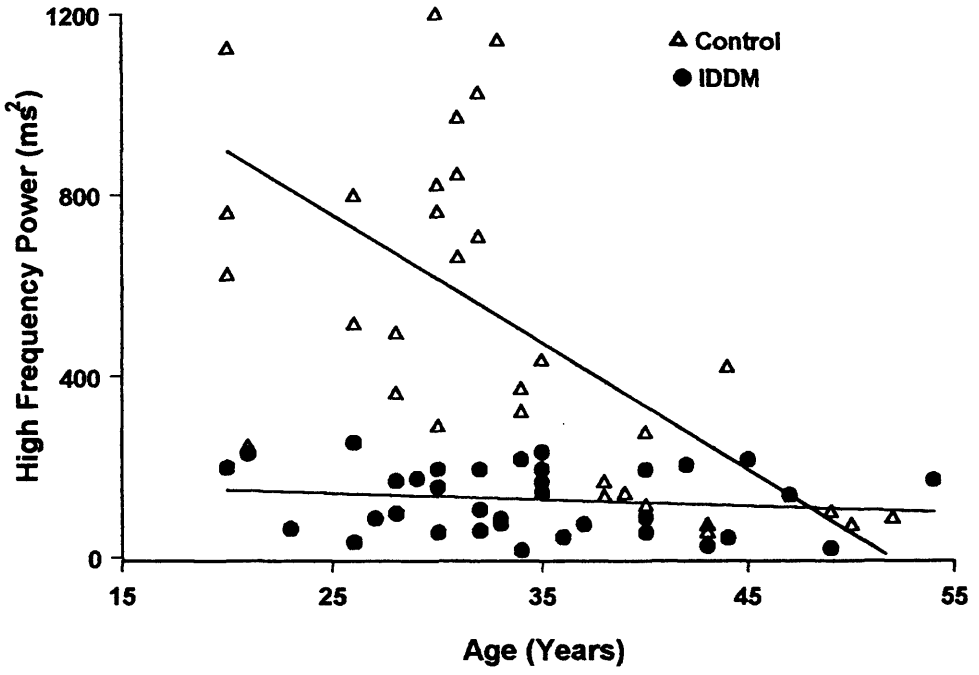

Fig 3.3: Relation between age and high frequency power. r= -0.15 p = 0.38 for IDDM group; r = -0.58 p < 0.01 for control subjects.

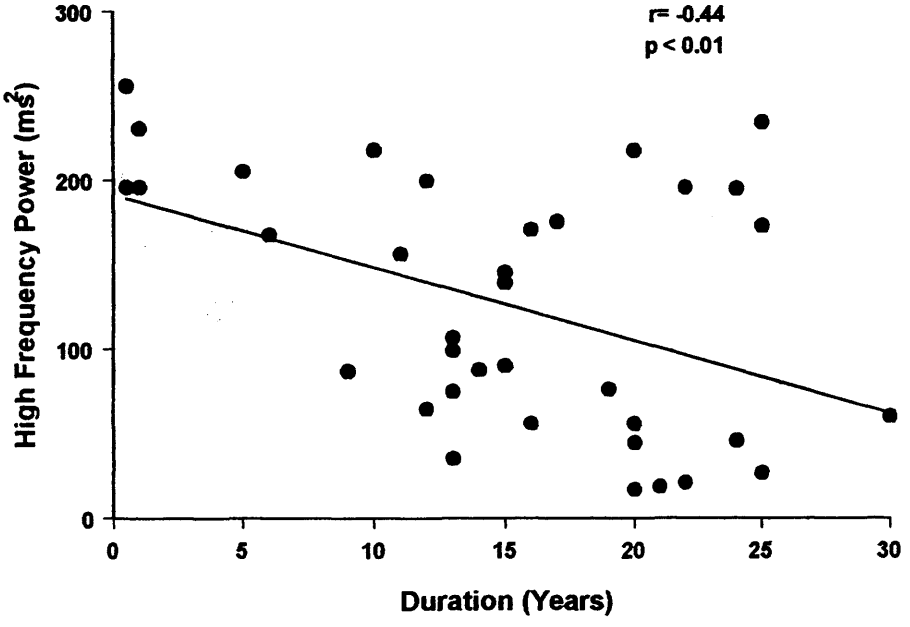

Fig 3.4: Relation between duration of diabetes and high frequency power.

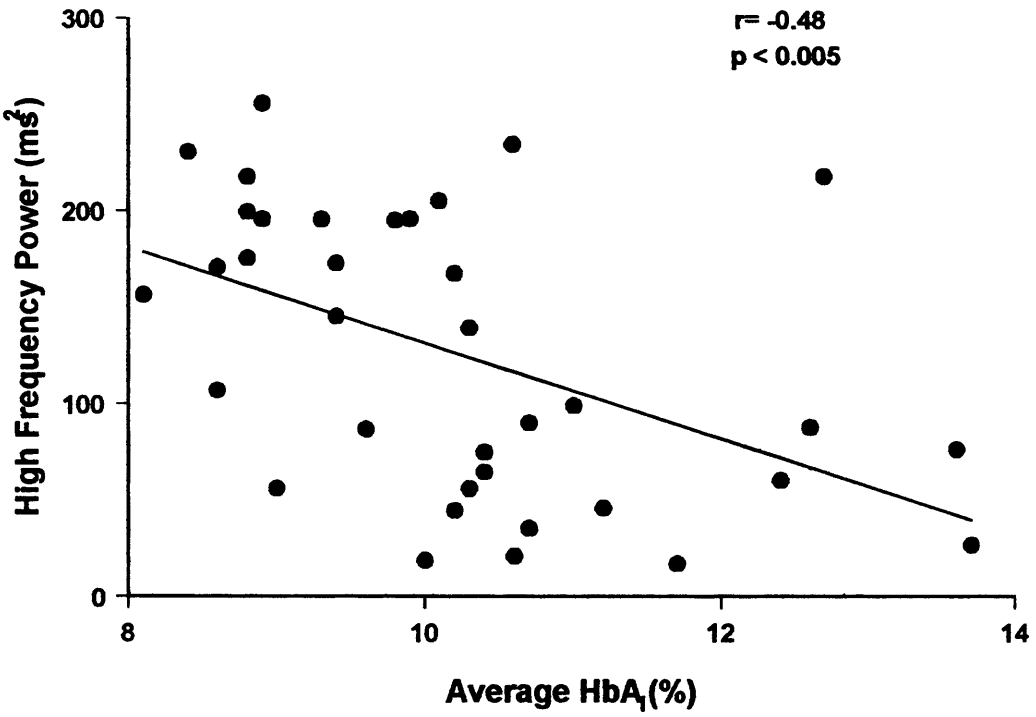

Fig 3.3: Relation between average HbA_1 and high frequency power.

There is continued debate as to the interpretation of the low frequency component of the power spectral density curve of heart rate variability. This component is considered by some to be a marker of sympathetic function (Malliani et al 1991, Bernardi et al 1994) whilst others consider it to comprise both sympathetic and parasympathetic influences (Akselrod et al 1981, Parati et al 1995). Added to this controversy is the finding that in conditions such as congestive cardiac failure where increased sympathetic activity is expected a paradoxical reduction in low frequency power can be seen (Sleight et al 1995). However, during sympathetic activation the resulting tachycardia is associated with a

marked reduction in total power and when the spectral components are expressed in absolute units there are reductions in both low frequency and high frequency powers dependent on the reduction in total power. The use of normalised units to express the powers of the components minimises the effect of total power and the very low frequency component on the values of low frequency and high frequency so allowing the balance between the sympathetic and parasympathetic systems to be studied (Malliani et al 1990). In the present study there was an increase in resting heart rate in the IDDM group (although not statistically significant) and a reduction in total power. Converting the absolute powers to normalised units reveals a reduction in high frequency power and increase in low frequency/high frequency ratio representing a reduction in parasymapthetic activity and a relative sympathetic predominance.

Abnormalities of the power spectra of heart rate variability have been reported previously in diabetic subjects (Lishner et al 1987, Pagani et al 1988, Comi et al 1990, Bellavere et al 1992). In contrast to the present study, in the studies by Lishner, Comi and Bellavere and colleagues the majority of the patients studied had abnormal bedside tests of autonomic function- in particular sinus arrhythmia was lost in the majority of their patients. In addition, the majority of the subjects had evidence of diabetic peripheral neuropathy. Bellavere and co-workers reported that in IDDM subjects with normal bedside tests of autonomic function (i.e. equivalent to the current study group) the power spectra of heart rate variability were not statistically different when compared to non-diabetic control subjects. However, reviewing their data shows a large reduction in total power, low

frequency and high frequency powers in the IDDM group although this did not reach statistical significance ($p < 0.06$). Possible explanations for this lack of statistical power are the small number of patients studied (15 in each group) and the short recording period of only 5 minutes. This increases the difficulty of assuming stationarity of data which is required for spectral analysis (Kay 1981) and short periods of data collection have poor reproducibility (Töyry et al 1995). Pagani and colleagues studied a mixture of IDDM and NIDDM patients and once again a majority of their patients had abnormal bedside tests of autonomic function or other vascular complications of diabetes.

Changes in sympathovagal balance, namely a decrease in the high frequency spectral band and a relative increase in the low frequency band have already been shown in diabetic patients with established autonomic neuropathy (Comi et al 1990, Bernardi et al 1992). Moreover, Bernardi and colleagues demonstrated a lack of the normal circadian pattern of autonomic function; at night there is usually an increase in parasympathetic and a relative reduction in sympathetic activity which was lost in the diabetic patients they studied. In the present study there was an increase in sympathovagal balance, as assessed by the ratio of low frequency/ high frequency power. It is proposed that this was due to a reduction of parasympathetic activity and a relative sympathetic predominance as will discussed in Chapter 5.

Power spectral analysis of systolic blood pressure variability has not previously been documented in IDDM subjects. In the present study, although in both groups the subjects were normotensive on clinic blood pressure recordings, there was an increased variability of systolic blood pressure in the low frequency band in the IDDM patients. It can be

postulated that this increased variability was due to an impairment of arterial baroreflex mechanisms (Siché et al 1993) and this will be discussed more fully in Chapters 4 and 8. The measures of heart rate variability showed significant negative correlations with duration of diabetes and average HbA_1. Previous studies of autonomic function have produced conflicting information about the association of duration of diabetes and autonomic function. A survey in 168 young diabetic patients showed that disease duration was not an important factor in the development of diabetic autonomic neuropathy (Maser et al 1990). However, in that study the standard clinical tests of autonomic function were used to diagnose autonomic neuropathy. Another study has recorded an association between duration and impaired autonomic function in IDDM but not non-insulin dependent diabetes (Masaoka et al 1985). Other studies using beat-to-beat heart rate variation found no such association between duration of diabetes and measures of autonomic function (Ziegler et al 1987). Similarly, in the current study no changes in autonomic function were seen with duration of diabetes using standard tests but total power and high frequency power of heart rate variability showed a significant negative correlation with duration of diabetes. However, even in IDDM subjects of less than five years duration high frequency power was impaired compared to that of age matched non-diabetic control subjects. Abnormalities in autonomic nerve function have been detected in newly diagnosed diabetics using the standard bedside tests (Fraser et al 1977, Ziegler et al 1991). In the study by Fraser and colleagues, patients were a mixed group of insulin requiring and non-insulin dependent diabetic subjects and were older than those in the present study group. In Ziegler's study the definition of insulin dependent diabetes (the

presence of ketonuria at the time of diagnosis) was not adhered to and the 'IDDM' subjects had a mean HbA_{1c} of greater than 11% implying significant hyperglycaemia for at least three months prior to testing. Furthermore, no follow up data was available to show whether these abnormalities progressively declined or, as suggested by Fraser and colleagues (1977) when studying peripheral diabetic neuropathy, revert to normal after approximately one year.

The degree of hyperglycaemia is an important factor in the development of microvascular complications in diabetes. Recently published results from the Diabetes Control and Complications Trial Research Group have shown that hyperglycaemia is an important factor in the development and progression of diabetic peripheral and autonomic neuropathy (Diabetes Control and Complications Trial Research Group 1993, 1995b). Similarly, the current studies found long-standing hyperglycaemia to be significantly associated with impairment of total and high frequency power although no relation was seen between glycaemic control and standard autonomic function tests.

Why were there no differences in the standard tests of autonomic function between the two groups and yet marked differences in power spectral analysis? Controlled respiration, such as that used to assess sinus arrhythmia in the traditional bedside tests, is associated with an increase in parasympathetic activity (Malliani et al 1991). It is possible that at rest, when the respiratory rate was greater that 15 breaths per minute, parasympathetic function was impaired whereas enhancement of parasympathetic tone during deep breathing resulted in a normal inspiration/expiration ratio. Thus, it would appear that subtle, early abnormalities of autonomic function can not be detected using the standard bedside tests

of autonomic function. There are other possible but unlikely explanations for the discrepancies in power spectral analysis between the two groups. Firstly, control subjects were recruited from hospital staff who could possibly have less of an alerting reaction when attending the clinical laboratory. This is unlikely to explain the differences in power spectral analysis found in this study since the IDDM patients were involved in regular research and were used to the laboratory setting and the equipment and techniques used. All of the investigations were performed by the author who had met the patients several times prior to the investigations and often recruited the patients in to the study from the Diabetic Clinic. The control subjects were less involved with clinical research programmes and to assume that working in a hospital equates to a loss of the alerting reaction in unfamiliar surroundings is naive. The non-significant increase in heart rate in the IDDM group is therefore a probable indication of early parasympathetic dysfunction rather than a 'stress response'. Secondly, respiration has a key role in modulating heart rate dynamics and it is possible that differences in respiratory rate could account for differences in heart rate power spectra. During data collection the respiratory rate was closely monitored by visually watching movement of the chest. If the respiratory rate fell below 15 breaths per minute then data was discarded. In reality none of the subjects had a respiratory rate that fell below 15 breaths per minutes and there were no differences in respiratory rate between the two groups. Some workers have found an association between diabetic autonomic neuropathy (defined as abnormal bedside cardiovascular reflex tests) and sleep apnoea syndrome (Guillminault et al 1981). However, other workers in larger and more controlled studies have failed to find any evidence for abnormal breathing patterns in diabetic patients

with autonomic neuropathy (Catterall et al 1984, Spallone et al 1994). The IDDM patients in the current study were young, normotensive and not obese so it is unlikely that there breathing pattern would be different from control subjects and this plays little or no part in the differences in pulse interval power spectra.

In summary, using spectral analysis of heart rate and blood pressure variability, the present study has identified abnormalities of cardiovascular neural control that were not detected by more traditional tests of autonomic function. There was a reduction in high frequency power of R-R variability and this may be indicative of an abnormality of parasympathetic function. There was also an increase in blood pressure variability without a sustained rise in blood pressure. Sympathovagal balance, as assessed by the low frequency/ high frequency ratio was increased amongst the IDDM group. Using these non-invasive methods it may be possible to identify IDDM patients at risk developing overt diabetic autonomic neuropathy prior to the development of symptoms who may warrant further treatment options such as intensification of their insulin regime. In the Diabetes Control and Complications Study intensification of insulin treatment resulted in a 53% reduction in the incidence of diabetic autonomic neuropathy (Diabetes Control and Complications Trial Research Group 1995) even when measured by the crude bedside tests of heart rate variability.

CHAPTER 4

ASSESSMENT OF BARORECEPTOR-CARDIAC REFLEX SENSITIVITY

4.1.1 Introduction

Based on the standard bedside tests of autonomic function, none of the IDDM subjects studied had evidence of autonomic dysfunction. However, a subgroup of these patients were shown to have abnormalities in blood pressure and heart rate variability. Central to the beat-to-beat autoregulation of heart rate and blood pressure are the arterial baroreflex mechanisms. If these reflexes are impaired then this may explain some of the observed abnormalities in heart rate and blood pressure spectra. As has been discussed in Section 1.7.2, traditionally, baroreceptor-cardiac reflex sensitivity is assessed by measuring the changes in R-R interval reflexly produced in response to acute pharmacologically induced changes in blood pressure (Bristow et al 1969, Gribbin et al 1971). Concerns have been expressed about these traditional pharmacological methods of assessing baroreceptor-cardiac reflex sensitivity. Phenylephrine has the theoretical disadvantage of altering the viscoelastic properties of the aorta and the supraphysiological changes in blood pressure induced pharmacologically have raised concerns that they do not represent the function of the baroreceptors in the 'resting' state, thus potentially influencing baroreflex sensitivity (Kircheim et al 1976, Omboni et al 1993). Recently, these difficulties have been overcome by using physiological manoeuvres such as the Valsalva manoeuvre to induce changes in blood pressure. A further method uses sequence and spectral analysis of resting heart rate and systolic blood pressure data to study baroreceptor-cardiac reflex sensitivity. Data can be collected non-invasively using the Finapres device.

The aim of these studies was to see if the observed changes in heart rate and systolic blood pressure variability in the IDDM group could be due to abnormalities in arterial baroreceptor-cardiac reflex sensitivity.

4.2 Assessment of baroreceptor-cardiac reflex sensitivity using the Valsalva manoeuvre.

4.2.1 Patients and methods

Baroreceptor-cardiac reflex sensitivity can be measured from the relation of systolic blood pressure change to R-R interval lengthening during phase 4 of the Valsalva manoeuvre i.e. from the lowest BP after release of the strain to the peak BP observed a few seconds later. (Palmero et al 1981, Smith et al 1987, Ferrer et al 1991).

Subjects

The thirty five insulin dependent diabetic subjects and thirty five control subjects described in the previous section were studied.

Protocol for the Valsalva manoeuvre

Subjects attended the test laboratory at least 2 hours post-prandial and the tests were performed by the author in a quiet room, with the temperature controlled between 20-24°C.

The Finapres cuff was applied to the middle finger of the left hand and the chest leads of a surface ECG was attached to record R-R interval. Subjects were seated with the left arm supported at the level of the right atrium. After at least five minutes of rest (to allow stabilization of the Finapres) all subjects performed the Valsalva manoeuvre- a forced expiration at 40 mmHg for 15 seconds. Mouth pressure was measured with a pressure transducer. After release of the strain data were collected for a further 60 seconds during

which time the subjects were asked not to talk. After a 'trial run' to familiarise the subjects with the equipment and the technique, three Valsalva manoeuvres were performed. The analogue data collected were converted to a digital signal using an on-line personal computer sampling at 200 Hz. A QRS detection algorithm automatically marked the R-R intervals (from the surface ECG) and phase 4 of the Valsalva manoeuvre was identified from the point of release of the strain (Fig 4.1). Baroreceptor-cardiac reflex was determined from the regression of R-R interval on systolic BP including all beats in phase 4 with no lag as described by Palmero and colleagues (Palmero et al 1981).

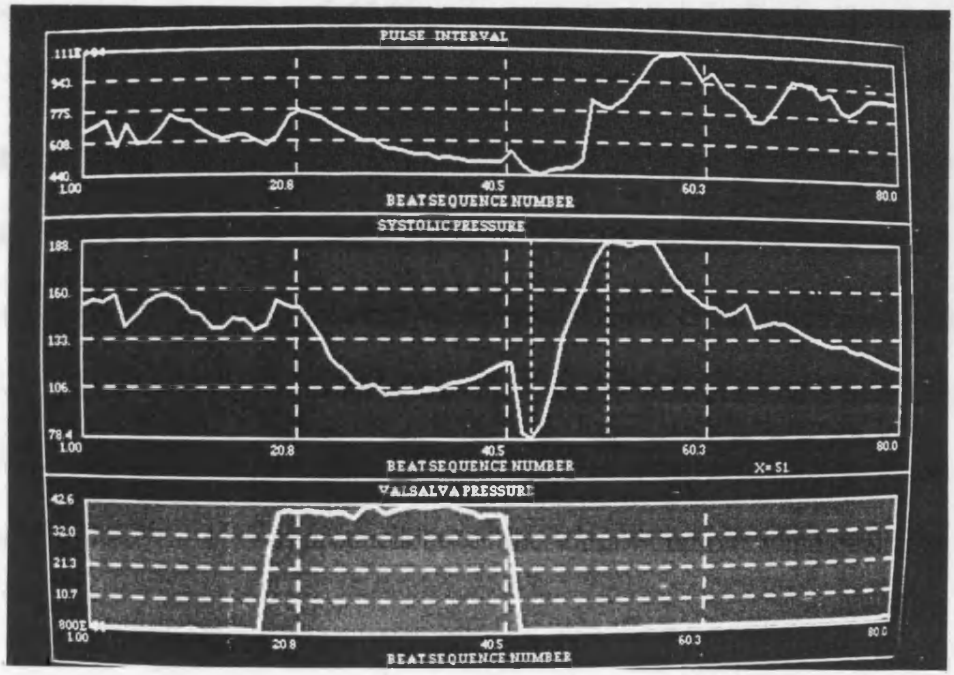

Figure 4.1 - All phases of the Valsalva manoeuvre presented for assessment of baroreceptor-cardiac reflex sensitivity from phase 4 of the Valsalva manoeuvre.

Only regression lines with a correlation coefficient of > 0.8 or significant at $p < 0.05$ were used. The average of the three Valsalva manoeuvres performed by each subject was taken as the baroreceptor-cardiac reflex sensitivity.

In the study all variables were normally distributed (normality was tested using the Shapiro-Wilk *W* test) therefore between-group comparisons were made using Student's two-tailed unpaired t-test. Results are expressed as mean ± standard error of the mean (SEM). The relations between BRS and continuous variables such as age, duration of diabetes and HbA_1 were assessed using Pearson's correlation coefficient and least squares regression analysis. A p value of < 0.05 was regarded as statistically significant.

4.2.2 Results

Reproducibility of BRS.

Ten control subjects and two IDDM patients agreed to repeat the tests after a median interval of 3 weeks (range 2-12 weeks). The intra-individual coefficient of variation between the two study days was 6.4%, which is comparable to the published reproducibility of the Valsalva ratio (Ewing et al 1985.)

Results

The IDDM patients and control subjects' characteristics were summarised in Chapter 3. Briefly, they were closely matched for age and systolic blood pressure with the 35 diabetic patients (Table 3.1). None of the subjects studied had any evidence of autonomic neuropathy as assessed by these standard tests of autonomic function (Table 3.2). Despite comparable Valsalva ratios between control and IDDM groups, baroreceptor-cardiac reflex sensitivity calculated from phase 4 of the Valsalva manoeuvre was reduced

significantly in the IDDM group (3.4 ± 0.3 vs 9.2 ± 0.4 ms/mmHg Fig 1 $p<0.00001$). There was a significant age-related decline in baroreceptor-cardiac reflex sensitivity (Fig 4.2 $r = 0.56$ $p<0.001$) in the control subjects which was not seen in the diabetic group. Furthermore, in the IDDM group baroreceptor-cardiac reflex sensitivity was lower in patients with a longer duration of diabetes (Fig 4.3 $r = 0.6$ $p<0.001$). This relationship of declining autonomic function with increasing duration of diabetes was not observed with the standard clinical tests ($p = 0.8$). There was a significant inverse relation between baroreceptor-cardiac reflex sensitivity and average HbA_1 in the diabetic subjects (Fig 4.4 $r = 0.5$ $p<0.005$) which again was not observed using standard autonomic function tests.

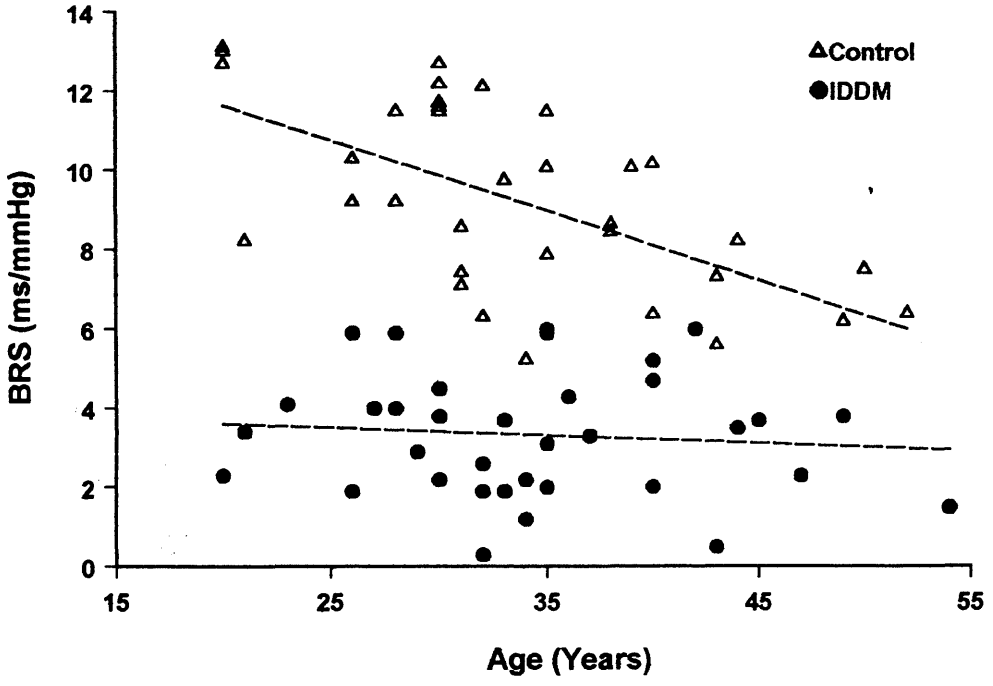

Fig 4.2: Relation between age and baroreceptor-cardiac reflex sensitivity derived from phase 4 of the Valsalva manoeuvre. ($r = -0.56$, $p < 0.001$ for control subjects; $r = -0.1$, $p = 0.5$ for IDDM patients).

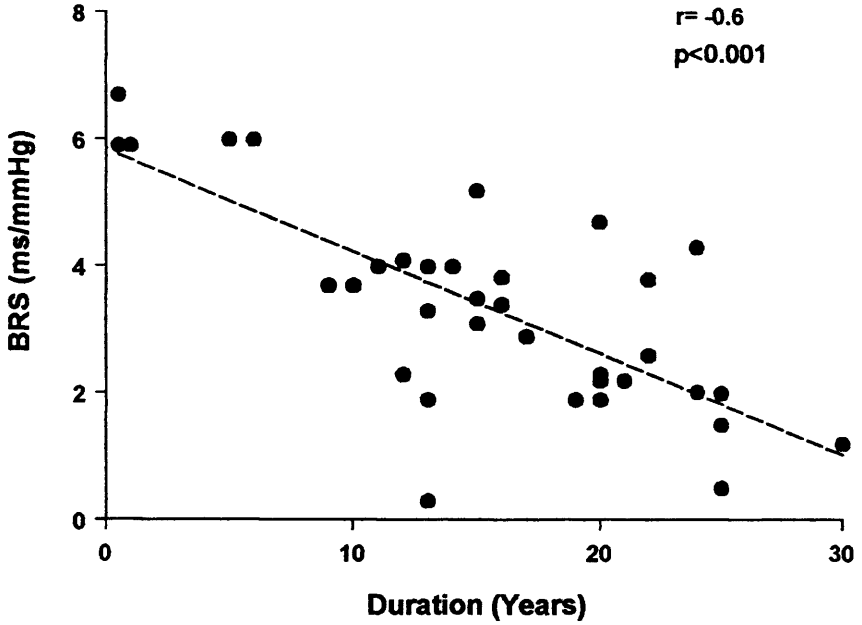

Fig 4.3: Relation between duration of diabetes and baroreceptor-cardiac reflex sensitivity.

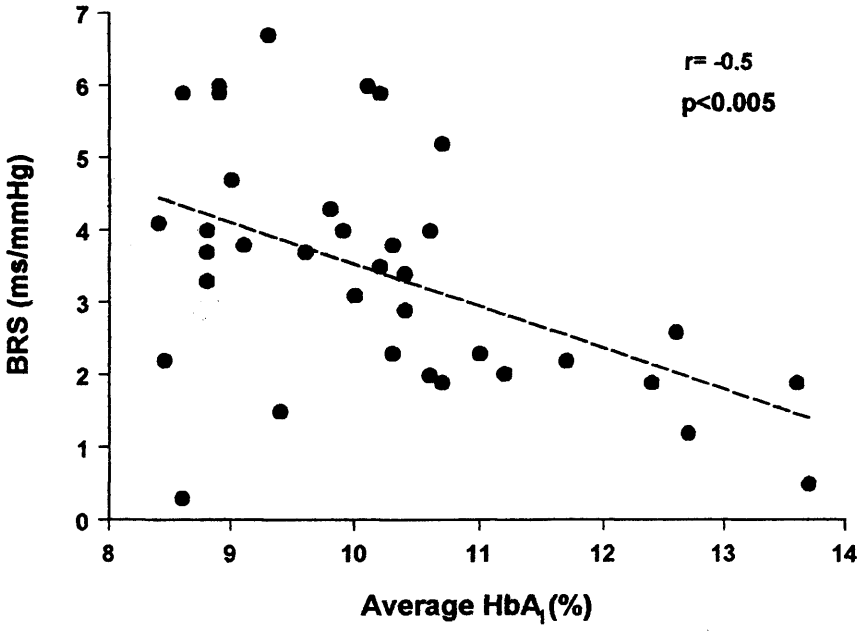

Fig 4.4: Relation between average HbA_1 and baroreceptor-cardiac reflex sensitivity.

4.3 Assessment of baroreceptor-cardiac reflex sensitivity using sequence analysis.

4.3.1 Introduction

Whilst subjects were well tutored in the technique for the Valsalva manoeuvre the possibility still remains that any detected differences in baroreceptor-cardiac reflex sensitivity could be as a consequence of technique rather than an actual impairment in the baroreflex. Utilising the non-invasive properties of the Finapres, alternative approaches to assessing baroreceptor-cardiac reflex sensitivity have been sought. One technique, which has been validated against intra-arterial recordings (Parati et al 1988, Bertinieri et al 1988), involves computer identification of sequences of three or more consecutive beats characterized either by a progressive increase in systolic blood pressure followed by a linearly related lengthening in pulse interval or by a progressive reduction in systolic BP followed by a linearly related shortening of pulse interval. The slope of the regression line between systolic BP and pulse interval changes is taken as an index of baroreceptor-cardiac reflex sensitivity (Parati et al 1988, Bertinieri et al 1988, Steptoe et al 1990). These sequences are virtually eliminated following sinoaortic denervation, indicating they result from baroreflex modulation of the sinus node (Minami et al 1993). Long periods of data (up to 30 minutes) have been recorded with the Finapres servo mechanism deactivated and the observed blood pressures are comparable to those obtained by brachial intra-arterial measurement with an offset (usually around 5mmHg) that remains constant throughout the recording (Parati et al 1989).

Accordingly, baroreceptor-cardiac reflex sensitivity was assessed using sequence analysis of non-invasive continuous systolic blood pressure and pulse interval data recorded supine and standing. The aim of the study was to confirm that the abnormalities in baroreceptor-

cardiac reflex sensitivity detected in Valsalva manoeuvre were not dependent on learned techniques.

4.3.2 Patients and methods

Subjects

The same subset of thirty five insulin dependent diabetic subjects and thirty five control subjects in the previous section were studied.

Protocol

Data were collected on the same visit as the Valsalva manoeuvre data and under the same conditions so subjects attended the test laboratory at least 2 hours post-prandial and the tests were performed in a quiet room with the temperature controlled between 20-24°C. The Finapres cuff was applied to the middle finger of the left hand and a surface ECG fitted to record R-R interval. Subjects were initially supine with the arm supported at the level of the right atrium and patients were asked not to talk. Subjects rested for ten minutes before recording was started. The Finapres was then calibrated and when stable, resting systolic blood pressure and R-R interval recorded for 15 minutes. During this time the self-servo mechanism of the Finapres was disabled. The subjects were then asked to stand, again with the arm supported at the level of the right atrium, and after a five minute resting period, during which time the Finapres was again calibrated, the systolic blood pressure and R-R interval recorded for a further 15 minutes. The respiratory rate was monitored throughout the recording periods and recording stopped if this fell below fifteen breaths per minute. If there was any interruption during the recording period, e.g. telephone ringing or a third party entering the room then recording was also stopped.

Data were converted from analogue to digital using an on-line personal computer sampling at 200 Hz. A QRS detection algorithm was employed to automatically mark the R-R intervals (from the surface ECG). The computer software detected increases and decreases in systolic blood pressure and compared these to changes in R-R interval. Where three or more consecutive systolic blood pressure readings increased by 0.5 mmHg or more pulse interval was automatically compared and if R-R interval lengthened by 6 ms this was counted as an 'up BRS' sequence. Similarly, where systolic blood pressure decreased by 0.5 mmHg or more for three or more consecutive readings and this was associated with a decrease in R-R interval of at least 6 ms this was counted as a 'down BRS' sequence. Baroreceptor-cardiac reflex sensitivity was determined from the regression of R-R interval on systolic BP for all up, down and non-BRS sequences (i.e. where changes in systolic blood pressure were not associated with changes in R-R interval). Only regression lines with a correlation coefficient of > 0.8 or significant at $p > 0.05$ were used and an average baroreceptor-cardiac reflex sensitivity for all of the up and down baroreflex sequences was calculated.

Results are expressed as mean ± standard error of the mean (SEM). Between-group comparisons were made using Student's two-tailed unpaired t-test (after testing for normality using the Shapiro-Wilk *W* test). The relations between BRS and continuous variables such as age, duration of diabetes and HbA_1 were assessed using Pearson's correlation coefficient and least squares regression analysis. A p value of < 0.05 was regarded as statistically significant.

4.3.3 Results

Reproducibility of sequence derived baroreceptor-cardiac reflex sensitivity.

Sequence analysis of baroreceptor-cardiac reflex sensitivity was measured again after a median interval of 3 weeks (range 2-12 weeks) in 12 subjects (10 controls and 2 diabetic patients). The intra-individual coefficient of variation between the two study days was 16.2% for the total baroreceptor-cardiac reflex sensitivity sequences and 16% for the 'up baroreceptor-cardiac reflex sensitivity' sequences, 17.3% for the 'down baroreceptor-cardiac reflex sensitivity' sequences.

Results

Baroreceptor-cardiac reflex sensitivity was again significantly reduced in the IDDM group compared to the control population for both 'up' and 'down' sequences regardless of position (Table 4.1, Fig 4.5). Furthermore, the IDDM subjects had significantly fewer up and down sequences than the control population independent of posture (Table 4.1).

In keeping with the Valsalva manoeuvre data there was a significant age-related decline in the control group baroreceptor-cardiac reflex sensitivity (Fig 4.5: $r = -0.6$, $p < 0.001$ while supine and $r = -0.67$ $p < 0.001$ while standing) which was not seen in the IDDM patients. Furthermore, in the IDDM group baroreceptor-cardiac reflex sensitivity was lower in patients with a longer duration of diabetes when measured supine (Fig 4.6: $r = -0.6$, $p < 0.001$ for up sequences and $r = -0.5$, $p < 0.004$ for down sequences) and standing ($r = -0.5$, $p < 0.001$ for up sequences and $r = -0.52$, $p < 0.001$ for down sequences). There was a significant inverse relation between baroreceptor-cardiac reflex sensitivity and average HbA_1 in the diabetic subjects and this was again seen supine (Fig 4.7: $r = -0.59$, $p < 0.001$ for up and down sequences) and standing ($r = -0.5$ $p < 0.001$ for up sequences and $r = -$

0.58 $p < 0.001$ for down sequences).

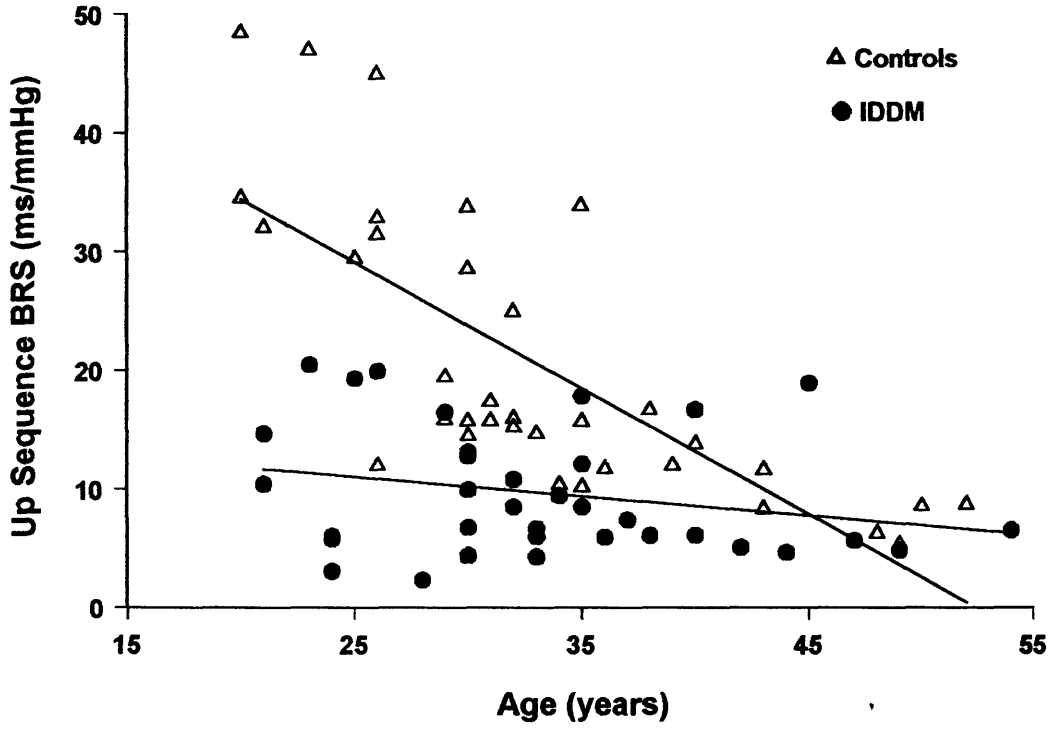

Fig 4.5: Relation between age and baroreceptor-cardiac reflex sensitivity derived from sequence analysis. ($r = -0.51$, $p < 0.001$ for control subjects; $r = -0.23$, $p=0.2$ for IDDM) .

Table 4.1: Comparison of baroreceptor-cardiac reflex sensitivity derived from sequence analysis in insulin dependent diabetic subjects compared to controls (Mean ± SEM).

	Diabetic up sequences	Control up sequences	p	Diabetic down sequences	Control down sequences	p
Supine BRS (ms/mmHg)	10.04 ± 1.1	22.4 ± 1.84	< 0.001	10.75 ± 1.0	23 ± 1.84	<0.001
Standing BRS (ms/mmHg)	3.1 ± 2.2	7.3 ± 2.2	< 0.001	3.4 ± 1.9	8.3 ± 2.5	<0.003
Total number supine	22.4 ± 2.2	54 ± 3.7	< 0.001	30 ± 2.9	50 ± 5.3	<0.001
Total number standing	16.2 ± 3.1	41.5 ± 5.7	< 0.001	28.4 ± 3.2	48.2 ± 7.1	<0.001

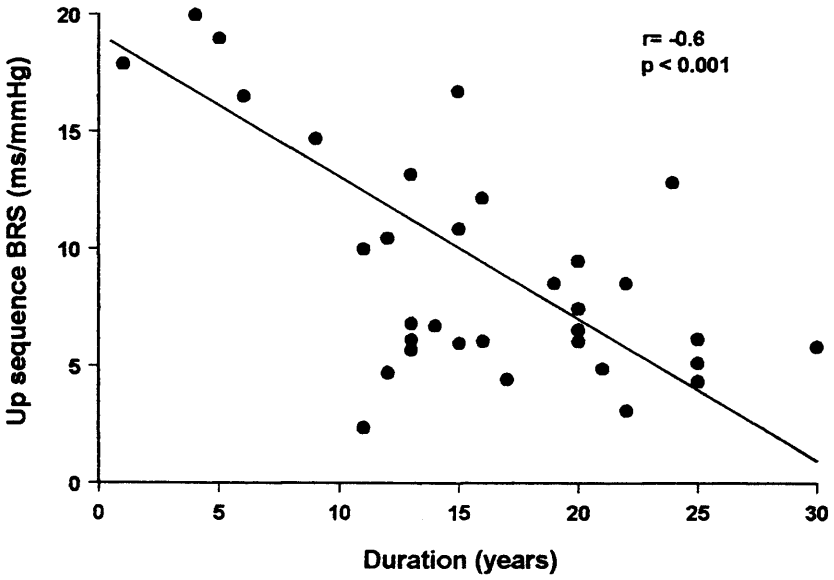

Fig 4.6: Relation between duration of diabetes and baroreceptor-cardiac reflex sensitivity.

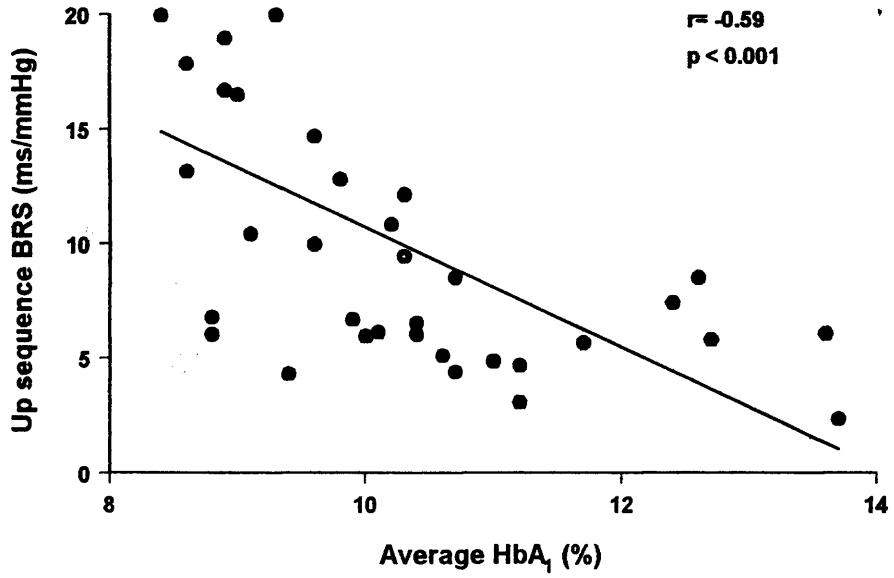

Fig 4.7: Relation between average HbA_1 and sequence derived baroreceptor-cardiac reflex sensitivity

4.4.1 The assessment of baroreceptor-cardiac reflex sensitivity using spectral analysis of pulse interval and systolic blood pressure variability

Introduction

The techniques described in the previous section can be described as open-loop (i.e. stimulus→response) methods of assessing baroreceptor-cardiac reflex sensitivity. Owing to concerns raised about this simplistic way of thinking of baroreflex mechanisms mentioned previously (Chapter 1) workers have attempted to use closed-loop methods to study baroreceptor-cardiac reflex sensitivity. One such technique is derived from spectral analysis data of pulse interval and systolic blood pressure variability. The average gain of the transfer function between variations in R-R interval and systolic blood pressure in both low and high frequency band widths can be used to represent baroreceptor-cardiac reflex sensitivity and is usually described as the α coefficient (Pagani et al 1988, Lucini et al 1994). This is calculated from the square root of the ratio of the spectral components of pulse interval and systolic blood pressure variability.

In the current study baroreceptor-cardiac reflex sensitivity was explored further from the overall gain of the baroreflex represented by the α coefficient. The aim of this study was to examine if differences baroreceptor cardiac reflex sensitivity between control and IDDM subjects calculated from open loop studies were also found in closed loop systems.

4.4.2 Patients and methods

Subjects

Once again the same thirty-five insulin dependent diabetics and the thirty-five control subjects matched for age, sex and systolic blood pressure were studied.

Protocol

The same data collected for the sequence analysis of baroreceptor-cardiac reflex sensitivity was used for the spectral analysis assessment of baroreceptor-cardiac reflex sensitivity. Sequences of R-R interval and systolic BP data were interpolated with a third order polynomal and re-sampled at 2 Hz to produce signals with a uniform time base. Three segments of data with 512 samples each were used to estimate the power spectra of R-R interval and systolic BP for each patients by means of a fast Fourier transform algorithm after removing linear trends and applying a cosine tapered window. The power spectra were smoothed with a 9-point triangular window producing estimates with 42 degrees of freedom per harmonic. The total powers of R-R interval and systolic blood pressure were computed for the low-frequency (0.05-0.15 Hz) and high frequency (0.2-0.35 Hz) spectral bands. The baroreceptor-cardiac reflex sensitivity index α was calculated from the square root of the ratio of the spectral components of pulse interval and systolic blood pressure variabilities. The mean of the low and high frequency power ratios was taken as the α index (Pagani et al 1988, Lucini et al 1994).

Results are expressed as mean $\pm$ standard error of the mean (SEM). Between-group comparisons were made using Student's two-tailed unpaired t-test (after testing for

normality using the Shapiro-Wilk *W* test). The determinants of spectral power and α were assessed using multiple regression analysis with age, systolic blood pressure and duration of diabetes as the independent predictor variables. A p value of < 0.05 was regarded as statistically significant.

The coherence function was computed from the cross spectra and used to assess the relationship between systolic BP and heart rate at each harmonic (Robbe et al 1987). Simulated sequences of Gaussian random variates were used to establish the 95% lower confidence limits for the squared coherence (γ^2) using the same procedure adopted for the spectral analysis. Only patients with γ^2 above the lower 95% confidence limit for both the low and high frequency bands were included in the study.

4.4.3 Results

Reproducibility of α coefficient

The reproducibility of the α coefficient as an index of baroreceptor-cardiac reflex sensitivity was assessed in a similar way to the before. The coefficient of variation for α coefficient was 19.2% standing and 18.1% supine.

Results

In the supine position, the α coefficient of overall baroreflex gain was significantly reduced in the diabetic population compared to controls (20.4 ± 1.9 vs. 9.4 ± 0.92

ms/mmHg $p < 0.0001$). A similar reduction in the α coefficient was again seen in the IDDM group when in the standing position (8.1 ± 2.1 vs. 2.9 ± 1.0 ms/mmHg $p < 0.001$). The ratio of low frequency power to high frequency power gives an approximation of sympathovagal balance and was increased in the IDDM group compared to controls (Table 3.3). As has been seen with other methods of assessing baroreceptor-cardiac reflex sensitivity, there were significant negative correlations between the α index and duration of diabetes (Fig 4.8 $r = -0.5$ $p < 0.005$) as well as overall glycaemic control (Fig 4.9 $r = -0.49$ $p < 0.01$).

4.5 Discussion

These studies are the first to describe impairment of baroreceptor-cardiac reflex sensitivity in a group of IDDM patients with no overt evidence of cardiovascular autonomic neuropathy or other complications of diabetes mellitus. The IDDM subjects studied had reduced heart rate variability as assessed by spectral analysis but no evidence of autonomic dysfunction, using the standard clinical tests. The reduction in heart rate variability described in the preceding Chapter may be due to impairment of arterial baroreflex mechanisms. Thus, using the techniques described in this study it is possible to detect significant abnormalities of cardiovascular neural control that are undetected using traditional clinical tests of autonomic function.

Surveys of autonomic dysfunction in diabetic subjects have, for the most part, depended upon the standard battery of bedside tests including the Valsalva manoeuvre (Smith 1984, Ewing et al 1985). From Chapter 2 there were no differences in the Valsalva ratios

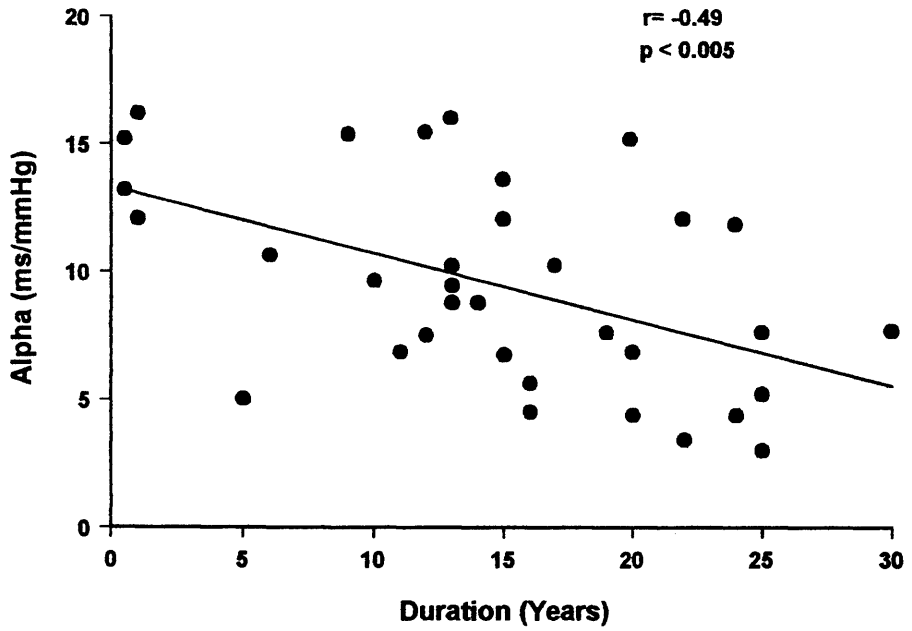

Fig 4.8: Relation between α index (from supine data) and duration of diabetes.

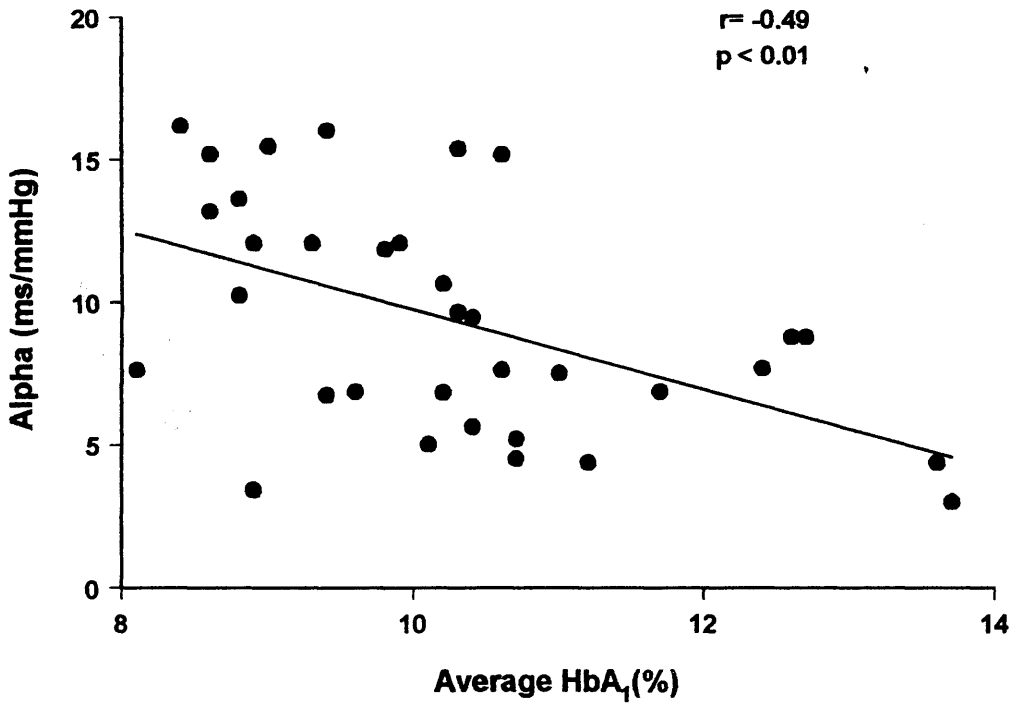

Fig 4.9: Relation between α index (from data recorded supine) and average HbA_1.

between the two groups. The cardiovascular responses to the Valsalva manoeuvre are complex with the rise of blood pressure after release of the strain resulting in stimulation of arterial baroreceptors and a vagally mediated slowing of the heart rate (Palmero et al 1981, Smith et al 1987, Ferrer et al 1991).In the present study, all of the subjects studied had a normal tachycardic response during phase 2 of the manoeuvre suggestive of normal sympathetic function (Bennett et al 1976). The Valsalva ratio simply measures the ratio of maximum to minimum R-R interval irrespective of where they occur during the manoeuvre whilst the computerised techniques employed in the current study allows identification of phase 4 specifically and allows the role of the parasympathetic system to be studied more closely. It is therefore apparent that significant abnormalities of cardiovascular autoregulation can be overlooked using the traditional methods of assessing autonomic function.

Abnormalities of baroreceptor-cardiac reflex sensitivity have been identified previously in diabetic patients (Bennett et al 1976, Ferrer et al 1991) although the suspected site of the lesion in the baroreflex arc has been unclear. Bennett and colleagues studied baroreflex sensitivity using phenylephrine and compared the results with cardiovascular responses during the Valsalva manoeuvre The majority of their study group had symptoms suggestive of severe autonomic neuropathy such as diarrhoea and postural hypotension. This autonomic failure was apparent from the response to the Valsalva manoeuvre: only 5 of the 16 diabetic patients studied had a normal phase 4 of the Valsalva manoeuvre i.e. a blood pressure overshoot and a reflex slowing of the heart rate. The 5 subjects with

normal Valsalva ratios had baroreflex sensitivities comparable to the control group, unlike the patients described in this Chapter. Bennett, however, performed the Valsalva manoeuvre in the supine position which is known to increase parasympathetic activity (See Chapter 5) and affect the reflex response to the Valsalva manoeuvre (Ten Harkel et al 1990). Thus, this enhanced parasympathetic activity may have masked the subtle impairment of vagal function which is possibly responsible for the reduction in baroreceptor-cardiac reflex sensitivity from phase 4 of the Valsalva manoeuvre.

Ferrer and colleagues (1991) used techniques which were similar to those of the present study to examine arterial baroreflexes in diabetic patients. The majority of Ferrer's patients again had severe secondary complications of diabetes mellitus including symptomatic autonomic neuropathy. Moreover, the IDDM patients were hypertensive compared to control subjects- a situation known to reduce baroreflex sensitivity (Smyth et al 1969). The majority of these patients had a reduced tachycardia during phase 2 of the Valsalva manoeuvre and all had a delayed or absent bradycardia during phase 4 of the manoeuvre. It is apparent, therefore, that the patients studied by Ferrer and colleagues had advanced autonomic neuropathy involving both arms of the autonomic nervous system.

In the current study all IDDM subjects demonstrated a normal tachycardic response during phase 2 of the Valsalva manoeuvre and all had a blood significant blood pressure overshoot after release of the strain. It is unclear whether the tachycardic response is due to an increase in sympathetic activity or a withdrawal of parasympathetic tone (Bennett et al 1976). Phase 4 of the Valsalva manoeuvre is regarded as a vagal (parasympathetic) response. The abnormal heart rate variability described in the IDDM group in Chapter 3

was suggestive of a selective reduction in parasympathetic activity and this explains, at least in part, the reduction in baroreceptor-cardiac reflex sensitivity. It is impossible to determine whether this is an impairment of afferent of efferent fibres of the baroreflex arc from these data and workers have emphasised their own opinions (Sharpey-Schafer et al 1960, Bennett et al 1976, 1980). It is difficult to see why there should be selective impairment of afferent or efferent pathways although the preservation of the response to phase 2 of the Valsalva manoeuvre may suggest a selective efferent lesion.

It has been well established that autonomic function declines with increasing age in non-diabetic subjects (O'Brien et al 1986). A similar reduction in baroreceptor-cardiac reflex sensitivity with age has been described previously (Gribben et al 1971) and this was confirmed in the control group of the present study. By contrast baroreceptor-cardiac reflex sensitivity was depressed in all of the diabetic patients with no further decline with increasing age. Presumably, the 'insult' of diabetes to the baroreflex mechanisms was greater than the gradual reduction in baroreceptor-cardiac reflex sensitivity associated with increasing age.

Baroreceptor-cardiac reflex sensitivity has been reported to be lower in the standing compared with the supine position (Pickering et al 1971)and this was confirmed in the current study using sequence analysis and the α index. Both IDDM patients and controls had lower baroreceptor-cardiac reflex sensitivity on standing.

In addition to an overall reduction in baroreceptor-cardiac reflex sensitivity derived from sequence analysis, there were also differences in the number and length of sequences in the IDDM group compared to control subjects. Sequences (both up and down) were

significantly shorter and there was a significant increase in the number of 'non-baroreceptor-cardiac reflex sensitivity' sequences -where changes in systolic blood pressure are not associated with appropriate changes in pulse interval- in the IDDM group. Thus, IDDM impairs the cardiac modulation that baroreflexes exert and this impairment is manifest not only as a reduced sensitivity but a reduced rate of engagement. A similar finding has been found in hypertensive subjects (Parati et al 1988) but has not been studied in diabetic subjects.

The association of duration of diabetes and autonomic function has been investigated in previous studies (Masaoka et al 1985, Ziegler et al 1987, Maser et al 1990). With the current study group, using standard tests, no changes in autonomic function were seen with duration of diabetes but baroreceptor-cardiac reflex sensitivity showed a statistically significant negative correlation with duration of diabetes. Although only four of the IDDM subjects had been diagnosed for less than five years, baroreceptor-cardiac reflex sensitivity in this subgroup was not significantly different from matched control subjects.

Abnormalities in autonomic nerve function have been detected in newly diagnosed diabetics using the standard bedside tests (Fraser et al 1977, Ziegler et al 1991). However, Fraser and colleagues compared a mixed group of insulin requiring and non-insulin dependent diabetic subjects and were older than the present study group. In the case of Ziegler's study the definition of insulin dependent diabetes (the presence of ketonuria at the time of diagnosis) was not strictly applied and the 'IDDM' patients had a mean HbA_{1c} of greater than 11% implying significant hyperglycaemia for at least three months prior to testing. Furthermore, there was no follow up information about whether these

abnormalities progressively declined or, as suggested by Fraser and colleagues (1977) when studying peripheral diabetic neuropathy revert to normal after approximately one year.

The degree of hyperglycaemia is an important factor in the development of microvascular complications in diabetes. Recently published results from the Diabetes Control and Complications Trial Research Group have shown that hyperglycaemia is an important factor in the development and progression of diabetic retinopathy, nephropathy and neuropathy (Diabetes Control and Complications Trial Research Group 1993. Diabetes Control and Complications Trial Research Group 1995). Similarly, the current studies found long-standing hyperglycaemia to be significantly associated with impairment of baroreceptor-cardiac reflex sensitivity although no relation was seen between glycaemic control and standard autonomic function tests.

As discussed in Chapter 1, there is continued debate as to the origin of the low frequency component of the power spectra. One hypothesis, championed by De Boer (1987) is the suggestion that oscillations around 0.1 Hz are a pure baroreceptor phenomenon. Whilst this hypothesis has been supported by others (Bernardi et al 1994, Sleight et al 1994) there are also those who believe that this is an oversimplification (Lucini et al 1994, Pagani et al 1995). It seems to this author that exclusively attributing a cardiovascular rhythm to any single neural circuit is an oversimplification of a complex interaction of mechanisms.

All of the three methods employed to assess baroreceptor-cardiac reflex sensitivity in this study showed reduced values in the IDDM patients. The results from the sequence analysis and α index yielded comparable results however the Valsalva manoeuvre derived

value was significantly lower than for the other two methods. This apparent anomaly also was observed in the control group. Others have demonstrated that sequence analysis derived baroreceptor-cardiac reflex sensitivity produces comparable to those derived from spectral analysis methods (Hughson et al 1993) but the Valsalva manoeuvre was not compared. If the first early beats of the Valsalva manoeuvre are excluded from the analysis of baroreceptor-cardiac reflex sensitivity then the results obtained are comparable to those of the phenylephrine method (Palmero et al 1982). However, examining the whole of phase 4, as used in the current study, gives a different baroreflex sensitivity to that from the phenylephrine method (Goldstein et al 1982). Early in phase 4 of the Valsalva manoeuvre other reflex systems such as the cardiopulmonary baroreceptors are activated so isolation of the arterial baroreceptors is more difficult (Smith et al 1986). Sequence and spectral derived baroreceptor-cardiac reflex sensitivity have been shown to be comparable to the phenylephrine 'gold standard' (Parlow et al 1995).

In summary, these studies are the first to describe impairment of baroreceptor-cardiac reflex sensitivity in a group of IDDM patients with no overt evidence of cardiovascular autonomic neuropathy or other complications of diabetes mellitus. The impairment of baroreceptor-cardiac reflex sensitivity may be due, in part, to an abnormality of parasympathetic activity and showed significant negative correlations with duration of diabetes and glycaemic control.

CHAPTER 5

EFFECTS OF ATROPINE ON HEART RATE VARIABILITY AND BARORECEPTOR-CARDIAC REFLEX SENSITIVITY

A discussion of the possible mechanisms for defective cardiovascular autoregulation

5.1 Introduction

Spectral analysis of heart rate variability has shown a significant reduction in high frequency power and an increase in sympathovagal balance in the IDDM group. It is tempting to assume that this implies a reduction in parasympathetic activity and thus a sympathetic predominance. However, at rest the individual contributions of the parasympathetic and sympathetic arms of the autonomic nervous system in cardiovascular autoregulation are difficult to assess. The techniques employed in these studies give only indirect information about the sympathetic component of the autonomic nervous system and its role in the baroreflex. Indirect evidence for cardiac sympathetic activity has come from muscle sympathetic nerve studies although correlation of this data with cardiac sympathetic activity is difficult (Fagius 1982, Hoffman et al 1993). More recently MIBG scans have been used to study organs, such as the heart, that are rich in sympathetic innervation. There is some evidence that MIBG scans are abnormal early in the natural history of IDDM (Mantysaaria et al 1993) suggesting an early sympathetic abnormality but the clinical significance of these findings is unclear. In addition, the use of a radioactive isotope precludes repetitive scanning to see if the abnormality progresses.

Cardiac vagal and sympathetic activity constantly interact whilst at rest and the relative contributions of the parasympathetic and sympathetic arms of the cardiac autonomic nervous system can be assessed in atropinised patients. Pomeranz and colleagues (1985) administered atropine and propranolol to healthy, normotensive volunteers and showed that atropine practically abolished the high frequency band of the power spectral density curve of heart rate variability. There was a similar reduction in the low frequency power whilst supine however this was much less dramatic when subjects

were standing. It seems likely, therefore, that whether in the supine or standing position the high frequency band is mediated almost entirely by the parasympathetic system with little or no sympathetic component. In the standing position, the low frequency band has a major contribution from the sympathetic nervous system although there is a smaller contribution from the parasympathetic system. Thus, the relative contributions of the sympathetic and parasympathetic arms of the autonomic system may be studied in standing, atropinised patients.

The aims of this study are to examine the effects of atropine on the parameters of cardiovascular autoregulation already described and to attempt to clarify the site of the defect in the baroreflex.

5.2 Subjects and methods

Subjects

Of the thirty-five IDDM patients and thirty-five control subjects who underwent assessment of heart rate and blood pressure variability, ten IDDM patients and six age sex and systolic blood pressure matched control subjects agreed to undergo further studies.

Methods

Further investigations were performed a median interval of six months (range 3-12 months) from the original study. All subjects gave written informed consent after full discussion with the author and having read a patient information sheet. The study was approved by the Ethical Committee of the Leicester Royal Infirmary.

An intravenous cannula was inserted into a right forearm vein and the Finapres fitted to the middle finger of the left hand. A surface ECG was fitted to record R-R interval. Once the Finapres recording was stable, all subjects then had fifteen minute blocks of supine and standing heart rate and blood pressure data recorded as described previously. The subjects then performed three Valsalva manoeuvres in the sitting position. From these data power spectral density curves for heart rate and systolic blood pressure variability were constructed. Baroreceptor-cardiac reflex sensitivity was calculated using sequence analysis of resting pulse interval and blood pressure data as well as from phase 4 of the Valsalva manoeuvre. In addition the α coefficient was calculated from the spectral analysis data as described in Chapter 4.

Atropine at a dose of 0.03 mg/kg was then infused intravenously over 5 minutes. After the infusion the Finapres was re-calibrated and once more data collected, as described above.

Statistics

Results are presented as mean ± SEM. The significance of changes was assessed with paired t-tests, unpaired t-tests or analysis of variance as appropriate. All data were normally distributed as assessed with the Shapiro-Wilk *W* test. A p value of < 0.05 was regarded as statistically significant.

The coherence function was used to assess the relationship between systolic BP and heart rate at each harmonic (Robbe et al 1987). Simulated sequences of Gaussian random variates were used to establish the 95% lower confidence limits for the squared coherence (γ^2) using the same procedure adopted for the spectral analysis (See Chapter 3). Only patients with γ^2 above the lower 95% confidence limit for both the

low and high frequency bands were included in the study. Using these criteria no patients were excluded from the study.

5.3 Results

The baseline characteristics for the two groups are presented in Table 5.1. They were closely matched for age and systolic blood pressure and there were no differences in the standard tests of autonomic function. Irrespective of the technique used, the IDDM group showed a statistically significant reduction in baroreceptor-cardiac reflex sensitivity before infusion of atropine (Table 5.2). In addition the total variance of heart rate variability and the individual low and high frequency powers of heart rate variability were significantly reduced in the IDDM group (Table 5.3). These findings mimic those from the larger studies reported in Chapters 3 and 4.

Irrespective of the analytical technique used, atropine significantly reduced baroreceptor-cardiac reflex sensitivity in both the control and IDDM groups (Table 5.2).

The 95% lower confidence limit of γ^2, as estimated by simulation, was 0.4. All IDDM and control subjects had values of γ^2 above 0.5 for both the low frequency and high frequency bands. γ^2 was reduced after atropine but values were still greater than 0.4. In the control subjects total variance of heart rate variability was significantly reduced after atropine. In addition, individual high and low frequency powers significantly reduced by atropine when subjects were supine (Table 5.3). These effects were less dramatic when subjects were standing, especially in the low frequency band (Table 5.3). IDDM subjects again showed a reduction in total power and a significant reduction in high and low frequency powers after atropine. However, on standing there

was a less marked response to atropine on the low and high frequency powers compared to control subjects (Table 5.4).

5.4 Discussion

The principal findings of this study were as follows: 1) a reduction in total variance of heart rate variability after atropine in both groups more evident in the control subjects. 2) A reduction in low and high frequency powers in both groups, when supine, after atropine. 3) On standing there were less dramatic effects of atropine on the low frequency band-widths in both groups more marked in the IDDM group (standing significantly reduced the high frequency power in the control group before atropine). 4) Baroreceptor-cardiac reflex sensitivity was universally reduced by atropine in control subjects. 5) No significant difference in baroreceptor-cardiac reflex sensitivity was seen in IDDM subjects standing after atropine.

Pomeranz and colleagues (1985) have described a similar reduction in total variance and high frequency power of heart rate variability after atropine in a healthy group of volunteer. They found a reduction in both low and high frequency power after atropine in the supine position although atropine had less effect on the low frequency power in the standing position. These findings are in keeping with the control group of the present study. In the Pomeranz study, increasing the pharmacological autonomic blockade with propranolol had a dramatic effect on the low frequency power whilst standing. This led them to conclude that in the supine position both the high and low frequency bands are mediated by parasympathetic activity.

Table 5.1: Characteristics and standard autonomic function tests of cases and controls. (Mean ± SEM)

	Controls (n = 6)	Diabetic subjects (n = 10)
Age (years)	29.2 ± 1.7	27.9 ± 2.5
Duration of diabetes (years)	N/A	10.3 ± 1.2
HbA_1 (%)	4.9 ± 0.5†	10.2 ± 0.2*
SBP (mmHg)	115 ± 2.7	110 ± 4.1
DBP (mmHg)	70 ± 1.9	72 ± 1.3
Valsalva ratio	1.77 ± 1.3	1.74 ± 1.9
Expiration/Inspiration	23.4 ± 1	20.9 ± 1.1
30:15 ratio	1.39 ± 0.2	1.39 ± 0.09
Handgrip BP (mmHg)	37 ± 1.2	39 ± 1.5
BP L-S**	2 ± 1.2	3 ± 0.6

**Average HbA_1 since diagnosis.*

*** Systolic blood pressure standing minus lying.*

Table 5.2: Baroreceptor-cardiac reflex sensitivity (ms/mmHg) before and after atropine. (Mean ± SEM).

	Controls	IDDM	p
SUPINE			
Valsalva manoeuvre	10.2 ± 1.0	4.5 ± 0.8	< 0.001
Up Sequences	23.2 ± 1.8	14.9 ± 1.9	< 0.01
Down Sequences	24.1 ± 2.3	11.7 ± 1.1	< 0.001
α index	19.6 ± 1.8	7.9 ± 1.1	< 0.002
STANDING			
Up Sequences	8.2 ± 2.2	3.2 ± 1.9	< 0.005
Down Sequences	9.1 ± 2.7	4.4 ± 2.9	< 0.01
α index	7.8 ± 3.5	3.5 ± 2.0	< 0.01
AFTER ATROPINE			
SUPINE			
Valsalva manoeuvre	0.3 ± 1.0	1.0 ± 0.5	0.09
Up Sequences	0.7 ± 1.7	1.2 ± 0.9	0.1
Down Sequences	1.1 ± 1.8	1.2 ± 1.0	0.1
α index	0.8 ± 0.7	0.6 ± 1.1	0.4
STANDING			
Up Sequences	0.4 ± 1.2	0.8 ± 0.5	0.07
Down Sequences	0.5 ± 1.5	0.3 ± 0.5	0.2
α index	0.9 ± 1.0	1.0 ± 0.7	0.6

Table 5.3: Comparison of spectral analysis results of resting, supine and standing pulse interval and systolic blood pressure for the control and IDDM populations before and after atropine. (Mean ± SEM). (Key as for Table 3.3)

	Control	Diabetic	p
SUPINE			
Power LF (ms^2)*	855.6 ± 54.3 (55 ± 3.9)	499.9 ± 77.3 (69 ± 3.5)	0.001 (0.05)
Power HF (ms^2)†	459.3 ± 79.3 (39 ± 5.1)	129.1 ± 12.1 (18 ± 7.1)	<0.001 (0.002)
LF/HF‡	2.34 ± 0.80 (2.3 ± 0.6)	4.2 ± 0.7 (4.1 ± 0.5)	0.001 (0.001)
STANDING			
Power LF (ms^2)*	734 ± 72.3 (68 ± 4.5)	680.4 ± 36.1 (95.8 ± 3.7)	0.02 (0.001)
Power HF (ms^2)†	167.1± 32.5 (15.2± 2.1)	89.1 ± 32.0 (11 ± 2.1)	<0.001 (<0.01)
LF/HF‡	4.5 ± 0.5 (4.1 ± 0.7)	9.1 ± 1.64 (8.6 ± 0.15)	<0.001 (<0.001)
AFTER ATROPINE			
SUPINE			
Power LF (ms^2)*	137.5 ± 27.1 (59 ± 1.3)	179.8 ± 12.47 (75 ± 0.9)	0.02 (0.02)
Power HF (ms^2)†	36.1 ± 16 (20 ± 4.1)	27.1 ± 6.3 (23 ± 1.1)	<0.04 (0.1)
LF/HF‡	1.4 ± 0.26 (2.2 ± 0.53)	2.76 ± 0.57 (3.1 ± 0.55)	0.02 (0.06)
STANDING			
Power LF (ms^2)*	110.1 ± 12.3 (78 ± 2.3)	271.2 ± 18.2 (85.8 ± 1.7)	0.002 (0.002)
Power HF (ms^2)†	10.3 ± 9.2 (19.9 ± 3.1)	25.1 ± 3.2 (15 ± 1.1)	<0.001 (0.004)
LF/HF‡	11. 7 ± 0.72 (3.8 ± 0.49)	15.6 ± 1.4 (6.6 ± 0.55)	<0.001 (<0.001)

Table 5.4: Percentage change in low and high frequency spectral powers after the administration of atropine. *P* values refer to the difference in percentage change between the two groups.

	Control	Diabetic	p
SUPINE			
Power LF (ms^2)	-86 ± 3.2	-65 ± 2.4	0.001
Power HF (ms^2)	-93 ± 4.2	-80 ± 2.1	0.001
STANDING			
Power LF (ms^2)	-85 ± 2.6	-60 ± 2.9	<0.001
Power HF (ms^2)	-96 ± 1.9	-72 ± 2.1	<0.001

On standing there is a strong sympathetic influence on the low frequency fluctuations. The current study confirms the findings of Pomeranz and colleagues in the control group and similar although less dramatic effects were seen in the IDDM group.

Little data is available in the literature as to the effects of atropine on baroreceptor-cardiac reflex sensitivity. There are, however, a number of studies which have examined the effects of β-adrenoreceptor blockade on baroreceptor-cardiac reflex sensitivity, although the results are conflicting. Floras and colleagues (1988) found that chronic β-blockade decreased the baroreflex gain assessed by the phenylephrine ramp technique. Conversely, other workers have found that chronic β-blockade increased overall baroreflex gain assessed non-invasively by measuring beat-to-beat changes in systolic blood pressure and pulse interval and calculating the α index (Lucini et al 1995). Invasive measurements decrease baroreflex gain which may be one source for discrepancy between these studies. Invasive techniques are naturally associated with a variable level of anxiety and stress and this has been shown to independently reduce the gain of the baroreflex (Pagani et al 1991).

Studies of β-adrenoreceptor blockade and those looking at the effect of standing on baroreceptor-cardiac reflex sensitivity suggest there is a negative correlation between changing levels of sympathetic drive and the gain of the baroreflex mechanisms. Thus, with β-blockade and when standing there is an increase in sympathetic drive to the sino-atrial node and a reduction in baroreceptor-cardiac reflex sensitivity. No similar studies are available to study the effects of muscarinic receptor blockade on baroreceptor-cardiac reflex sensitivity.

Sympathetic outflow to the sino-atrial node increases from very low levels on rising from supine to standing and therefore 'pure' sympathetic modulation can be studied in

standing, atropinised patients. In the present study, the IDDM patients and control subjects showed similar changes in spectral parameters and baroreceptor-cardiac reflex sensitivity after atropine when supine although changes were less in the IDDM group. On standing the IDDM group showed a less marked effect of atropine on heart rate variability and in baroreceptor-cardiac reflex sensitivity compared to the control subjects. This suggests that in this situation there was a marked impairment of residual parasympathetic activity and hence a smaller change following muscarinic blockade. Therefore, it is possible to speculate that the reduction in baroreceptor-cardiac reflex sensitivity seen in the IDDM group results from, at least in part, an abnormality of parasympathetic activity. This view is supported by the resting low frequency/high frequency ratio observed on spectral analysis indicating the IDDM subjects have a sympathetic predominance. By contrast, the less sensitive tests of bedside autonomic function described in Chapter 2 failed to identify an abnormality of parasympathetic activity. Controlled respiration such as that used to assess sinus arrhythmia has been shown to increase parasympathetic function (Malliani et al 1991) so it is possible that at rest, when the respiratory rate is at least 15 breaths/minute, the resting parasympathetic activity was impaired whereas parasympathetic tone was enhanced during deep breathing resulting in a normal inspiration/expiration ratio. The difference in spectral powers after atropine confirms that, in the present study, some residual parasympathetic activity was present in the IDDM group.

Although there is evidence of reduced parasympathetic activity, the possiblity remains that impairment of the baroreflex reflects afferent dysfunction of the baroreceptor itself. Baroreceptors are found at the junction of the medial and adventitial layers of the arterial wall and loss of baroreceptor sensitivity may reflect either involvement of

vasa vasorum by early microangiopathy, or an early loss of large artery compliance which has been demonstrated in IDDM (Lehman et al 1993). Baroreceptors are modulated by other factors such as noradrenaline and vasoactive substances released by the endothelium (Chapleau et al 1991). Early endothelial dysfunction has been demonstrated in small arteries from IDDM patients with no obvious microvascular disease, although background diabetic retinopathy was present in the majority of patients in this study (McNally et al 1994). One of the IDDM subjects described in Chapter 4 had significant impairment of baroreceptor-cardiac reflex sensitivity (Valsalva manoeuvre derived BRS 0.5 ms/mmHg versus 9.4 ms/mmHg for age, sex matched control) but had normal endothelial function as assessed by resistance artery response to vasoactive substances (Lawrence et al 1997).

It follows that any pathophysiological approach to explain the impaired baroreceptor-cardiac reflex sensitivity would need to be in keeping with the observation that a significant impairment of baroreceptor-cardiac reflex sensitivity can be found in IDDM subjects studied within a few months of diagnosis (Chapter 4). Lehmann and colleagues have found an initial *increase* in aortic compliance in the first 5 years of diagnosis (Lehmann et al 1993)- but one would imagine that this would increase rather than reduce arterial wall stretch with increases in blood pressure. Furthermore, a recent study looked at several sites throughout the arterial tree and failed to find evidence of reduced arterial compliance in a group of uncomplicated IDDM patients (Kool et al 1995). Theoretically, the differences found between IDDM and control populations in the markers of cardiovascular regulation could be due to vessel wall abnormalities of the digital arteries in IDDM patients. This would result in inaccurate readings from the Finapres- (for discussion see Chapter 1). Studies in non-diabetic subjects comparing

Finapres data with simultaneous intra-arterial recordings and have demonstrated good correlations although with an off-set that remains reasonably constant (Parati et al 1989). Furthermore, calculations of the spectral bands from data recorded with both systems are comparable (Parati et al 1989, Omboni et al 1993). Unfortunately, no comparisons for the Finapres and intra-arterial recordings are available for IDDM patients but in the current study there was close correlation between syphgmomanometer blood pressure readings and the Finapres. In addition, the assessment of heart rate variability was performed using data derived from the surface ECG not from the Finapres.

Afferent cardiovascular information, including that originating from the baroreceptors is processed in the nucleus tractus solitarius in the dorsal medulla of the brain stem. Efferent information eminates from the same region (Chalmers et al 1994). There is increasing evidence that the central nervous system and not only the peripheral nervous system is damaged by the metabolic changes associated with diabetes mellitus. However, although changes in the peripheral and autonomic nervous systems have been studied extensively, the difficulty of investigating changes in the central nervous system means that the question as to whether and to what extent a functional impairment of central nervous system function exists remains unanswered. However, both hypoglycaemia and prolonged hyperglycaemia have been shown to be associated with changes in the electroencephalogram (EEG) of diabetic patients (Glaser 1976). More recently, brain stem evoked potentials have shown significant differences between IDDM patients and control subjects (Donald et al 1984). Moreover, there was a good correlation between peripheral nerve velocity and brain stem potentials although this relationship is questioned by other workers (Fedele et al 1984). Similarly,

Dejgaard and colleagues (1991) have shown that the abnormality in brain stem evoked potentials is increased in patients with longer duration of diabetes- the so called diabetic encephalopathy. Interestingly, in view of the findings in baroreceptor-cardiac reflex sensitivity in IDDM of short duration reported in Chapter 4, brain stem evoked potentials in IDDM patients of less than one year duration were also abnormal although, only in 5% of cases. Other workers have reported a positive correlation with glycaemic control, as assessed by HbA_{1c}, and brain stem evoked potentials (Pozzessere et al 1988). These observations suggest that anatomical/functional damage to the brain stem structures does exist in IDDM patients and is dependent on duration of diabetes and glycaemic control. Furthermore, these electrophysiological anomalies are in keeping with the anatomical and pathological findings in diabetic subjects (Reske-Nielsen et al 1965). The abnormalities of brain stem function could, in theory, result in impaired autonomic function and in reduced baroreceptor-cardiac reflex sensitivity. Structural and pharmacological blockade of the brain stem in animal models has resulted in a reduction in the arterial baroreceptor reflex and an increase in blood pressure (Doba et al 1973, Blessing 1989) although it is unclear if these abnormalities persist and cause sustained hypertension or if the baroreflex resets. Far less is known about man because of logistic difficulties. Patients with chronic complete high cervical cord lesions have intact efferent vagal and sympathetic neurone to the sino-atrial node but the spinal sympathetic neurons are deprived, however, of baroreflex supraspinal inhibitory inputs. Thus in these patients the supraspinal mechanisms involved in baroreceptor-cardiac reflex sensitivity and heart rate variability can be studied. One study in these patients reported a total loss of the low frequency component of the

heart rate power spectrum (Inoue et al 1990). However, subsequent research has contradicted this and has reported the presence of a low frequency component in some tetraplegic patients although presented conflicting arguments about the origin of this oscillation (Koh et al 1994, Guzzeti et al 1994). Whilst little work is available on the role of brain stem function and its regulation of blood pressure and heart rate variability in IDDM patients there is convincing evidence of structural change. These changes may well be responsible for part of the impairment of baroreceptor-cardiac reflex sensitivity observed in the current study.

In summary, atropine reduced the total variance of heart rate variability in IDDM and control subjects. Whilst supine both low frequency and high frequency powers were reduced equally, however, on standing the control subjects showed a selective reduction in high frequency power. By contrast, atropine had less effect on the spectral powers when the IDDM group were standing. Thus, it can be concluded that, in part, the reduction in baroreceptor-cardiac reflex sensitivity seen in the IDDM group results from an impairment of parasympathetic nerve function and and/or a relative sympathetic predominance although other mechanisms also may be involved.

CHAPTER 6

QT INTERVAL, QT DISPERSION AND CARDIOVASCULAR AUTOREGULATION IN IDDM

6.1 Introduction

There is mounting evidence to suggest an important role for the autonomic nervous system in cardiac repolarization (Ahnve et al 1983, Higham et al 1994).Since it is likely that the abnormalities of heart rate variability and baroreceptor-cardiac reflex sensitivity found in the IDDM group are attributable, in part, to a reduction in parasymapthetic activity then this may be reflected in abnormalities of ventricular repolarisation. Such abnormalities of cardiac repolarisation may be expressed as lengthening or increased dispersion of the QT interval. Although controversial (see Section 1.4) prolongation of the QT interval has been documented in diabetic patients with severe autonomic dysfunction (Bellavere et al 1988, Veglio et al 1995). Moreover, other workers have proposed a close linear relationship between QT interval and worsening autonomic function in IDDM patients (Ewing et al 1991).

It is now recognised that QT intervals across the surface ECG are not of uniform length and the interlead variability of QT intervals, the QT dispersion, may provide more detailed information about cardiac repolarisation. Also, it has been suggested that dispersion of the QT interval can be used as a measure of arrhythmic risk in a number of settings (Higham et al 1994). Little, and conflicting, information is available about QT dispersion in IDDM patients and, furthermore, the role of the autonomic nervous system in QT dispersion unclear.

The aim of the current study was to measure the association between sympathovagal balance and baroreceptor-cardiac reflex sensitivity with QT intervals and QT dispersion in

insulin dependent diabetic patients with evidence of defective cardiovascular autoregulation but with normal bedside tests of autonomic function.

6.2 Patients and Methods.

Subjects and Methods

All of the 35 IDDM subjects who underwent assessment of heart rate variability were studied. All patients had a 12 lead simultaneous ECG (Hewlett Packard Pagewriter) recorded at rest. Each ECG contained at least 10 leads in which a QT interval could be accurately measured. A single observer, blinded to all other data, analysed all ECG recordings.

Electrocardiograms were scanned by a flatbed scanner interfaced with a personal computer. Each image was then cut and copied into 12 files, corresponding to the 12 leads of the electrocardiogram. Specially designed software skeletonised and joined each image. Each image was magnified on the computer monitor for optimal QT interval measurement using a 'mouse'. This method has been validated elsewhere (Bhullar et al 1993) and QT interval measurements were made using standard criteria. Leads were excluded from QT dispersion analysis if the T wave, or its terminal portion, were too flat to determine accurately the T wave end; or if a U wave so interfered with a T wave end that the end of the T wave could not be accurately identified. Maximum and mean QT intervals were rate corrected using Bazett's formula ($QT/\sqrt[2]{R\text{-}R}$) to give QT_c. QT dispersion is the difference

between the longest and shortest non-rate corrected QT interval across the surface electrocardiogram. Of the 35 patients, 4 of them had ECG recordings that were of poor quality and scanning was not possible. They were, therefore, excluded from the study.

Statistics

Results are expressed as mean ± standard deviation (mean ± SD). After confirming normality of data with Shapiro-Wilks' *W* test data were compared by Pearson's correlation coefficient and least squares regression analysis. The determinants of QTc and QT dispersion were assessed using multiple regression analysis with age, systolic blood pressure, duration of diabetes and baroreceptor-cardiac reflex sensitivity as the independent predictor variables. A p value of < 0.05 was regarded as statistically significant.

6.3 Results

Mean and standard deviation of QTc intervals and QT dispersion are presented in Table 6.1. Table 6.2 shows the correlation between measures of cardiac autonomic function and mean QTc intervals. None of the subjects had prolonged QTc (> 440ms).

There were significant negative correlations between mean QTc intervals and sequence derived baroreceptor-cardiac reflex sensitivity whether measured standing or supine (Fig 6.1). Furthermore the α coefficient derived from spectral studies (Fig 6.2) and the ratio of low frequency to high frequency power showed significant negative correlations with mean QTc. There were no associations between either maximum QTc intervals or QT

dispersion and all measures of autonomic function. The relations between QTc and baroreceptor-cardiac reflex sensitivity were independent of age, sex, systolic blood pressure and duration of diabetes.

6.4 Discussion

This study suggests a link between global measurement of cardiac repolarisation, as expressed by prolonged mean QTc intervals, and measures of cardiovascular autoregulation in a group of IDDM subjects with normal standard clinical tests of autonomic function. By contrast, dispersion of cardiac repolarisation, suggested as an important mechanism in the development of cardiac arrhythmias, does not appear to be associated with the tests of autonomic function studied.

There is considerable evidence that the QT interval on the surface ECG is to some extent determined by the autonomic nervous system (Rosen et al 1992). Manipulation of the autonomic nervous system by procedures such as left stellate ganglionectomy results in QT interval prolongation (Abildskov 1991). Moreover a direct vagal activity on the QT interval has also been demonstrated (Ahnve et al 1982). More specifically, QT interval prolongation has been demonstrated in diabetic patients with clinical autonomic neuropathy, and it is suggested that this has a possible role in sudden cardiac death in these patients (Bellavere et al 1988, Ewing et al 1990, Veglio et al 1995). Conversely, one study has suggested that diabetic neuropathy is associated with a decrease in QTc intervals (Ong et al 1993).

Table 6.1: Measures of autonomic function, QTc and QT dispersion (Mean ± SD).

Autonomic Function	
VBRS* (ms/mmHg)	3.2 ± 1.4
Supine sequence BRS† (ms/mmHg)	12.1 ± 3.7
Standing sequence BRS† (ms/mmHg)	5.2 ± 2.6
LF Power‡ (ms^2)	582.9 ± 36.1
HF Power** (ms^2)	77.9 ± 20.2
LF/HF††	8.8 ± 2.7
Alpha‡‡ (ms/mmHg)	5.3 ± 2.1
QT measurements	
Mean QTc (ms)	406 ± 22
Maximum QTc (ms)	428 ± 22
QT dispersion (ms)	44 ± 13

* Valsalva derived baroreceptor-cardiac reflex sensitivity

† Sequence analysis derived baroreceptor-cardiac reflex sensitivity

‡ Total power of low frequency band (0.05-0.15 Hz) when supine

** Total power of high frequency band (0.2-0.35 Hz) when supine

†† Ratio of low frequency to high frequency power.

‡‡ Alpha values. Mean baroreceptor-cardiac reflex sensitivity for supine low and high frequency

Table 6.2: Correlation of mean QTc intervals with autonomic function measurements

	r	p
VBRS* (ms/mmHg)	-0.02	0.9
Supine BRS† (ms/mmHg)	-0.4	0.009
StandingBRS† (ms/mmHg)	-0.36	0.05
LF Power‡ (ms^2)	-0.42	0.06
HF Power** (ms^2)	-0.42	0.09
LF/HF††	-0.4	<0.01
Alpha‡‡ (ms/mmHg)	-0.42	0.008

* Valsalva derived baroreceptor-cardiac reflex sensitivity.

† Sequence analysis derived baroreceptor-cardiac reflex sensitivity.

‡ Total power of supine low frequency band (0.05-0.15 Hz).

** Total power of supine high frequency band (0.2-0.35 Hz).

†† Ratio of low frequency to high frequency power.

‡‡ Mean baroreceptor-cardiac reflex sensitivity for low and high frequency bands when supine.

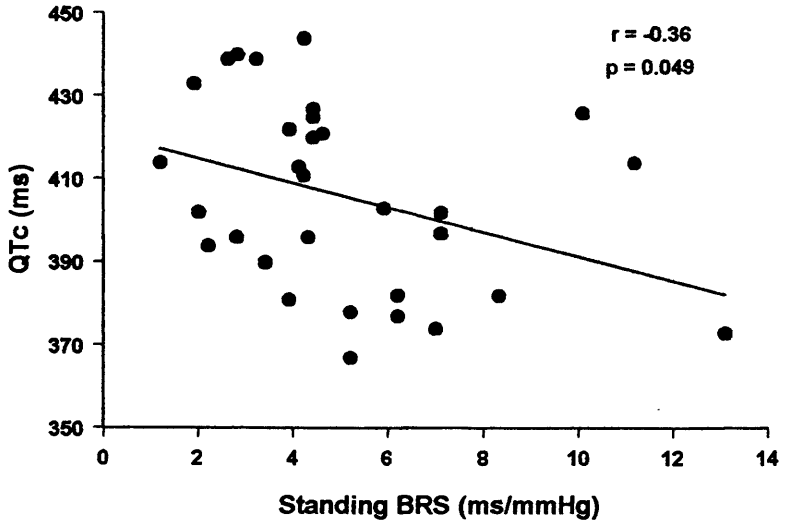

Fig 6.1: Relation between the standing, sequence derived baroreceptor-cardiac reflex sensitivity and QTc.

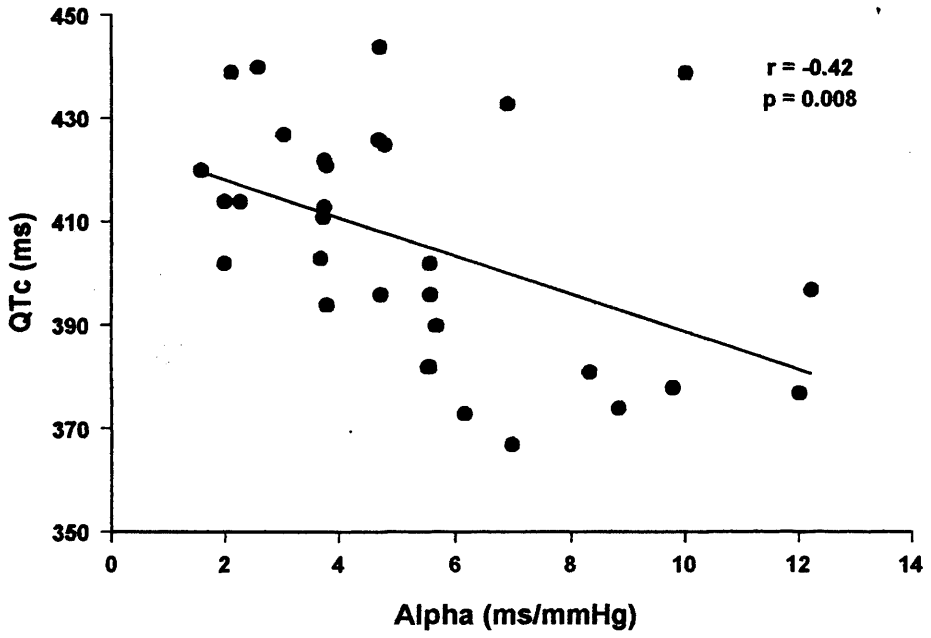

Fig 6.2: Relation between overall α index and QTc.

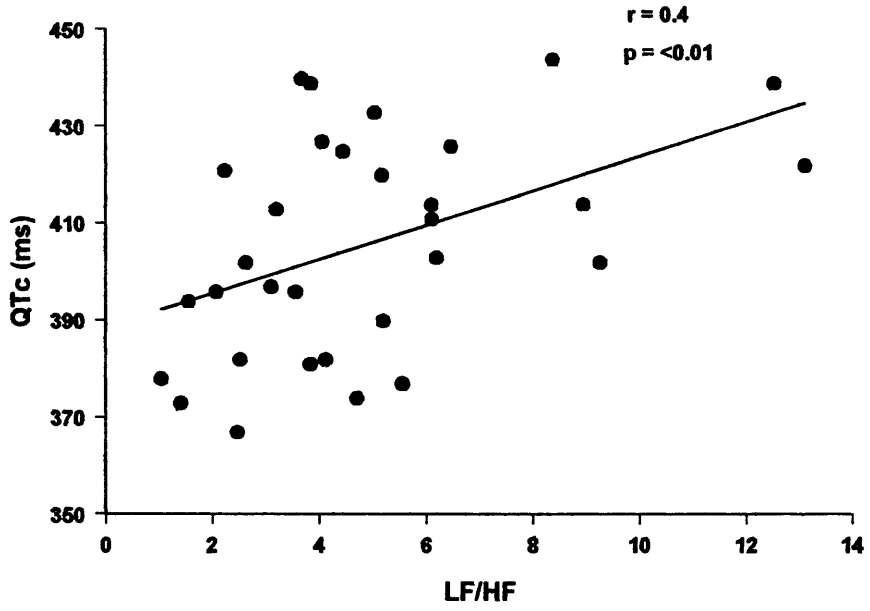

Fig 6.3: Relation between sympathovagal balance, as assessed by the low frequency/high frequency ratio (LF/HF) from spectral analysis data of heart rate variability and QTc.

This study, however, only looked at a small number of diabetic subjects who were a mixture of NIDDM and IDDM patients. In addition, there was a difference in the heart rates between the diabetic and the control groups studied which may have lead to overcorrection of QT intervals by Bazett's formula.

The data presented in the current study are in agreement with the majority of evidence that abnormalities of autonomic function in diabetic patients are reflected in global prolongation of cardiac repolarisation and potentially prolonged QT intervals. The upper limit of 'normal' QTc duration is a matter of debate because normal ranges for QTc length

have never been produced. A value of 0.44s has been accepted as the upper limit of normal for QTc since 1978 (Schwartz et al 1978) and this has recently been validated from a large follow-up study in normal subjects (Shouten et al 1991). This study reported that patients with QTc greater than 0.44s had an increased risk of death. In the current study, None of the subjects of the present study had prolonged QTc by this definition but there was a linear relationship with increasing sympathovagal balance and lengthening of QTc. A linear relationship between autonomic function and QTc has been reported previously in diabetic subjects with progressively increasing scores for the standard tests of autonomic function (Ewing et al 1991) but has not previously been reported in diabetic subjects with normal bedside autonomic function tests.

The literature contains little information about the role of the autonomic nervous system in dispersion of the QT interval. One recent study has described QT dispersion in patients with primary autonomic failure, showed that while QT and QTc intervals were prolonged in the patients with primary autonomic failure, QT dispersion was no different between the patients studied and controls (Lo et al 1996). By contrast, in a study of 13 patients with diabetic nephropathy it has been suggested that severe autonomic neuropathy is associated with increased QT dispersion (Kirvela et al 1994). However, these patients also had a high incidence of coronary artery disease, and other cardiac problems, which could have been responsible for the observed increase in QT dispersion. Another study by Wei and colleagues (1995) of diabetic patients with abnormal bedside tests of autonomic function, showed QT dispersion was increased compared to a smaller group of non-diabetic subjects. However, the patients studied by Wei and colleagues were older than the present

study group (mean age 56 years old) and this increases the likelihood of silent myocardial ischaemia affecting QT dispersion. The present study suggests that the autonomic nervous system is not important in determining dispersion of cardiac repolarisation, and therefore in QT dispersion measurement.

The patients in the present study all had clinically normal autonomic function, therefore it seems likely that the more subtle abnormalities of autonomic function demonstrated in the apparently healthy diabetic patients might also be expressed in globally prolonged cardiac repolarisation. Thus, it can be postulated that abnormalities of autonomic function are can be reflected in prolongation of the QT interval whereas QT dispersion abnormalities may only develop as a result of myocardial damage.

There was a significant negative correlation between overall baroreceptor gain (α) and QTc as well as baroreceptor-cardiac reflex sensitivity derived from sequence analysis which has been postulated to be due, in part, to be a reflection of autonomic dysfunction and more specifically parasympathetic function. In addition, increasing sympathovagal balance was associated with a linear lengthening of QTc.

There were no significant relations between QT dispersion or QTc and baroreceptor-cardiac reflex sensitivity derived from the Valsalva manoeuvre. However, the Valsalva manoeuvre is difficult to perform and relies on an intact baroreceptor mechanism as well as parasympathetic and sympathetic function. The patients studied were all asymptomatic with no clinical evidence of autonomic dysfunction so that subtle abnormalities of sympathovagal balance may go undetected in such a crude test.

In conclusion these data suggest that abnormal baroreceptor-cardiac reflex sensitivity, due in part to impaired parasympathetic function, may be reflected in the heart by global prolongation of ventricular repolarisation, but not by dispersion of ventricular repolarisation. This may represent an increased risk of developing ventricular arrhythmias and, as will be discussed in Chapter 8, may be associated with an increased risk of sudden death.

CHAPTER 7

CIRCADIAN BLOOD PRESSURE CHANGES AND LEFT VENTRICULAR MASS INDEX IN IDDM

7.1 Introduction

In Chapter 3 it was mentioned that autonomic function has a well recognised diurnal pattern with an increase in sympathetic function during daytime and a parasymapthetic predominance during sleep. Just as there is a diurnal pattern of autonomic function so is there a diurnal pattern of blood pressure variation. Blood pressure levels tend to be higher in the morning than the evening with the lowest level during sleep at night (Millar-Craig et al 1978). Although the basic day-night change is well recognised, there is continuing debate about the physiological mechanisms underlying this rhythm. Recently, studies have been performed to determine the role of the autonomic nervous system in the diurnal variation of blood pressure. The absence of a fall in blood pressure at night has been shown in patients with denervated hearts following cardiac transplantation (Reeves et al 1986). Similarly, in patients with severe non-diabetic autonomic neuropathy blood pressure has been shown to rise at night (Mann et al 1983) and diabetic patients with severe autonomic neuropathy also have a reversal of the circadian pattern of blood pressure change (Hornung et al 1989, Liniger et al 1991, Felici et al 1991).

Blood pressure varies markedly throughout the day so clearly a single blood pressure reading is a poor representation of an individuals 'blood pressure'. For this reason twenty-four hour non-invasive blood pressure monitoring has become widely used in the diagnosis and treatment of hypertension (Mancia et al 1993). It has major advantages over traditional methods of blood pressure measurement, especially in the case of the alerting reaction (Mancia et al 1993) and has a closer association with target organ damage. The absence of a night-time fall in blood pressure has been shown to be associated with

increased left ventricular hypertrophy (Consensus Document 1990, Verdecchia et al 1990) and increased risk of cerebrovascular disease (Kobrin et al 1984). Furthermore, twenty four hour blood pressure monitoring has been shown to be more reproducible than single clinic measurements (James et al 1988).

Previous studies have used a definition of diurnal blood pressure change as a 10% reduction in nighttime systolic or diastolic blood pressure compared to daytime. Subjects have been classified as 'non-dippers' where there is less than a 10% difference between day and night blood pressures (Pickering 1990, Verdecchia et al 1991). Most workers utilise standard time periods of 7.00-22.00 hours for daytime and 22.00-7.00 hours for nighttime (Consensus Document 1990) which are set into the program of the 24-hour blood pressure monitors. Analysis of the data collected from these automated devices is usually performed without reference to the subjects individual sleep pattern. Since sleep is the primary determinant of the nocturnal decline of blood pressure it is obvious that the use of fixed time period for blood pressure data analysis has major disadvantages (Rosansky et al 1995). Because of the problems of analysing twenty four hour blood pressure data using fixed time periods other techniques have been applied including Fourier analysis, square wave fit analysis (Thijs et al 1994) and the use of cumulative sums (cusum) analysis. Of these only the latter technique is time independent (Stanton et al 1992).

This study was designed to assess the diurnal rhythm of blood pressure in the study groups described in Chapter 2 and to compare the day-night differences in systolic blood pressure

with measures of autonomic function. In addition, the association between the diurnal blood pressure change and left ventricular mass index was also examined.

7.2 Subjects and Methods

Subjects

Of the 65 patients and control subjects described in Chapter 2, all underwent 24 hour ambulatory blood pressure recording. This included the subset of 35 patients in whom tests of heart rate and systolic blood pressure variability were performed.

Methods

Twenty-four hour blood pressure monitoring was performed using the SpaceLabs 90207 ambulatory blood pressure monitor (Redmond, Washington, USA). This device has been extensively evaluated and is approved by the British Hypertension Society (O'Brien et al 1991). A cuff of the appropriate size was applied to the non-dominant arm and the monitor was programmed to record every 15 minutes between 7.00-22.00 hours ('daytime') and 30 minutes between 22.00-7.00 hours ('nighttime'). Subjects were asked to document their activities during the period of recording and to note the time at which they retired to bed and arose in the morning. They were also asked to comment on the diary if their sleep was disturbed or, in the case of the IDDM subjects, if they experienced hypoglycaemia, especially during sleep. No limitations were applied to the subjects daily routine The monitors were returned the following day and downloaded into a standard

IBM type personal computer. For inclusion into the study a minimum of 80% of attempted BP readings had to be successful and based on these criteria four recordings were excluded but were successfully repeated one week later.

In addition, IDDM patients underwent transthoracic 2D-echocardiography to assess left ventricular mass index. Measurements were obtained from M-mode echo's derived from 2-D echocardiograms using the Hewlett Packard Sonos 1500 system (Hewlett-Packard Co, Boise, Idaho). The patients were placed in the left lateral decubitus position with the left arm raised supporting the head. Left ventricular mass was calculated using the formula described Devereux et al (1977) and was corrected for body surface area to provide the left ventricular mass index . Three consecutive cycles were taken for measurement and the average of the three used as the left ventricular mass index. The echocardiographic studies were performed by a single trained technician unaware of the results of other parts of the study.

7.2.1 Analysis

Hourly mean systolic and diastolic blood pressures were obtained for the 24 hour period. Subjects were categorised as 'dippers' or 'non-dippers' according to the presence or absence of a 10% fall in blood pressure values based on the fixed time periods.

Cusum analysis was used as a more reliable, time independent method of assessing diurnal variation in blood pressure (Stanton et al 1992, Weston et al 1996). This involves subtracting mean 24 hour systolic blood pressures from the hourly systolic blood pressure readings and the differences are then added. The differences are plotted against time to

give the cusum plots (Fig 7.1). Crest systolic blood pressure is calculated from the slope of the plot during the 6 hours or more when it rises most steeply, the trough systolic blood pressure from the maximum decrease over at least 6 hours. The difference between crest

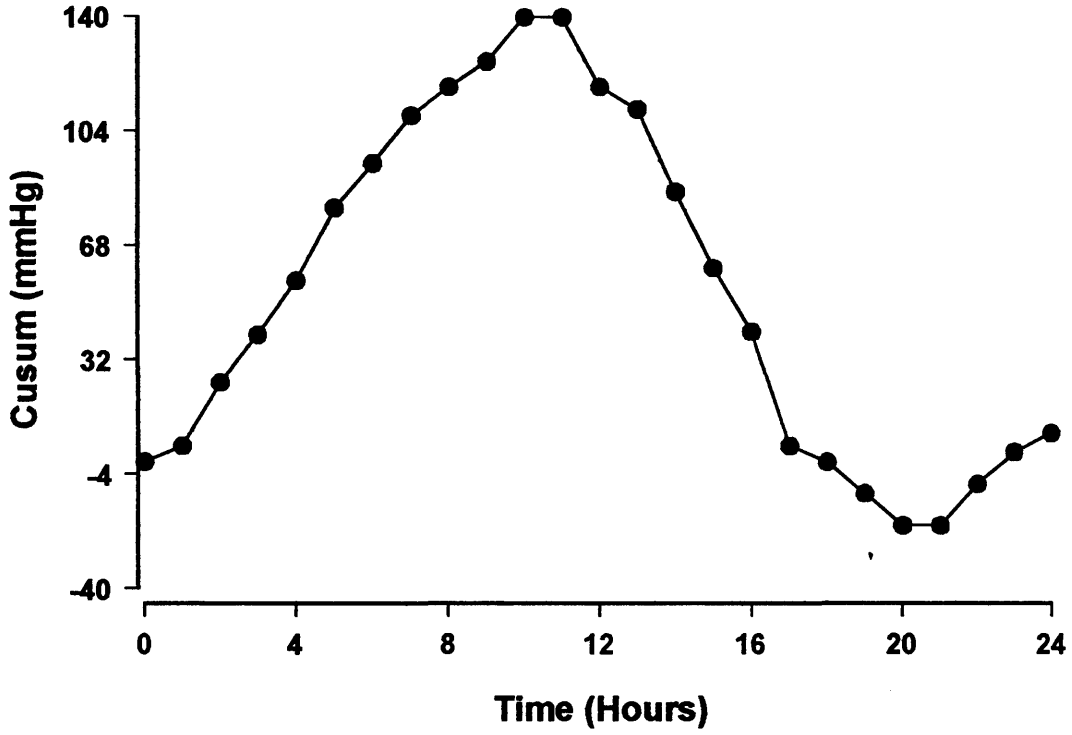

Fig 7.1: A typical example of a Cusum plot taken from a control subject in the study group.

and trough systolic blood pressure is the cusum derived circadian alteration of magnitude (CDCAM)- equivalent to the day/night difference and quantifies the extent of the diurnal blood pressure change. Cusum plot height (CPH) is the amplitude of the entire plot

calculated from the difference between the maximum and minimum values of the plot. This reflects the magnitude and duration of the circadian blood pressure change. The cusum derived statistics are therefore time independent.

All data were shown to be normally distributed by the Shapiro-Wilk *W* test. Results are therefore reported as mean ± SD and differences between groups were assessed by unpaired Student's t-test. The relationship between ambulatory blood pressure and dependent variables (duration of diabetes, age, HbA_1, and markers of autonomic function) were determined by least squares linear regression and multiple regression analysis.

7.3 Results

Blood Pressure

There were no differences between clinic blood pressure readings for the two groups (Table 7.1). All subjects were normotensive with a mean clinic blood pressure on three occasions of < 140/90. All control subjects showed a ≥ 10% change in systolic blood pressure between day and night i.e. were classified as 'dippers'. Only one of the IDDM group failed to show a > 10% fall in systolic blood pressure at night using the fixed time periods as discussed above.

No significant differences were observed between mean 24-hour blood pressure for IDDM patients and control subjects (Table 7.1). Although day time heart rate was higher in the IDDM group these differences were not statistically significant. This was also seen from the heart rate data collected during the assessment of heart rate variability (see Chapter 3).

Mean nocturnal heart rate was, however, higher in the IDDM group than in the control subjects (53 ± 10.2 vs. 62 ± 8.4 beats per minute $p = 0.05$).

There was an increased variability in day time systolic blood pressure in the IDDM group as assessed by the standard deviation (Table 7.1). The standard deviation of systolic blood pressure showed a significant positive correlation to low frequency/ high frequency ratio of spectral bands of heart rate variability in the IDDM group ($r = 0.43$ $p < 0.03$) although no such correlations were observed in the control group.

Simple day-night differences were not related to duration of diabetes or glycaemic control. By contrast, after controlling for other variables, CDCAM showed significant negative correlations with duration of diabetes ($p < 0.05$) and HbA_1 ($p < 0.05$).

CDCAM showed a significant negative correlation with low frequency/ high frequency ratio after controlling for other variables but did not show any relationship to standard tests of autonomic function.

Echocardiographs

Of the 65 IDDM subjects asked to have echocardiography 15 subjects refused the examination. A further 10 were not measurable due to technical reasons. Of the remaining 40 patients (23 females) the left ventricular mass index showed a significant negative correlation with CDCAM. From the IDDM group who undertook assessment of heart rate and systolic blood pressure variability 31 had left ventricular mass index determined. There was a significant negative correlation with baroreceptor-cardiac reflex sensitivity derived

from sequence and spectral analysis and left ventricular mass index (Fig 7.1 r = -0.56 p < 0.001 for up-sequences when supine).

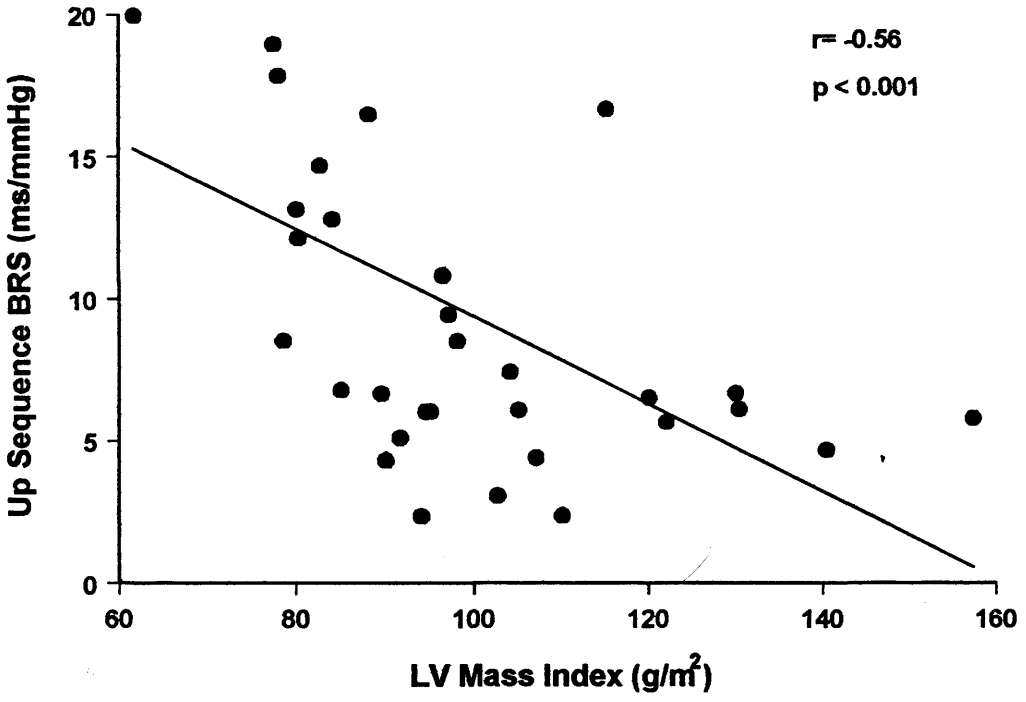

Fig 7.1: Relation between left ventricular mass index and baroreceptor-cardiac reflex sensitivity derived from sequence analysis.

Table 7.1- Systolic blood pressure (mmHg) data between control and IDDM groups (Mean ± SD).

	Controls (n= 65)	Diabetics (n= 65)	p‡
Clinic SBP	122 ± 13	126 ± 12	0.1
Mean 24HR SBP	119 ± 9	126 ± 17	0.07
Day SBP	125 ± 11	128 ± 21	0.2
Night SBP	109 ± 5	115 ± 12	<0.05
D-N	18 ± 4.1	14 ± 3.7	0.07
CDCAM	26.8 ± 8.4	18.5 ± 9.9	<0.03

SBP= Systolic blood pressure.

D-N= Day-night difference in systolic blood pressure.

CDCAM= Cusum derived circadian alteration of magnitude.

Sympathovagal balance, as assessed by the low frequency / high frequency ratio, showed a significant positive correlation with left ventricular mass index (Fig 7.2 r = 0.7 p <0.002) but there was no relation between left ventricular mass index and standard tests of autonomic function.

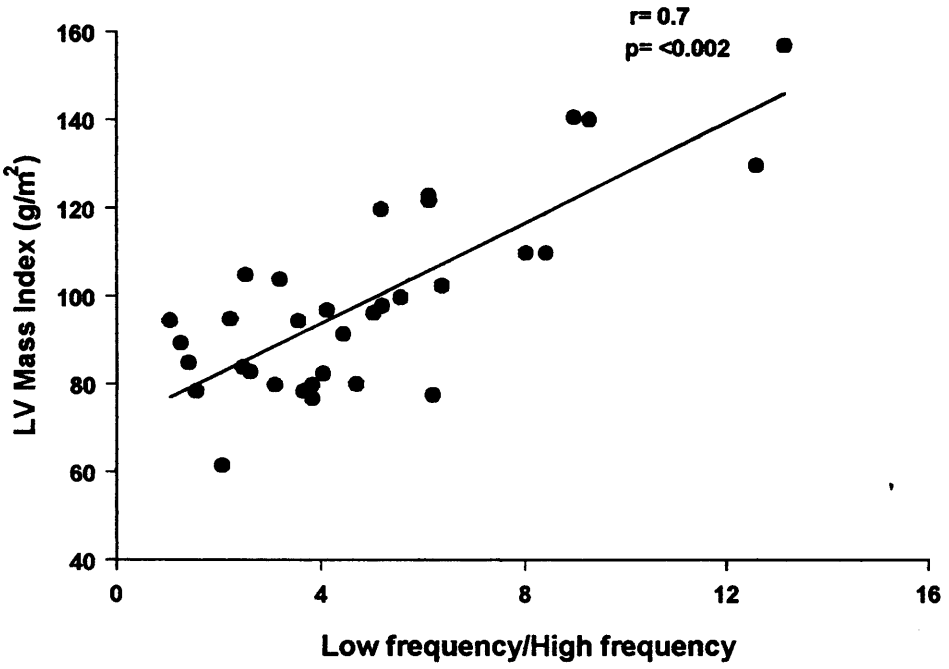

Fig 7.2: Relation between sympathovagal balance and left ventricular mass index.

7.4 Discussion

This study demonstrated evidence for an increased variability in day-time blood pressure in a group of IDDM patients with normal bedside tests of autonomic function. This increase in variability showed a significant positive correlation with sympathovagal balance (as

assessed by the low frequency / high frequency ratio) and a significant negative correlation with baroreceptor-cardiac reflex sensitivity. Only one of the IDDM subjects studied failed to show a > 10% reduction in systolic blood pressure between day and night. However, using the more reliable Cusums method of assessing circadian blood pressure variation, the IDDM patients had a reduced diurnal variation in blood pressure as assessed by the CDCAM compared to the control group. CDCAM showed a significant negative correlation with duration of diabetes and HbA_1. In addition, there was a positive correlation with low frequency / high frequency ratio and CDCAM.

Whilst the clinic measurement of blood pressure is important, 24 hour blood pressure profiles have been shown to be more strongly correlated to markers of end-organ damage, especially to left ventricular hypertrophy and cerebrovascular disease (Kobrin et al 1984, Verdecchia et al 1990, White 1991). None of the IDDM subjects were hypertensive on clinic or 24 hour blood pressure recordings but 5 subjects had left ventricular mass indices greater than $120g/m^2$. There was a significant negative correlation between CDCAM and left ventricular mass index. A negative correlation was seen between CDCAM and baroreceptor-cardiac reflex sensitivity derived from sequence and spectral analysis (Chapter 5). Furthermore, a significant positive correlation between left ventricular mass index and sympathovagal balance was observed.

Twenty four hour power spectral analysis of heart rate variability has documented a circadian oscillation in autonomic activity with a prevalence of sympathetic tone during the day and vagal tone at night (Furlan et al 1990); i.e. it parallels the changes in circulating catecholamines and vasoactive hormones (Richards et al 1986). In a mixed population of

IDDM and NIDDM patients with abnormal clinical tests of autonomic function Spallone and colleagues (1993) identified an impairment of this autonomic circadian rhythm. Abnormalities of twenty-four hour blood pressure variability have also been described in diabetic subjects with abnormal bedside cardiovascular reflex tests (Spallone et al 1993, Phong Chau et al 1994). These abnormalities have been postulated to be due to the impaired circadian rhythm of autonomic function and an abnormal nocturnal sympathetic predominance. The earliest documented abnormality of circadian blood pressure variability is a loss of the normal nocturnal fall in blood pressure with normal blood pressure variability during the daytime (Liniger et al 1991, Spallone et al 1993, Lurbe et al 1993, Phong Chau et al 1994). Reviewing the data of Phong-Chau and co-workers reveals that they did find an increase in day-time systolic blood pressure variability although this did not reach statistical significance ($p = 0.06$). Another group, using an increased frequency of blood pressure recordings during the day time, have shown an increased variability of systolic blood pressure in a population of non-insulin dependent diabetic patients with no historical evidence of autonomic dysfunction (McKinlay et al 1994). However, in this study, there was no formal assessment of autonomic function.

In the current study daytime systolic blood pressure showed a significantly increased variability as assessed by the standard deviation of recordings taken every 15 minutes. This finding is mirrored in the spectral powers of systolic blood pressure variability which showed a significant increase in the low frequency power in the IDDM group compared to control subjects (Chapter 3). Variability of blood pressure at rest is dependent on the sensitivity of the intact arterial baroreflex (Siché et al 1993). It is possible, therefore, that

the increased variability of daytime systolic blood pressure was due to the reduction in baroreceptor-cardiac reflex sensitivity described in Chapter 4. Baroreflexes buffer changes in arterial pressure (Ferguson et al 1985), and failure of the baroreflex results in a loss of this buffering mechanism and thus increased variability in blood pressure (Robertson et al 1993). Baroreceptor-cardiac reflex sensitivity is lower in patients with essential hypertension than normotensive controls (Bristow et al 1969). Traditionally, it has been held that baroreceptor-cardiac reflex sensitivity declines as a consequence of essential hypertension (possibly by increasing compliance and abnormal endothelial function) rather than impaired baroreceptor-cardiac reflex sensitivity causing hypertension. The converse has recently, however, been suggested- that hypertension develops as a result of impaired baroreceptor-cardiac reflex sensitivity (Ramirez et al 1985). Studies in humans with carotid baroreceptor denervation, in some cases, have shown an initial increase in blood pressure variability and a later persistent blood pressure elevation (Robertson et al 1993). It is possible, therefore, that the diabetic group in the present study may be at an increased risk of developing persistent hypertension and that the earliest evidence for this is the reduced nocturnal decline in systolic blood pressure.

The current study showed a significant impairment of the diurnal heart rate variation in the diabetic group. Also, as reported in Chapter 3, the IDDM patients had an increased daytime heart rate but this did not reach statistical significance. Ewing and colleagues (1983) have reported abnormal diurnal changes in heart rate in diabetic subjects with abnormal bedside tests of autonomic function. They postulated that this may be due to

reduced vagal tone at night and so an increasing sympathetic predominance as reported by Bernardi et al (1992).

The present study is the first to describe a relationship between diurnal blood pressure changes, sympathovagal balance and left ventricular mass index in IDDM patients with normal bedside autonomic function tests. Subjects with symptomatic diabetic autonomic neuropathy have been shown to have abnormal ventricular systolic function on radionuclide ventriculography in the absence of ischaemic heart disease (Zola et al 1986) Furthermore, in IDDM patients with poor metabolic control echocardiographic evidence of abnormal left ventricular function has been observed and associated with adrenergic hypersensitivity (Maraud et al 1990). Autonomic neuropathy, defined as 2 or more abnormal bedside tests of autonomic function, has been associated with increased left ventricular mass index (Gambardella et al 1993). Similarly, deficits in the baroreceptor-cardiac reflex was highly correlated with the level of cardiac hypertrophy in animal models (Minami et al 1993). There was a significant increase in left ventricular mass index in the IDDM patients in the current study as baroreceptor-cardiac reflex sensitivity declined. Furthermore, left ventricular mass index showed a significant positive correlation with sympathovagal balance. Whilst one is reluctant to draw conclusions from data with no control group it is possible to speculate that the increase in left ventricular mass index mass index could be due to the increase in blood pressure load associated with a reduced diurnal rhythm of systolic blood pressure which in turn may be due to a relative sympathetic overactivity. Other factors than circadian changes in autonomic function may be responsible for the diurnal change in systolic blood pressure and these include the renin-

angiotensin-aldosterone axis (Delea 1979) and atrial naturetic peptide (Colantanio et al 1988). These factors have not been assessed in the present study and it is probable that the pathogenesis of the altered circadian rhythm of blood pressure is multifactorial. It is also possible that changes in left ventricular mass index are in some way responsible for the changes in autonomic parameters in particular a lack of responsiveness to changes in systolic blood pressure could be due to myocardial dysfunction rather than a defect in the reflex arc.

In summary, abnormalities of circadian blood pressure change are detectable in 'normotensive' IDDM patients. It is interesting to speculate that impaired baroreceptor-cardiac reflex sensitivity, possibly due to an impairment of parasympathetic function and a relative sympathetic predominance, may result in an increased blood pressure load at night. In other clinical settings, an reduced nocturnal fall in systolic blood pressure is associated with end-organ damage including left ventricular hypertrophy. Some of the patients in this study had left ventricular hypertrophy despite being 'normotensive' on clinic blood pressure readings and this may be due to an increased blood pressure load over 24 hours. Twenty-four hour ambulatory blood pressure monitoring is patient friendly and should be used more frequently in IDDM to identify those patients at risk from end-organ damage.

CHAPTER 8

SUMMARY AND SUGGESTIONS FOR FURTHER RESEARCH

8.1 Summary of Principal Study Findings

Chapter 2 comprised of a study using the five traditional tests of autonomic function and the development of an automated device to allow accurate assessment of the changes in heart rate during these tests. Ewing and colleagues (1981, 1982, 1985) were the first to formalise these tests into a package that, with a scoring procedure for abnormal tests, provided a technique to allow serial autonomic function testing and detect the development of autonomic neuropathy. Work based on this methodology has contributed considerably to the assessment of the epidemiology and natural history of diabetic autonomic neuropathy (Ewing et al 1985, Sampson et al 1990). The mortality rate of 56% at five years has been reported for patients with symptomatic diabetic autonomic neuropathy. The screening study described in Chapter 2 revealed no difference in autonomic function between a well matched control group and 64 IDDM patients. Furthermore, the values of the five tests of autonomic function were similar to age adjusted reference values in both groups (O'Brien et al 1986). Whilst these tests of autonomic function are easy to perform it should be remembered that a sizable fraction (13%) of the normal population may have one abnormal test and there is a wide variation in the reproducibility of the results (O'Brien et al 1986). With the advent of convincing evidence showing improved glycaemic control can result in a reduction in the incidence and a slowing in the progression of autonomic neuropathy early detection is clearly important (Diabetes Control and Complications Trial Research Group 1993, 1995) but the standard bedside tests of autonomic function seem inadequate to this task.

In an attempt to detect early cardiovascular autonomic neuropathy other workers have developed further techniques of assessing autonomic function. Amongst these is the use of spectral analysis to assess beat-to-beat changes in heart rate and systolic blood pressure (Akselrod et al 1981, Pomeranz et al 1985, Pagani et al 1986, Cerutti et al 1987). Chapter 3 describes the application of these newer techniques of assessing autonomic function in a randomly selected subgroup of the study population. Despite absence of differences in standard tests of autonomic function, there was considerable differences in power spectra of heart rate and blood pressure spectra in the IDDM group compared to the control subjects matched for age, sex and systolic blood pressure. Briefly, there was a reduction in total variance of pulse interval spectra in the IDDM group mostly due to a reduction in the high frequency spectral band. There was a consequent increase in the low frequency/high frequency ratio which has been proposed as a measure of sympathovagal balance (Pagani et al 1986, Bernardi et al 1992). Chapter 3 also describes the first study of spectral analysis of systolic blood pressure variability in IDDM patients showing a significant increase in the low frequency power of the systolic blood pressure spectra in IDDM subjects compared to control subjects. There is continuing debate as to the interpretation of the low frequency component of the power spectral density curve. This component is regarded by some to be a marker of sympathetic function (Malliani et al 1991) whilst others consider it to include both sympathetic and parasympathetic influences (Akselrod et al 1981, Parati et al 1995). Also, the finding of a paradoxical reduction in low frequency power in conditions where increased sympathetic activity is expected (such as congestive cardiac failure) adds to this controversy (Sleight et al 1995). However, during sympathetic

activation the resulting tachycardia is associated with a marked reduction in total power and when the spectral components are expressed in absolute units there are reductions in the reductions in both low frequency and high frequency powers dependent on the reduction in total power. The use of normalised units to express the powers of the components minimises the effect of total power and the very low frequency component on the values of low frequency and high frequency so allowing the balance between the sympathetic and parasympathetic systems to be studied (Malliani et al 1991). In the present study, there was an increase in resting heart rate in the IDDM group (although not statistically significant) and the absolute powers were converted to normalised units the reduction in high frequency power and increase in low frequency/high frequency ratio was apparent. This represents a reduction in parasymapthetic activity and a relative sympathetic predominance. Similar changes in sympathovagal balance, namely a decrease in the high frequency (vagal) spectral band and a relative increase in the low-frequency (mainly sympathetic) band has already been shown in diabetic patients with established autonomic neuropathy (Pagani et al 1988, Bernardi et al 1991, Bernardi et al 1992). The present study extends these observations showing there was a decrease in the high frequency power and a relative increase in the low frequency power of R-R interval spectra in a group of IDDM patients with no clinical evidence of autonomic dysfunction and with normal standard tests of autonomic function. Thus, the use of spectral analysis methods in the present study has detected significant abnormalities of autonomic function in a group of asymptomatic insulin dependent diabetic patients that went undetected by the traditional tests of autonomic function.

Beat-to-beat regulation of heart rate and blood pressure are under the control of arterial baroreceptors and therefore abnormal baroreceptor-cardiac reflex sensitivity may explain some of the differences seen in power spectra. Chapter 4 reports the assessment of baroreceptor-cardiac reflex sensitivity using three non-invasive methods. Traditionally, baroreceptor-cardiac reflex sensitivity assessment requires the use of pharmacological agents to induce changes in blood pressure and intra-arterial monitoring which significantly limits the clinical usefulness of the technique. With the availability of non-invasive measurement systems such as the Finapres and the development and validation of non-pharmacological means of assessing baroreceptor-cardiac reflex sensitivity (Palmero et al 1981, Parati et al 1988, Lucini et al 1994) the measurements become of clinical relevance. Baroreceptor-cardiac reflex sensitivity derived from phase 4 of the Valsalva manoeuvre and during sequence analysis of resting heart rate and systolic blood pressure showed marked reductions in the baroreceptor-cardiac reflex sensitivity for the IDDM patients compared to controls. The use of spectral parameters to assess the α coefficient, an index of the overall gain of the baroreflex, again showed a significant reduction in IDDM subjects. Baroreceptor-cardiac reflex sensitivity showed significant negative correlations with duration of diabetes and average HbA_1. Control subjects showed a significant negative correlation between baroreceptor-cardiac reflex sensitivity and age but this was not found in the IDDM group. Thus, these methods uncovered evidence of significant impairment of arterial baroreflex mechanisms which was undetected by the standard bedside tests of autonomic function. This impairment of baroreceptor-cardiac

reflex sensitivity may, in part, be responsible for the reduction in heart rate variability found in the IDDM population and possibly predispose them to sustained hypertension. The sympathetic and parasympathetic arms of the autonomic nervous system constantly interact so that it is incorrect to artificially isolate the individual influences from the power spectra. On method of studying the sympathetic and parasympathetic components of the spectra involves pharmacological blockade of the parasympathetic arm with atropine. Chapter 5 describes the effects of atropine on the heart rate power spectra and baroreceptor-cardiac reflex sensitivity in a subgroup of the study patients. This demonstrated that heart rate variability was reduced after atropine in both the control and IDDM groups although the effects were less evident in IDDM patients. When standing, a manoeuvre known to increase sympathetic activity, atropine had less effect on the total variance in both groups but again the reduction in power with atropine was less in the IDDM group. Baroreceptor-cardiac reflex sensitivity was assessed by the three methods after atropine and was reduced in the control group in all situations. IDDM patients however showed no significant change in baroreceptor-cardiac reflex sensitivity, when standing, after atropine. These data suggest that the reduction in spectral power and baroreceptor-cardiac reflex sensitivity seen in the IDDM group can, at least in part, be attributed to a defect in parasympathetic activity although there are other possible mechanisms which were discussed.

Cardiac repolarisation is dependent on the autonomic nervous system and it follows that autonomic dysfunction may be associated with abnormalities of QT interval and dispersion (Ewing et al 1990, Veglio et al 1995). Chapter 6 explored this possibility in a subgroup of

the total IDDM population. The study demonstrated that QTc but not QT dispersion showed a significant negative correlation with sympathovagal balance and with baroreceptor-cardiac reflex sensitivity derived from sequence and spectral studies. It can be concluded that impaired baroreceptor-cardiac reflex sensitivity, possibly due to impaired parasympathetic function is associated with a global prolongation of ventricular repolarisation but not dispersion of ventricular repolarisation.

Chapter 7 showed that as baroreceptor-cardiac reflex sensitivity declines in the IDDM group then there is a reduction in the day-night difference in 24 hour blood pressure control despite the fact that subjects were 'normotensive' based on clinic blood pressure measurements. In addition, there was an increase in left ventricular mass index as sympathovagal balance increased in the IDDM group. There was no correlation between day-night blood pressure differences and baroreceptor-cardiac reflex sensitivity in the control subjects who were matched for age, sex and clinic blood pressure. It can be hypothesised that as the sympathetic predominance increases the day-night difference in systolic blood pressure declines in the IDDM patients. Thus, the increase in blood pressure load may then be responsible for the increase in left ventricular mass index.

8.2 Implications of these findings for IDDM patients

8.2.1 Sudden Death

Diabetic patients with symptomatic autonomic neuropathy have a well documented increased mortality rate. Epidemiological studies have shown that the five year mortality is between 23-56% (Ewing et al 1985, Sampson et al 1990). The majority of these deaths are attributed to macrovascular disease but a significant minority of them are sudden and unexplained.

In the 1980s concern was expressed about the increased number of sudden deaths among young, apparently healthy, IDDM patients associated with a lack of hypoglycaemic awareness following the introduction of human insulins. To investigate this further, the British Diabetic Association funded a study looking at all of the deaths amongst IDDM patients in 1989 (Tattersall and Gill 1991). There were 53 deaths reported, 3 of whom were over 50 years old. It was decided to study only those deaths in patients less than 50 years old, based on the assumption that patients above 50 would have some evidence for coronary heart disease. Of the remaining 49 cases 11 others had a definite cause of death at post mortem. The researchers undertook extensive investigation in an attempt to find a cause of death in the remaining 34 cases. After reviewing case notes and interviewing friends and relatives it was discovered that 22 of the cases were found 'dead in bed'. These patients had been seen, apparently well, the day before their death but they were all found the next morning lying dead in an undisturbed bed and post mortem examination was unable to identify a cause for death (in particular there was no structural cardiac

abnormality). Only a small minority of these patients had documented ante-mortem evidence of diabetic complications and only in one patient was there documented evidence of autonomic neuropathy. None of the patients had convincing post-mortem evidence of hypoglycaemia although this is notoriously difficult to diagnose. Owing to glycogenolysis, blood taken from the right side of the heart at post mortem has a spuriously high glucose value. By contrast, blood glucose concentrations from left sided blood samples are often low due to continued glycolysis. Some pathologists rely on vitreous humor glucose levels but these also are unreliable. Tattersall and Gill found historical evidence of nocturnal hypoglycaemia in at least 14 of the 'dead in bed' patients concluding that the 'dead in bed' syndrome was due to hypoglycaemia predisposing to respiratory depression or cardiac dysrhythmia. Following on from this study, other workers have reported a similar incidence of the 'dead in bed' syndrome amongst young, apparently healthy IDDM patients (Thordarson et al 1995, Sartor et al 1995).

The blood glucose nadir most commonly occurs at 2-3am and nocturnal hypoglycaemia is a common occurrence amongst IDDM patients. However, massive insulin overdose rarely results in sudden death (Critchley et al 1984) so some other factor(s) must predispose patients to sudden death.

Sudden death is observed in a number of other clinical settings such as infancy, in epileptic patients (Jay et al 1981) and following myocardial infarction. In the latter example similar data regarding heart rate variability and baroreceptor-cardiac reflex sensitivity are available as for the IDDM patients. Moreover, a reduced total power of heart rate

variability following acute myocardial infarction is associated with an increased mortality; over a 31 month follow-up period, mortality in patients with lower total power of heart rate variability was increased five fold (Kleiger et al 1987). Increased low frequency power has also been shown to be associated with an increased mortality (Bigger et al 1992) and reduced high frequency power has been postulated as a marker of increased risk of sudden cardiac death in patients following a myocardial infarction (Hartikainen et al 1995). In all of these studies heart rate variability was assessed from 24-hour Holter recordings. In addition, all made the oversimplification that high frequency power = parasympathetic function and low frequency power = sympathetic function. As a result it was concluded, therefore, that a reduced parasymapthetic activity and consequently an increased sympathetic activity is associated with an increased risk of sudden death following acute myocardial infarction. This oversimplification limits the value of these studies although it is interesting that all show similar changes, and similar risk profiles, for the data derived from power spectra. In a recent study, the more robust measure of sympathovagal balance the low frequency/ high frequency ratio has been studied post acute myocardial infarction (Singh et al 1996). It was shown that the low frequency/ high frequency ratio measured within 24 hours of an acute myocardial infarction was predictive of 30 day mortality. In other words, a relative increase in sympathetic activity is associated with an increased mortality in patients after acute myocardial infarction.

A lower baroreceptor-cardiac reflex sensitivity has also been associated with a poor prognosis in patients with myocardial infarction (Billman et al 1982, De Ferrari et al 1995). Billman and colleagues induced myocardial ischaemia in dogs and studied

baroreceptor-cardiac reflex sensitivity using the phenylephrine technique. They found that there was a significantly increased risk of ventricular fibrillation in animals with the lowest baroreflex slope. Once again, this was thought to be due to impairment of parasympathetic function exposing a relative over activity of the sympathetic nervous system therefore predisposing to ventricular arrhythmias. However, no assessment of individual components of the autonomic nervous system were made. De Ferrari and colleagues also used the phenylephrine technique to study baroreceptor-cardiac reflex sensitivity as well as performing spectral analysis of heart rate variability in patients after acute myocardial infarction. They found that reduced baroreceptor-cardiac reflex sensitivity and a reduction in parasympathetic activity (once again oversimplifying the interpretation of the individual frequency bands of the power spectra) were associated with an increased risk of sudden death from ventricular arrhythmias.

As described in the Chapter 3, the IDDM patients studied have shown more convincingly a reduced heart rate variability and a relative sympathetic predominance. This was also associated with a reduction of baroreceptor-cardiac reflex sensitivity due to, at least in part, a reduction in parasympathetic activity. Whilst one has to be cautious in extrapolating from acute myocardial infarction studies to diabetic patients, the present findings could predispose IDDM patients to ventricular arrhythmias.

QT interval on the surface ECG is to some extent determined by the activity of the autonomic nervous system (Rosen et al 1992). Prolongation of the QT interval has been associated with sudden cardiac deaths amongst IDDM patients with abnormal bedside tests of autonomic function (Bellavere et al 1988, Ewing et al 1990). The study described

in Chapter 6 shows that as sympathetic predominance increased so the QTc lengthened, although none of the subjects studied had prolongation of the QTc (>0.44 s). Dynamic changes in QT interval have been reported with changes in blood glucose concentration (Heller et al 1995). Thus during hypoglycaemia there is prolongation of the QTc. Hypoglycaemia has also been associated with an increase in sympathetic activity as assessed by plasma catecholamine measurements.

Drawing these data together it is possible to hypothesise why apparently healthy IDDM patients should be at risk from sudden cardiac death. At-risk patients may already have impaired parasympathetic activity and a sympathetic predominance, resulting in reduced baroreceptor-cardiac reflex sensitivity. During sleep there is usually a reduction in sympathetic and relative increase in parasympathetic activity (Furlan et al 1990). In diabetic patients with abnormal bedside tests of autonomic function a relative sympathetic overactivity at night has already been reported (Bernardi et al 1992) as have abnormalities of circadian blood pressure and heart rate change (Ewing et al 1983, Lurbe et al 1993). Ewing and colleagues (1983) have reported an increase in ventricular ectopic activity at night in diabetic patients with autonomic neuropathy. Night-time hypoglycaemia would further increase the imbalance between sympathetic and parasympathetic arms of the autonomic nervous system . Furthermore, hypoglycaemia, impaired baroreceptor-cardiac reflex sensitivity and increased sympathovagal balance are all associated with lengthening of the QTc- a further risk factor for ventricular arrhythmia. Thus, it is possible that the susceptibility to sudden death in diabetic patients depends upon an increased risk of developing ventricular arrhythmias from over activity of the sympathetic nervous system

relative to parasympathetic tone. This hypothesis is supported by the powerful secondary preventative effects of beta-blockers in diabetic patients following myocardial infarction (Kjekshus et al 1990). Further direct evidence for sympathetic over-activity in diabetic patients comes from studies which have found raised plasma noradrenaline levels in poorly controlled IDDM patients (Eckberg et al 1986). Finally, the normal circadian pattern of cardiovascular events is lost in diabetic subjects. In non-diabetic patients, acute myocardial infarction has a circadian variation with a significant morning peak. There is loss of this circadian rhythm in the general diabetic population (Fava et al 1995).

As described in Chapter 7, there was an association with increasing left ventricular mass index and reduced diurnal change in systolic blood pressure. This could possibly represent early end-organ damage due to an increased blood pressure load. Data from the Framingham study has shown that non-diabetic patients with evidence of left ventricular hypertrophy have a 2 fold increase in 5 year mortality rate when compared to subjects with normal left ventricular mass (Kannel et al 1986). Furthermore, a significant association between electrocardiographic evidence of left ventricular hypertrophy and sudden death and echocardiographic evidence of left ventricular hypertrophy (Kannel et al 1969, Levy et al 1990) and ventricular arrhythmias (Messerli et al 1984) has been observed. Whilst only 2 of the IDDM subjects studied had left ventricular hypertrophy (left ventricular mass index > 120 g/m^2) there was an overall trend for an increased left ventricular mass index as sympathovagal balance increased. Thus, echocardiography to assess left ventricular mass index and possibly left ventricular function could be used to identify IDDM at risk from sudden death.

8.2.2 Hypertension and IDDM

Hypertension and diabetes often occur together, which increases the likelihood of target organ damage including nephropathy, retinopathy and cardiovascular disease (Klein et al 1996). The work for this thesis has identified an increased blood pressure load in 'normotensive' IDDM patients. There was early evidence for increasing left ventricular mass index in association with an increased blood pressure load. Early recognition and treatment of hypertension in IDDM patients is therefore important to lessen the risk of end-organ damage. Work on diabetic nephropathy has shown a beneficial effect of angiotensin-converting enzyme inhibitors and non-dihydropyridine calcium channel blockers in slowing the progression of the renal disease. It is felt that it is not simply a blood pressure lowering effect of these agents that slows progression of renal disease but an additional effect of these agents (Bakris 1993). Is it possible to recommend specific treatment for hypertensive IDDM patients with evidence for defective heart rate variability and impaired baroreceptor-cardiac reflex sensitivity? If an IDDM patient is known to have impaired baroreceptor-cardiac reflex sensitivity it would seem foolish to treat that patient with pharmacological agents that would lower baroreceptor-cardiac reflex sensitivity further or avoid drugs that can improve heart rate variability and baroreceptor-cardiac reflex sensitivity. Animal studies have shown that diuretics and ß-blocker agents have little effect on baroreceptor-cardiac reflex sensitivity whereas angiotensin-converting enzyme inhibitors increase baroreceptor-cardiac reflex sensitivity and heart rate variability (Ichikawa et al 1995). In patients with ischaemic heart disease ß-blockade has shown little effect on baroreceptor-cardiac reflex sensitivity but reduced the Valsalva ratio (Airaksinen et al 1994) whereas angiotensin-converting enzyme inhibitors have been shown to increase

heart rate variability (Osterziel et al 1990) and baroreceptor-cardiac reflex sensitivity (Veerman et al 1996) in patients with congestive cardiac failure. The effect of angiotensin-converting enzyme inhibitors on autonomic function is to increase parasympathetic activity probably by preventing the attenuating effect of angiotensin II on vagal modulation of heart rate (Townend et al 1995). Angiotensin II has also been shown to have an centrally acting inhibitory effect on baroreceptor-cardiac reflex sensitivity (Mace et al 1985). The improved survival in patients on ACE inhibitor agents with congestive cardiac failure is thought to be due, in part, to this autonomic modulation. Furthermore, ACE inhibitors have been shown to be more potent in reducing left ventricular mass index than ß-blockers or diuretics in patients with essential hypertension (Schmeider et al 1996). Some caution needs to be given to these findings, however, as survival data in hypertensive patients is only available for those patients treated with ß-blockers and/or thiazide diuretics. Whilst there are no studies looking at the effects of ACE inhibition on autonomic function and baroreceptor-cardiac reflex sensitivity in IDDM patients there would seem to be circumstantial evidence that ACE inhibitors are suitable agents for treating hypertension in IDDM patients especially if there is evidence for impaired heart rate variability, reduced baroreceptor-cardiac reflex sensitivity or left ventricular hypertrophy.

8.3 Suggestions for further studies

As one expects from new avenues of investigation, the research conducted for this thesis raises more questions than provides answers. Significant abnormalities in heart rate and systolic blood pressure variability have been detected in a group of asymptomatic IDDM patients, despite 'normal' bedside tests of autonomic function. This was associated with evidence for impaired circadian modulation of blood pressure, and a possible increase in end-organ damage as assessed by left ventricular mass index.

The obvious next question is to see if these changes in cardiovascular autoregulation progress, or if they return to normal. Ewing has stated that once autonomic dysfunction is present it usually deteriorates further and never returns to normal (Ewing et al 1982). Conversely, peripheral nerve dysfunction has been shown to revert back to normal (Fraser et al 1977). No prospective studies are available looking at baroreceptor-cardiac reflex sensitivity or heart rate variability in IDDM patients. The tests used are reproducible, non-invasive and easy to perform so are suited to repeated measurement.

Symptomatic diabetic autonomic neuropathy is associated with increased mortality. In other clinical settings, particularly after acute myocardial infarction, impaired baroreceptor-cardiac reflex sensitivity and reduced heart rate variability are also associated with increased mortality. Whilst an attempt has been made in the discussion of this thesis to suggest that IDDM patients with impaired baroreceptor-cardiac reflex sensitivity are an 'at-risk' group, no clinical evidence for this is available. Impaired baroreceptor-cardiac reflex sensitivity was associated with increasing left ventricular mass index and reduced circadian change in systolic blood pressure, both of which are suggestive of increased

cardiovascular risk. A prospective study would allow the relative risk of these abnormalities of cardiovascular autoregulation to be studied.

These studies have concentrated on the assessment of the arterial baroreceptors. The low pressure baroreceptors, situated in the cardiac walls and pulmonary arteries have not been studied in IDDM patients although Bennett et al (1980) have studied these in a mixed group of diabetic subjects with severe microvascular disease. The cardiopulmonary volume receptors exert a tonic restraint on sympathetic activity and are involved in blood volume homeostasis via effects on sympathetic modulation of renin release and the release of vasopressin from the hypothalamus (Grassi et al 1988, Robertson et al 1976). Abnormal nocturnal fluid overload has been suggested as a cause for the loss of the night time reduction in systolic blood pressure in IDDM patients (Mulec et al 1995). Study of the cardiopulmonary baroreceptors in IDDM patients using lower body negative pressure could, therefore, yield interesting information.

Further work is also required to establish the site and nature of the lesion(s) causing the impairment of baroreceptor-cardiac reflex sensitivity. The atropinisation of the IDDM patients suggested an abnormality of parasympathetic function although it would be churlish to assume that a multi-system disorder such as IDDM would selectively affect parasympathetic function only. Further work looking at pharmacological blockade with ß-blocker agents and clonidine (a centrally acting sympathoinhibitory drug) would provide useful information and help clarify the site of the defects in the baroreflex. The brain stem is central to the autoregulation of blood pressure and there is little information about brain stem function and baroreceptor-cardiac reflex sensitivity in IDDM subjects. With

improved technology, brain stem evoked potentials and possibly positive emission tomography may allow brain stem function to be studied.

The arterial baroreceptors are situated in the wall of the great vessels of the neck and thorax. Whilst compliance of these vessels has been studied detailed information about the compliance of the carotid sinus and baroreceptor-cardiac reflex sensitivity is lacking. Newer techniques such as pulse wave velocity measurement or the use of magnetic resonance imaging may allow the effects of compliance on baroreceptor-cardiac reflex sensitivity to be studied. Furthermore, endothelial dysfunction and the effects on baroreceptor-cardiac reflex sensitivity and heart rate variability need closer study.

In preliminary studies hypoglycaemia has been shown to have effects on cardiac repolarisation, at assessed by QTc measurements. The effects of hypoglycaemia on the other measures of autonomic function, in particular baroreceptor-cardiac reflex sensitivity has not been studied. With the reporting of the DCCT there is, for the first time, evidence that tight glycaemic control can lessen the risk of developing diabetic complications including diabetic autonomic neuropathy. This is however at the cost of increasing the risk of hypoglycaemia. Controlled hypoglycaemia in the laboratory, using a hypoglycaemic clamp may identify those patients at risk of cardiac arrhythmia during hypoglycaemia, particularly at nighttime when cardiovascular risk is increased in IDDM patients compared to the general population.

Finally, it is potentially possible to identify IDDM patients at risk of sudden death but is it possible to recommend specific treatment? The studies presented in this work have suggested an abnormality of parasympathetic function and an increase in sympathovagal

balance. The effect of angiotensin-converting enzyme inhibitors on autonomic function is to increase parasympathetic activity probably by preventing the attenuating effect of angiotensin II on vagal modulation of heart rate (Townend et al 1995). Angiotensin II has also been shown to have an centrally acting inhibitory effect on baroreceptor-cardiac reflex sensitivity (Mace et al 1985). Further studies are needed to look at the potentially beneficial effects of angiotensin-converting enzyme inhibitors on baroreceptor-cardiac reflex sensitivity and heart rate variability in IDDM patients.

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9.2 Published work from the thesis

Articles

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