Cardiovascular autonomic responses in pre- and post-ganglionic models of chronic autonomic failure

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Imperial College
University of London

Dr. Tim Young

Department of Neurovascular Medicine
Imperial College London
University of London
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ABSTRACT

Multiple System Atrophy (MSA) and Pure Autonomic Failure (PAF) are contrasting models of Chronic Autonomic failure. PAF primarily involves the post-ganglionic autonomic nervous system, whilst in MSA the pre-ganglionic structures are impaired. My central hypothesis is that this underlying neuropathological difference between MSA and PAF will lead to differing cardiovascular responses.

I will assess the cardiovascular effects of known pressor and vasomotor stimuli (mental arithmetic, cold pressor test, isometric exercise, water ingestion, inhaled CO_2 and inspiratory gasp) in MSA and PAF. Neurohormonal aspects will be explored by comparing the cardiovascular effects of the α_2-adrenoceptor agonist clonidine with serum noradrenaline levels in these groups, as well as comparing supine antidiuretic hormone (ADH) levels after head up tilt and correlating these with supine blood pressure (BP). As well as contrasting the cardiovascular responses, I will use the water ingestion studies to examine effects on orthostatic hypotension, a common complication of both MSA and PAF.

To measure cardiovascular responses during these studies I have used the Portapres II device to obtain continuous, non-invasive, beat-to-beat measurements of BP and heart rate (HR). Subsequent Model flow analysis using Beatscope software has then been used to calculate further cardiovascular indices, including cardiac output (CO), stroke volume (SV) and total peripheral resistance (TPR). In addition, intermittent BP and HR measurements have been obtained with an automated sphygmomanometer (Dinamap). Finally, peripheral vasomotor responses have been recorded by means of the Laser Doppler perfusion meter.

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List of Abbreviations:

A……...Amps

AD…….Autonomic Dysreflexia

AC…….…...Alternating Current

ACE……..Angiotensin Converting Enzyme

Ach……Acetylcholine

AchR…….Acetylcholine Receptor

Ad……..Adrenaline

ADC……..Apparent Diffusion Coefficient

ADH…….Antidiuretic Hormone

AF…….……Autonomic Failure

ANS……..……Autonomic Nervous System

ASIA…….American Spinal Injury Association

AVP……..Arginine Vasopressin

B-CIT……....^{123}I-2β-carboxymethoxy-3β-4-iodophenyl-tropane

BP…….Blood Pressure

BLE……..Bilateral Leg Elevation

CAF…….....Chronic Autonomic Failure

CC……Cutaneous Cold

Cho……...Choline

CISC……Clean Intermittent Self-Catheterisation

CO……....Cardiac Output

CP……Cold Pressor

CPAP……...Continuous Positive Airway Pressure

Cr…….Creatinine/phosphocreatine

CSF……..Cerebrospinal Fluid

CT……Computed Tomography

CVS……..Cardiovascular System

DA……Dopamine

DB……Deep Breathing

DBP……..Diastolic Blood Pressure

DC……Direct Current

DDAVP……1-desmaino-8-d-argine Vasopressin

DLBD……...Diffuse Lewy Body Disease

EF…….Ejection Fraction

EGG…….Electrogastrogram

EMG……Electromyography

EPO……..Erythropoietin

ET…….Endothelin

FDG……..Fluorodeoxyglucose

GCI……...Glial Cytoplasmic Inclusion

GH……Growth Hormone

Hg…….Mercury

hPa…….....Hectopascals

HR……Heart Rate

HUT……..Head up tilt

IBZM……....1,2,3-Iodobenzamide

IE……..Isometric Exercise

IML……..Intermediolateral Column

LCD……..Liquid Crystal Display

L-DOPS……L-threo 3,4-dihydroxyphenylserine

LED……..Light Emitting Diode

L-NMMA…….NG-monomethyl L-arginine

MA……Mental Arithmetic

MAP…….Mean Arterial Pressure

MRI……..Magnetic Resonance Imaging

MRS…….Magnetic Resonance Spectroscopy

MS……Miliseconds

MSA…….Multiple System Atrophy

MSA-C…….Multiple System Atrophy (Cerebellar onset)

MSA-P…….Multiple System Atrophy (Parkinsonian onset)

MSNA……..Muscle Sympathetic Nerve Activity

MTI……..Magnetization Transfer Imaging

MTR…….Magnetization Transfer Ratio

NA……Noradrenaline

NAA…….N-acetylaspartate

NCI……..Neuronal Cytoplasmic Inclusion

NFL……..Neurofilament Light Chain

NIDDM……Non-Insulin Dependent Diabetes

NMDA……..N-Methyl-D-aspartic acid

NO……Nitric Oxide

NOS……..Nitric Oxide Synthase

NSAID……..Non-steroidal Anti-inflammatory Drug

OBT……..Octanoid Breath Test

OH……Orthostatic Hypotension

OPCA……..Olivopontocerebellar Atrophy

PD…….Parkinson's Disease

PAF……...Pure Autonomic Failure

PAG……..Periaqueductal Grey

PET……..Positron Emission Tomography

PoTS……Postural Orthostatic Tachycardia Syndrome

PU……Perfusion Units

RBD…….REM Sleep Behaviour Disorder

REM……Rapid Eye Movement

rVLM……...rostral Ventrolateral Medulla

SBP…….....Systolic Blood Pressure

SCA……..Spino-Cerebellar Atrophy

SCI……...Spinal Cord Injury

SD……Standard Deviation

SE…….Standard Error

SE…….Standard Error of the Mean

SkBF…… Skin Blood Flow

SkVR…..Skin Vasomotor Reflex

SkRVR…….Skin Regional Vascular Resistance

SPECT…….Single Photon Emission Computed Tomography

SND……..Striatonigral Degeneration

SSR……...Sympathetic Skin Response

SV…….Stroke Volume

TS……..Tactile Stimulation

TDS……...ter die sumendum (three time daily)

TPR……..Total Peripheral Resistance

V……...Volts

VC……Vital Capacity

W……..Watts

Chapter 1: General Introduction

1.1: The Autonomic Nervous System in Health and Disease

The term "Autonomic Nervous System" was first coined by Langley over a century ago. It is an extensive neural network, innervating every organ in the body and plays an important role in regulation of a number of vital bodily functions such as the control of blood pressure (BP) heart rate (HR), sweating, digestion, micturition and sexual function. The autonomic nervous system (ANS) may be broadly divided into sympathetic and parasymapthetic divisions, each with important central (cerebral and spinal cord) and peripheral components. The ANS can be impaired either secondary to other conditions such as diabetes, or as a primary, idiopathic, process resulting in autonomic failure (AF) such as multiple system atrophy (MSA) and pure autonomic failure (PAF). The clinical consequences in these disease states are related to the underlying autonomic damage, and clinical investigations are based around different components of the ANS.

Historical Background concerning the Autonomic Nervous System

Langley and Gaskell revolutionised understanding of the autonomic nervous system with their work at the end of the nineteenth century. Gaskell was especially important in recording the anatomical layout of the autonomic nervous system; together with Langley he traced the outflow of preganglionic nerves from the spinal cord and described fundamental differences in the layout of the sympathetic and parasympathetic autonomic nervous systems [Gaskell 1916; Langley 1921]. Langley went on to describe the contrasting action of different tissues to adrenaline. He speculated that the reason for these differences to the same stimulus resulted from different types of "receptor substances" on the affected tissues [Langley 1921]. Elliot, a student of Langley, noted that both the adrenaline secreting (chromaffin) cells of the adrenal

medulla and the postganglionic sympathetic neurons were derived from the same ectodermal sympathoblasts. He proposed that postganglionic sympathetic nerves may produce their effects by secretion of adrenaline (although he believed that this was not made in the nerve terminals but rather sequestered from adrenaline released by the adrenal glands). He went on to suggest that both postganglionic parasympathetic nerves and spinal motor nerves innervating skeletal muscle both secreted similar substances, two decades before acetylcholine (Ach) was shown to a neurotransmitter common to these nerves. Henry Dale (who followed Elliot as Research Fellow to Langley) noted that Ach had many similarities in action to those of the parasympathetic nervous system (Dale coined the terms sympathetic and parasympathetic nervous systems). Although he correctly postulated the presence of an endogenous cholinesterase which rapidly degraded Ach, he did not suggest that Ach was secreted by the parasympathetic nervous system. It was Dale's close friend Loewi who finally deduced that a transmitter substance was released from the nerves to result in the effect. His classic 1921experiment on isolated frog hearts proved that this was the case. In this study he compared two frog hearts, the first with intact sympathetic and parasympathetic nerves, and the second with the nerves cut. He demonstrated that in the innervated heart electrical stimulation of the parasympathetic nerve led to a reduction in HR, and stimulation of the sympathetic nerve caused an increase in HR. He then showed that, if Ringers solution used to perfuse the first heart during stimulation of the parasympathetic nerve was then applied to the second, denervated heart, a reduced HR resulted. Similarly, when the sympathetic nerve of the first heart was stimulated, the perfusing Ringers solution, if applied to the denervated heart resulted in increased HR. Loewi originally described the substances as Vagusstoff and Acceleransstoff [Loewi 1921]. Follow-up work both by Loewi and Dale confirmed that Vagsstoff was in fact Ach, and Dale went on to show that Ach also was released by motor nerves supplying skeletal muscle as well as in other areas of the parasympathetic ANS, such as from gastric branches of the vagus [Dale & Feldberg, 1934]. Feldberg went on to show that Ach was also released as a neurotransmitter at the autonomic ganglia and from the splanchnic sympathetic nerves innervating the adrenals [Feldberg and Gaddum 1934].

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Organisation of the Autonomic Nervous System

The ANS is classically thought of as comprising two broadly antagonistic divisions, with sympathethetic system activation resulting in increased BP, HR, sweating, pupillary dilation and blood flow to skeletal muscle ("fight or flight"). By contrast, parasympathetic activation leads to reduced BP and HR, pupillary constriction and enhanced digestion Whilst it is true that sympathetic activation usually occurs in conjuction with parasympathetic inhibition (and vice versa), the two divisions may work in complentary fashion. As well as its two major divisions, the ANS may also be divided into afferent and efferent components. Afferent components allow feedback control, especially important for the rapid adjustments to changes in the cardiovascular systems. Such afferent signals include chemo- and mechanoreceptors in the heart atria and ventricles as well as in the aortic arch and carotid sinus. Signals travel in the vagus and glossopharyngeal nerves to the brain stem where synapses with the vasomotor centres can result in rapid changes in BP regulation by increasing or decreasing sympathetic and parasympathetic nervous activity. Thus a transient increase in BP is detected by mechanoreceptors in the wall of the aortic arch and the carotid sinus, resulting in a reflex inhibition of the tonic sympathetic outflow, and enhancement of the parasympathetic ANS leading to reduced peripheral vasoconstriction, reduced HR and reduced cardiac contractility. Although the ANS is often considered to be largely autonomous system, higher cerebral centres can strongly influence it. For example, mental stress results in cortical and limbic activation and thence to activation of the sympathetic nervous system. This is capable of eliciting an integrated physiological response. Thus there is an increase in BP and cutaneous vasoconstriction (via NA release from postganglionic sympathetic nerves), increased HR (via both parasympathetic withdrawal and adrenaline release), increased blood flow in skeletal

muscle (via adrenaline release), and increased sweating (via Ach release from postganglionic sympathetic nerves).

Neurotransmitters and the ANS

The major neurotransmitters involved are noradrenaline (NA) and acetylcholine (Ach). Both are involved centrally as well as peripherally. In the periphery Ach is the principal neurotransmitter at synapses in the ganglia for both sympathetic and parasympathetic divisions, acting as an agonist on nicotinic receptors on the postganglionic neurone. Postganglionic nerves differ, with the principle substance being Ach in the parasympathetic, and NA in the sympathetic nervous system (an important exception being sympathetic postganglionic nerves innervating the sweat glands where Ach is the principle neurotransmitter). The adrenal gland is a special case, being an endocrine gland intimately involved in the sympathetic nervous system response. It is innervated by preganglionic nerves which release Ach. When released the Ach causes release of adrenaline from the adrenal medulla into the blood stream. Other neurotransmitters are increasingly being recognised in both the sympathetic and parasympathetic nervous systems. Endorphins, substance P and calcitonin-gene related peptide are examples which are known to be released in varying amounts as co-transmitters in different sites within the ANS.

The substances thus released by the postganglionic ANS nerves and the adrenal gland produce their varying effects by acting as agonists on various ligand-gated receptors throughout the body. The type and number of these receptors in a particular tissue will be an important factor in determining the final effect at that tissue. Thus adrenaline results in bronchodilation in the airways and vasodilatation in skeletal blood vessels because of β_2 adrenoreceptors on smooth muscle, but causes tachycardia in the heart because of greater concentrations of β_1 receptors on cardiac pace maker myocytes. NA has much greater affinity

for α than β-adrenoceptors, thus NA results in greater vasoconstriction (via α_1 receptors on blood vessel smooth muscle) than adrenaline, but does not cause tachycardia.

ANS Dysfunction

The ANS may be damaged by a variety of insults, including co-existing diseases (such as diabetes or amyloidosis), trauma (including spinal cord injury and damage resulting from surgery), toxins and drugs. Chronic Autonomic Failure (CAF) represents 3 different disease states where AF occurs without a clear cause (primary). These 3 disease states are MSA, PAF and Parkinson's disease (PD) with AF [Mathias & Bannister 2002]. Whilst both major divisions of the ANS are affected in these 3 conditions, the lesion site differs between them. In PAF the lesion site is peripheral and postganglionic. Symptoms and signs are largely confined to the ANS, and the prognosis is generally good if the BP is well managed. By contrast, in MSA there is central, preganglionic impairment of the ANS with functional and morphological sparing of the postganglionic, peripheral autonomic nerves [Matthews 2002, Dotson et al 1990]. Unlike in PAF, additional features unrelated to the ANS are common. Parkinsonism and cerebellar dysfunction are frequently seen in MSA.

The final disease state comprising CAF is that of PD with AF. This group has both pre-and postganglionic impairment of the ANS. The degree of impairment appears heterogeneous. For these reasons I have studied the clearly differentiated disease states of MSA and PAF.

All 3 groups with CAF exhibit symptoms and signs of AF. These can be as varied as the organs that the ANS innervates. Thus problems can range from urinary or gastrointestinal tract dysfunction to sweating abnormalities or visual impairment. Subjects frequently have symptoms related to the cardiovascular system (CVS), most commonly orthostatic

hypotension (OH), which can develop with pre- or postganglionic lesion site damage and thus is common to both MSA and PAF. OH not only is among the most limiting of autonomic symptoms of MSA and PAF, but also is an important background to many of the studies outlined in this thesis, and as such will now be considered in more detail here.

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ORTHOSTATIC HYPOTENSION AND ITS MANAGEMENT

Orthostatic hypotension (OH or postural hypotension) is defined as a drop in blood pressure (BP) after standing for 3 minutes of greater than 20mmHg Systolic OR 15mmHg Diastolic [Mathias & Bannister 1999]. Although these values can provide a useful guide, it is important to make sure that treatment is not just based on BP readings, but also takes into account symptomatology. The fundamental problem of OH is that there is a reduced perfusion pressure to organs and tissues. Often the most marked effect of this is when the cerebral circulation is compromised, causing symptoms varying from "light-headedness" or "dizziness" to visual impairment or loss of consciousness. Other areas commonly affected include the neck and shoulder musculature. If perfusion to these muscles is compromised a claudication type pain (termed "coathanger pain" after the area affected) can develop [Mathias & Bannister 1999]. Importantly all the above symptoms rapidly subside if the patient lies flat as the perfusion is restored. This is an important point to elicit from the history to help in the differential diagnosis. It is also important in immediate treatment of these patients; if the patient loses consciousness but is unable to resume a lying posture (e.g. propped against a wall in a small toilet or being held up by well meaning friends) then irreversible effects of underperfusion may ensue. OH can cause problems after any length of stand. Indeed, in some patients the problem is so severe that even sitting upright is impossible. Other factors such as eating meals high in carbohydrate, dehydration or exercise may further exacerbate the disorder.

In essence any condition that compromises the circulating blood volume or reduces the ability to prevent pooling of blood in the lower extremities on upright stance can result in OH. Dehydration (or blood loss), vasodilatation (as seen in systemic infections) and certain medications (anti-hypertensives and anti-Parkinsonian medication) are often factors in OH. The major group affected are the elderly, in whom more than one of these factors may be important in the aetiology. The most severely affected patients tend to be those in whom there

is autonomic failure (AF). This can be primary (in conditions such as Pure Autonomic Failure, PAF, Multiple System Atrophy, MSA) or secondary (diabetes, amyloid and others) [Mathias & Bannister 1999]. The autonomic nervous system is important in regulation of the blood pressure in all positions. Because of this, in patients with AF, problems can also arise when lying flat. When supine, AF patients tend to be unable to prevent a rise in blood pressure, leading to various degrees of supine *hypertension*, which may be severe enough to require treatment in its own right, and certainly has to borne in mind when trying to treat the OH component. In such cases where medication is used to increase the blood pressure, the ideal is to use agents which have their main action during the day to help combat postural hypotension whilst not exacerbating supine hypertension at night.

NON-PHARMACOLOGICAL TREATMENTS OF ORTHOSTATIC HYPOTENSION

Various methods exist to help combat OH before resorting to drugs. These are often enough to control symptoms, and should still be considered in conjunction with drugs even in the more severe cases.

1) AVOIDANCE: as explained above some factors will tend to exacerbate OH. Medication should always be considered as a possible factor in OH, but it is often not practical to stop these or reduce the dose. More readily adjustable factors include exercise and eating meals (especially early in the day) high in carbohydrate. Exercise could be tailored to coincide with the patient's "best" times of the day (usually later in the day). Moderate exercise should be encouraged, but where this still appears to be causing a problem then the patient and relatives

should be aware of this. Supervised exercise or ensuring physical support is at hand (such as carrying a fold-up chair) should be considered.

Food high in carbohydrate appears to cause exacerbation of OH via an insulin-mediated mechanism. Symptom diaries may help the patient find the ideal meal composition. Usually it is breakfast and lunch which cause most problems. For these meals sugar and complex carbohydrates (such as rice, bread, potato, and pasta) should be kept to a minimum. The daily nutritional requirements can then be made up in the evening meal. Alcohol can also exacerbate symptoms (initially through vasodilatation, and subsequently due to increased diuresis). Finally, a special case exists for those whose postural hypotension is caused by diabetic autonomic failure. In these patients hyperglycaemia leads to increased diuresis with the risk of dehydration, which will tend to worsen OH.

2) FLUID INTAKE: It is important that patients are taking an adequate fluid intake to prevent dehydration. Detailed enquiries of the exact number of cups etc consumed in a day are often helpful. This is especially important in cases where fluid losses are likely to be increased such as with increased exercise, hot weather, pyrexia, vomiting and diarrhoea. In one special group at risk of OH, namely renal dialysis patients, it is important to remember that fluid intake will be tightly regulated by their renal physicians. A more specific technique, explored in this thesis is the drinking of specific volumes of water for rapidly improving OH & its symptoms.

3) MECHANICAL AIDS: BED TILT, CHAIRS, CROSS LEGS, STOCKINGS, And SUITS: A variety of simple manoeuvres can be learnt by patients to improve OH. These 'physical countermeasures' include crossing the legs whilst standing or squatting. Such methods probably work by increasing vascular resistance or venous return [Ten Harkel et al 1994]. In our experience, however, most patients with significant OH will have discovered some of these techniques themselves before seeking medical attention. Counter pressure

support garments may be applied to the legs [Sheps et al 1976] or even to the abdomen [Tanaka et al 1997]. However, these measures are often uncomfortable for the patient and may worsen peripheral pooling of blood on standing if they are subsequently not used. Head-up tilt of the patient's bed at night helps increase extracellular fluid volume [Bannister et al 1969]. This occurs both because renal perfusion pressures are lowered, reducing nocturnal diuresis, and because head-up tilt helps activate the renin-angiotensin system, causing fluid retention [Bannister 1977].

PHARMACOLOGICAL TREATMENTS OF ORTHOSTATIC HYPOTENSION

If the above non-pharmacological steps are insufficient to overcome the problems of OH, then drug therapy can be considered. It is important to remember that the above steps should not be abandoned if drug therapy is instituted, as they may still have a valuable role to play in combination with medication. Drug treatments for OH may be broadly divided into those which promote fluid volume expansion and those which cause vasoconstriction of resistance arterioles and capacitance veins.

I) drugs promoting fluid retention:

a) Fludrocortisone: Fludrocortisone (Florinef; 9-alpha flurohydrocortisone) is the most commonly used drug to alleviate OH, being first reported to alleviate OH in 1959 [Hickler et al 1959]. It is usually initiated at a dose of 100μg at night. It is a synthetic mineralocorticoid and as such promotes sodium and water retention in a similar way to aldosterone. Studies have shown that there is indeed an increase in orthostatic cardiac output in AF patients treated with fludrocortisone [van Lieshout et al 2000]. There may be additional components to its

action. The fluid-retaining actions also seem to apply to the vessel walls themselves, and it has been shown that the pressor effects of amines in healthy subjects are increased by fludrocortisone [Tobian & Redleaf 1958; Schmidt et al 1966]. Fludrocortisone does not have direct pressor action but may increase both α- and β-adrenoceptors in AF patients [Bannister et al 1981; Davies et al 1982]. This enhancement of adrenergic pressor activity rather than the fluid retention may be the major factor in the treatment of OH in AF at therapeutic doses [Davies et al 1979].

Fludrocortisone is usually fairly well tolerated if the total daily dose is no greater than 200μg. However, a significant number of patients will need to discontinue this drug because of side-effects. A study of 64 patients with OH found that 33% had had to discontinue fludrocortisone within 5 months of starting, most of these because of hypertension, cardiac failure or peripheral oedema [Hussain et al 1996]. It is interesting that although 24% of these patients developed hypokalaemia, in no case was the fludrocortisone stopped because of this. These side-effects help to emphasis that it is important to take into account the underlying condition causing OH. Whilst diabetic patients are prone to develop both OH and heart failure, patients with primary autonomic failure (such as MSA and PAF) are unlikely to develop cardiac failure, but are especially vulnerable to supine hypertension, which can be exacerbated by fludrocortisone.

Liquorice (containing glycyrrhizic acid) increases blood pressure, sodium and fluid retention by a mineralocorticoid action. The mechanism involves inhibition of the enzyme 11 (beta)-hydroxysteroid dehydrogenase which normally converts cortisol to cortisone [Sigurjonsdottir et al 2001; Quaschning et al 2001]. Cortisol but not cortisone has a marked mineralocorticoid action resulting in enhanced sodium and water retention. Although we have encountered patients who have found that taking liquorice improves their OH symptoms, there are marked risks of hypokalaemia which can be severe and occur at low doses [Olukoga et al 2000;

Stormer et al 1993; Cumming et al 1980] . For these reasons self medication with liquorice is probably best avoided unless regular checks of potassium levels are made.

b) DDAVP: DDAVP (desmopressin) is a synthetic vasopressin analogue. Vasopressin is an agonist at both V1 and V_2 receptors, causing vasoconstriction (of arterioles and venous capacitance vessels), and fluid retention (by action on the renal tubules) respectively. DDAVP however, is a selective agonist at V_2 receptors, and so promotes fluid retention without vasoconstriction. These points allow DDAVP to be given at night to reduce nocturia in AF patients without exacerbating supine hypertension. Thus, extravascular and intravascular volumes will be less depleted, and so OH is improved, especially in the early morning when OH is normally at its worst [Mathias et al 1986]. DDAVP is normally taken at night by tablet or intranasal spray at a dose of 200μg orally or 20μg intranasally. The major complication is that of hyponatraemia, which can develop soon after initiation, and so it is important to monitor serum sodium levels when commencing DDAVP [Mathias et al 1986, Mathias & Young 2003].

c) NSAIDS. Non-steroidal anti-inflammatory drugs (NSAIDS) are known to promote sodium retention, and thus fluid retention. There are likely to be several mechanisms, including reduction of prostaglandin dependant chloride reabsorption and increased sodium re-uptake in the renal tubules [Oats et al 1988a; Oats et al 1988b]. This fluid retention manifests as clinical oedema in up to 5% of all patients on NSAIDS [Whelton & Hamilton 1991]. In autonomic failure other factors, such as increased sensitivity to angiotensin II and noradrenaline, may contribute towards a pressor effect [Davies et al 1980]. Studies have suggested a possible role for NSAIDS in OH. Interestingly the type of NSAID used may be important, with indomethacin appearing to have the most promising results, being even more effective than fludrocortisone in treating postural hypotension in Shy-Drager patients in one small study.

The significant morbidity associated with chronic NSAID use tends to limit the use of these agents [Kochar & Itskovitz.1978; Bjarnason & Macpherson 1989; Pirson et al 1986]

II) drugs enhancing vasoconstriction

a) Ephedrine. Ephedrine is an orally active alkaloid with agonist action at both α and β adrenoreceptors [Hardman & Limbird 2001; Parfitt 1999]. Pressor effects occur both through activation of α_1 receptors on resistance vessels, and via β_1 receptor action causing increased heart rate. This lack of specificity of action means that ephedrine is contraindicated in patients with tachyarrhythmias and increases the risk of myocardial damage in susceptible individuals [Cockings & Brown 1997]. Ephedrine crosses the blood brain barrier, and insomnia and restlessness are well recognised side-effects [Hardman & Limbird 2001; Parfitt 1999]. Ephedrine has been shown to improve OH in AF, but as it also increases supine blood pressure, it is best to avoid taking in the hours before bed time [Davies et al 1978]. It has a half life of between 3-6 hours, and so is normally taken 3 times daily, with each dose being 15mg-45mg [Parfitt 1999].

b) Noradrenaline. Noradrenaline has been recently shown to be of value in PAF and MSA with OH refractory to other treatment [Oldenburg et al 2001; Kribben et al 1998]. In these cases patient-controlled ambulatory noradrenaline infusion via a port-a-cath system has been shown to dramatically improve control of OH, although such invasive treatment would clearly require careful patient selection. Attempts have been made to enhance endogenous noradrenaline concentrations in sympathetic ganglia using a combination of tyramine (which releases catecholamine from pre-synaptic storage vessels) and monoamine oxidase inhibitors

(which enhance this action of tyramine). Such an interaction has been shown to improve control of OH but this effect may be marginal and associated with marked exacerbation of supine hypertension [Nanda et al 1976; Davies et al 1978].

c) Midodrine. Midodrine (2',5'-dimethoxyphenyl-beta-glycinamide-ethanol hydrochloride) is an oral agent leading to peripheral vasoconstriction by activation of α_1 adrenergic receptors on veins and arterioles [Wright et al 1998; McClellan 1998]. Its actions are mediated by the active metabolite desgymidodrine, which is produced by hydrolysis of midodrine in the circulation. Maximal effect is reached 1 hour after taking midodrine. It has been shown to cause a significant increase in standing BP in patients with OH caused by a number of mechanisms [McClellan et al 1998; Young & Mathias 2004]. The risks of supine blood pressure in patients with OH associated with AF can be greatly reduced if the final daily dose is taken at least 4 hours before going to bed because of the short half life of midodrine, and a TDS regime is usually employed with individual doses between 2.5mg to 12.5mg [Wright et al 1998; McClellan 1998]. Although there is considerable experience of the use of midodrine over the past decade it is still not formally licensed for the treatment of OH in the UK. Side-effects are usually mild, most commonly involving piloerection, parasesthesia, scalp pruritis, cutis anserina ('Goose bumps'), formication and altered taste and smell perception [McClellan et al 1998; Young & Mathias 2004]. Overall midodrine is well tolerated and has been shown to be more effective than ephedrine in the treatment of OH [Fouad-Tarazi et al 1995; Low et al 1997; McClellan et al 1998].

d) Octreotide: Octreotide is a synthetic analogue of somatostatin, and like this endogenous compound it causes vasoconstriction of the splanchnic vascular bed, reduction of secretion of gastroenteropancreatic hormones, including insulin, and reduction of secretion of anterior pituitary hormones such as growth hormone [Battershill & Clissold 1989]. Octreotide has a longer half life (1.5 hours) than somatostatin, but has the disadvantage of requiring subcutaneous injection, reaching peak plasma values 30 minutes after injection [Parfitt 1999].

Postprandial exacerbation of OH can be a debilitating problem for patients, especially those with AF. The mechanism is thought to involve enhanced insulin release and dilation of the splanchnic vasculature after meals rich in carbohydrates [Mathias 1997]. Octreotide has indeed been shown to improve post-prandial hypotension, especially when used in combination with midodrine [Armstrong & Mathias 1991; Hoeldtke et al 1998]. However, octreotide also appears to improve OH not related to food intake [Hoeldtke et al 1991; Alam et al 1995; Bordet et al 1995]. Usually octreotide is prescribed for severe post-prandial hypotension, with the patients injecting themselves just before meals. The main side-effects relate to abdominal pain and diarrhoea.

e) Glypressin: Glypressin (triglycyllyseine-vasopressin) is a synthetic vasopressin analogue acting as a V_1 agonist without action on V_2 receptors. The resultant vasoconstriction of venous capacitance and arteriolar resistance vessels has been shown to improve OH in AF [Kochar et al 1985; Rittig et al 1991]. This pressor effect has been shown to be related to increased total peripheral resistance (TPR) rather than cardiac output [Rittig et al 1991]. Interestingly there may be a link between reduced endogenous vasopressin levels and the degree of OH in AF [Zeber et al 1983].

f) L-Threo-DOPS: L-Threo-DOPS (L-DOPS; L-threo 3,4-dihydroxyphenylserine; Doxidropa) is a synthetic noradrenaline precursor which is active both orally and intranasally [Suzuki et al 1982; Ando et al 1995]. L-DOPS has been clearly shown to reduce OH and its symptoms in MSA, PAF and haemodialysis patients [Tohgi et al 1993;Yoshizawa et al 1999; Mathias 2001]. Whilst not changing BP in healthy controls, L-DOPS leads to a peak increase in serum noradrenaline 3-5 hours [Suzuki et al 1982; Parfitt 1999]. Interestingly, L-DOPS also appears to have a central action, increasing muscle sympathetic activity (as measured by tibial nerve microneurography) in both healthy subjects and MSA patients [Nordenfelt & Mellander 1972; Tohgi et al 1993]. The MSA patients showed a rise in muscle sympathetic activity 30 minutes to 3 hours post L-DOPS ingestion, after which time the OH returned

despite peak levels of serum NA occurring at this time [Tohgi et al 1993]. These results suggest that the central actions of L-DOPS may be more important to its pressor effect than its peripheral production of NA. An additional factor in OH control may be the increase in Angiotensin II levels and rennin activity observed 2 weeks into L-DOPS treatment in MSA patients [Tohgi et al 1993]. Overall, L-DOPS appears to be well tolerated and in Parkinsonian subjects has additional benefits of reducing episodes of freezing. L-DOPS is usually taken orally, with the dose of 300mg twice daily being recommended as optimum [Tohgi et al 1993; Mathias 2001}.

g) Dihydroergotamine: Dihydroergotamine is a semi synthetic ergot alkaloid which has occasionally been used to treat OH via its vasoconstrictive actions [Nordenfelt & Mellander 1972; Parfitt 1999]. The major draw back to Dihdroergotamine is its poor bioavailability; whilst it can be delivered by parenteral routes, this tends to be more appropriate for its other main indication, that of acute migraine treatment, than several times daily chronic use as for OH [Bobik et al 1981]. Supine hypertension is exacerbated and nausea, vomiting and vasospasm are recognised side-effects [Parfitt 1999].

iii) drugs working via other mechanisms

a) **Erythropoietin:** Erythropoietin (EPO) increases haematopoiesis in response to hypoxia and has been shown to improve OH in autonomic failure [Hoeldtke & Streeten 1993; Biaggioni et al 1994; Winkler et al 2002]. Autonomic failure can be associated with erythropoietin deficiency anaemia, presumably because renal sympathetic activity stimulates erythropoietin secretion [Beynon 1977; Biaggioni et al 1994; Winkler et al 1999; Winkler et al 2001; Winkler et al 2002]. Although EPO increases haematocrit in these patients, there was not an associated increase in plasma

volume [Hoeldtke & Streeten 1993; Winkler et al 2002]. The major mechanism appears to be related to an increase in total peripheral resistance, likely to be in turn due to increased oxygen tension reducing NO-mediated vasodilatation [Rao & Stamler 2002]. The available data suggests that anaemic patients with AF and OH could benefit from EPO. However, EPO is not without risks, notably of cardiovascular morbidity and mortality, is expensive, and a formal randomised trial of its effectiveness in OH is still awaited [Besarab et al 1998; Rao & Stamler 2002].

b) **Pyridostigmine:** The reversible cholinesterase inhibitor pyridostigmine has recently been proposed as an alternative treatment for orthostatic hypotension. Pyridostigmine has long been used for myasthenia gravis as it delays breakdown of acetylcholine (Ach) at the skeletal neuromuscular junction. It produces a similar effect at sympathetic ganglia, thus enhancing existing sympathetic drive. It thus has been proposed as a "smart" drug that acts mainly when required (when the subject is upright leading to increased sympathetic activity) [Singer et al 2006]. Thus the theoretical attraction is that increased vasoconstrictive effects would not occur when the subject is supine (as existing sympathetic activity is less), reducing the risk of supine hypertension. In practice the effect on orthostatic hypotension in MSA and PAF is modest at 60mg and concerns remain about side-effects with 29% of subjects who initiated maintenance pyridostigmine bromide discontinuing therapy [Gales BJ 2007].

iv) Future drugs to aid postural hypotension

a) NOS inhibitors: Nitric Oxide (NO) is a powerful vasodilator produced from L-Arginine by the enzyme Nitric Oxide Synthase (NOS). In healthy humans NO is involved in the basal

regulation of vascular tone [Palmer et al 1987]. NO-mediated vasodilatation has been shown to be exaggerated in humans after sympathectomy [Charkoudian et al 2002]. NOS inhibition by agents such as L-NMMA may also cause increase in blood pressure in healthy young subjects. Some studies have shown that infusions of L-Arginine lower BP in normal and hypertensive subjects, and in patients with AF L-Arginine has a profound hypotensive effect [Ebel et al 1993; Kimber et al 2001]. It is possible that this marked hypotension is caused by an increase in NO production caused by the L-Arginine, but there are some questions regarding this.

b) **Endothelin agonists/antagonists**: Endothelin (ET) is one of the most potent vasoconstrictors known. The normal increase in serum ET on assuming upright posture may be impaired in OH [Kaufmann 1991]. Its vasoconstrictor actions are primarily mediated via ETA receptors. ETB receptors modulate this action increasing ET breakdown and also leading directly to vasodilatation by an NO related mechanism. Inhibition of ETB receptors by the ET-B receptor antagonist BQ788 leads to vasoconstriction but without increase in blood pressure in healthy subjects by enhancing the action of ET [Strachan et al 1999]..

An additional way to enhance the vasoconstrictor effect of ET is to reduce its breakdown by inhibition of Neutral Endoeptidase (NE). NE is a membrane bound enzyme involved in the breakdown of ET. It is also important in metabolism of Angiotensin II, ADH and bradykinin. However, studies in healthy patients show that not only is the predominant action of NE inhibitors to cause vasoconstriction without increase in blood pressure, but that only the inhibition of ET (with ETA inhibitor) is sufficient to prevent this vasoconstriction. ACE inhibition does NOT prevent the vasoconstriction implying that ET action is the predominant mechanism. The Neutral Peptidase inhibitor Candoxatril does indeed cause vasoconstriction in normal subjects [Ferro et al 1998].

Neither of the above agents has yet been evaluated for use in treating OH, but provides an interesting future area of research.

CONCLUSIONS: OH is a common and debilitating problem. Exclusion of treatable causes such as medication, hypovolaemia (including rare pathologies such as Addison's disease and common ones such as dehydration) should be initially performed. Most other cases will respond to non-pharmacological methods. If OH persists despite these, then the possibility of AF should be considered and, if suspected, evaluated with formal autonomic function tests. The next stage of treatment will normally take the form of fludrocortisone 100μg once daily, increasing if needed to 200μg once daily, with vigilance for hypokalaemia being required. Excessive fluid retention is the other major problem which may be encountered, and for this reason fludrocortisone is not recommended for dialysis patients with OH, and leg oedema may be considered an unacceptable side-effect in some other patients. If fludrocortisone alone is insufficient ephedrine is usually added at a dose of 15mg-45mg three times daily. Contra-indications include tendency to tachyarrhythmias. Midodrine can be added in if required at a dose of 2.5mg-12.5mg three times daily. Both midodrine and ephedrine should be avoided in the hours before bedtime due to the risk of exacerbating supine hypertension.

If early morning hypotension is a major problem, especially when associated with nocturia, DDAVP (typical doses 200μg orally or 20μg intranasally) may be tried at night. Serum sodium should be checked for evidence of hyponatraemia. Post-prandial hypotension, if not improved by the reduction of carbohydrate component of the meal, may respond to octreotide subcutaneous injections, typically 50-100μg up to three times daily just before a meal. Finally, in AF OH is sometimes exacerbated by anaemia. If these patients exhibit resistant

OH, then erythropoietin can be tried by subcutaneous injection (such as 50units/kg body weight 3 times weekly). The various other medications described in this review and elsewhere in the literature are probably best only considered in cases resistant to the medications described above.

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1.2: Multiple System Atrophy (MSA)

Introduction

MSA is a sporadic, progressive adult-onset disorder characterized by autonomic dysfunction, Parkinsonism and ataxia in any combination [Gilman et al 1998 & 1999]. The condition we now know as MSA has undergone several changes in nomenclature over the years. It is even possible that James Parkinson's first case in his classic 1817 paper 'An Essay on the Shaking Palsy' was in fact a case of MSA [Quinn 1994], although on reading the original paper, reprinted recently, this distinction is less clear [Parkinson 1817]. The first report of sporadic olivopontocerebellar atrophy was in 1900 [Dejerine and Thomas 1900], with additional pathological involvement of the basal ganglia confirmed in 1918 by Stauffenberg [Stauffenberg 1918]. At the beginning of the 1960s two separate subtypes of MSA were described with striatonigral degeneration [van der Eecken et al 1960; Adams et al 1961] and orthostatic hypotension predominant MSA [Shy & Drager 1960]. The term MSA itself was introduced by Graham & Oppenheimer [Graham & Oppenheimer 1969], although it was twenty years later before the hallmark glial cytoplasmic inclusion bodies were described, giving neuropathological justification for the description of MSA as a group [Papp et al 1989]. Nine years later sub-cellular description of these inclusion bodies as being consistently α-synuclein positive was the next major step forward [Wakabayashi et al 1998 and Spillanti et al 1998]. Classification of MSA has been modified to incorporate these new findings from Quinn's first proposal for a unifying classification in 1989 [Quinn 1989] to the Consensus guidelines of Gilman et al [Gilman et al 1998 and 1999]. Originally 3 sub-groups of MSA were described: MSA-C (cerebellar type), MSA-P (Parkinsonian-type) or Shy Drager Syndrome (where autonomic dysfunction is the major feature). The new Gilman

criteria have encouraged a generalized diagnosis of 'MSA' in which initial presentations may be broadly divided into two, namely MSA-P for striatonigral degeneration with predominant Parkinsonian features (SND) and MSA-C for olivopontocerebellar atrophy with predominant cerebellar atrophy (MSA-C), with the older Shy-Drager syndrome with prominent autonomic features being discouraged. Both SND and MSA-C have similar life expectancies [Ben-Shlomo et al 1997] with SND being more common in the West (49% of cases) [Geser et al 2005], although up to two-thirds had additional cerebellar dysfunction [Gilman et al 2005]. Increasingly it appears that both the final clinical and neuropathological endpoint is similar no matter what the presentation [Ozawa 2004] and as such MSA can be treated as a unified group. It is possible that MSA still represents a heterogeneous group of aetiologies, but there is not clear evidence that clinical features of investigations can reliably split the diagnosis further at present. For this reason the studies described in this thesis, all using clearly established cases of MSA established not only with the Gilman criteria but also in most cases with additional investigations such as Clonidine or sphincter EMG testing, have addressed MSA subjects as a group rather than attempting to further subdivide them along arbitrary lines. Sub-set analysis of my studies with MSA separated into MSA-C, MSA-P or Shy Drager failed to show any significant differences in results during my studies, further supporting this view. Studies, mainly based in the USA, have attempted to clarify the epidemiology of MSA. An example is a 14-year survey [Bower et al 1997], which followed people over the age of 50 in Minnesota. No convincing evidence of a potential environmental toxin has been yet suggested, although the possibility of increased risk amongst farmers exposed to pesticides, as with PD, remains, with a weakly protective effect apparently provided by a history of smoking as with PD [Vanacore 2005].

Clinical Features of MSA

The incidence of MSA has been estimated at 3 new cases per 100,000 per year [Bower et al 1997] placing it as an uncommon but not rare condition on a par with Huntington's disease and Motor Neuron disease. Point prevalence is similar at 1.9-4.9 per 100,000 [Chrysostome et al 2004]. Males may be slightly more affected than females (1.3:1) [Wenning et al 1997]. Although still referred to as a sporadic disease, the possibility of rare cases with family history has recently been raised [Soma et al 2006]. Unfortunately there is a relatively poor prognosis following diagnosis, with a mean survival of 6-9 years with onset usually in the 5th-6th decade of life [Ben-Shlomo et al; Watanabe et al 2002]. Cases can present as young as the late 30's however, and there is substantial variation of disease progression with survival of more than 15 years after diagnosis being possible. The main features include autonomic failure, Parkinsonism, cerebellar ataxia, and pyramidal signs in any combination. In a prospective study of 87 MSA subjects in the first year of their diagnosis in the USA, there were 56 male and 31 female subjects with a mean age of 63+/-8.6 years (mean+/-standard deviation) [Gilman et al 2005]. In this group, Parkinsonian and autonomic symptoms were the commonest presentations. About a third had experienced postural syncope with more experiencing presyncope events in the upright position. Erectile dysfunction was almost universal in the male subjects and this is invariably one of the earliest symptoms in male subjects. Examination confirmed Parkinsonian features in the majority of these patients, with bradykinesia, postural instability of the Parkinsonian type and rigidity affecting most subjects. Two-thirds showed postural tremor, one third had a resting tremor with symptoms being persistently asymmetrical in up to a third. Unsteadiness and falls with ataxia of gait, speech and limbs related to cerebellar dysfunction were present in most patients. Dysphagia was common with urinary incontinence and incomplete bladder emptying with constipation reported in a large majority. Sleep disorders occurred in approximately two-thirds of patients, with vivid dreams in about half, often occurring years before other symptoms. Interestingly in

view of emerging cognitive research in MSA, almost one-third of subjects complained of memory impairment. Sweating impairment may also be present but is variable and will usually not be a symptom volunteered by the patient unless directly asked for.

Although pathological and clinical studies show that MSA is ultimately a fairly homogenous condition whatever the initial symptoms, two major initial presentations are still described. In MSA-C the initial symptoms reflect the prominent cerebellar involvement with dysfunction leading to ataxia of gait, kinetic limb movements, speech (scanning dysarthria) and eye movements. Pure cerebellar disorders of course may present in a similar manner, but in MSA the additional features, including autonomic and Parkinsonian features will eventually declare themselves. Between 29-33% of patients with apparently isolated cerebellar ataxia will ultimately go to develop MSA [Gilman et al 2000; Abele et al 2002]

An apparent limb kinetic tremor can develop in MSA-P presentations of MSA, although this actually reflects a jerky postural tremor. Resting tremor, characteristic of PD, occurs less commonly in MSA, although akinesia and rigidity is often present. Despite this and the frequent presence of OH, early falls would be more characteristic of progressive supranuclear palsy (PSP). Orofacial or craniocervical dystonia when present can be severe, and other related disorders such as head drop (antecollis) would be atypical for idiopathic PD [Wenning et al 2003]. Dystonia of the vocal cords may also occur, leading not only to a high-pitched strained voice but to a danger of aspiration pneumonias. Finally, although the Parkinsonian features may initially be responsive to levodopa in nearly a third of cases, up to half of those so treated develop dyskinesias [Boesch 2002].

Clinical diagnostic criteria

Quinn first proposed clinical diagnostic criteria to subdivide cases into both possible and probable MSA, and then into striatonigral-degeneration type (SND) or olivopontocerebellar-atrophy type (OPCA) MSA based on the dominant motor symptom- Parkinsonism or cerebellar ataxia respectively [Quinn 1989]. A definite diagnosis of MSA could only be made with neuropathological confirmation. Unfortunately, despite providing a good specificity, the Quinn criteria have since been shown to offer relatively poor sensitivity based on clinicopathological studies [Litvan et al 1998]. Therefore internationally accepted consensus criteria have been developed for the clinical diagnosis of MSA [Gilman et al 1998 & 1999]. The Consensus criteria are used worldwide for clinical studies involving MSA, and have been used throughout this thesis in establishing MSA subjects for the studies. As with the Quinn criteria, MSA is categorized into possible, probable or definite MSA (pathologically confirmed by the presence of a high density of glial cytoplasmic inclusions in association with a combination of degenerative changes in the nigrostriatal and olivopontocerebellar pathways.). The further subdivision into MSA-C or P is no longer key to the diagnosis, reflecting the ultimate clinical and neuropathological similarities.

In practice these criteria may be applied to the diagnosis of MSA as follows [adapted from Gilman et al 1999]:

Exclusion Criteria: a) HISTORY: If onset is under 30 years of age, a family history of similar condition, another significant systemic condition which could explain the features, or hallucinations unrelated to medication.

b) EXAMINATION: DSM IV criteria for dementia, prominent slowing of vertical saccades or vertical supranuclear gaze palsy or evidence of focal cortical dysfunction such as aphasia, alien limb syndrome, and parietal dysfunction.

c) INVESTIGATIONS: Metabolic, molecular genetic and imaging evidence of an alternative cause of features

B) <u>Clinical domains, features, and criteria used in the diagnosis of MSA:</u>

Possible MSA *requires 1 criterion plus 2 features from separate other domains. When the criterion is Parkinsonism, a poor levodopa response qualifies as one feature (hence only one additional feature is required).*

Probable MSA *requires criterion for autonomic failure or urinary dysfunction plus poorly levodopa-responsive Parkinsonism or cerebellar dysfunction.*

I) Autonomic and urinary dysfunction Domain

Features: Orthostatic hypotension (by 20 mm Hg systolic or 10 mm Hg diastolic), urinary incontinence or incomplete bladder emptying.

Criteria: Orthostatic fall in blood pressure (by 30 mm Hg systolic or 15 mm Hg diastolic) or urinary incontinence (persistent, involuntary partial or total bladder emptying, accompanied by erectile dysfunction in men) or both

II) Parkinsonism Domain

Features: Bradykinesia (slowness of voluntary movement with progressive reduction in speed and amplitude during repetitive actions) or Rigidity or Postural instability (not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction) or Tremor (postural, resting or both)

Criteria: Bradykinesia AND at least one other Parkinsonian Feature

III) Cerebellar Dysfunction Domain

Features: Gait ataxia (wide based stance with steps of irregular length and direction) OR Ataxic dysarthria OR Limb ataxia OR Sustained gaze-evoked nystagmus

Criteria: Gait ataxia AND at least one other cerebellar dysfunction feature

IV) Corticospinal tract dysfunction Domain

Features: Extensor plantar responses with hyper-reflexia

Criteria: NIL

Additional "Red Flags" suggestive of MSA [adapted from Wenning et al 2004]
Atypical spontaneous or levodopa induced dystonia or dyskinesia mainly affecting orofacial muscles, occasionally resembling risus sardonicus of cephalic tetanus.
Axial dystonia (Pisa syndrome (subacute axial dystonia with a severe tonic lateral flexion of the trunk, head, and neck) or early severe camptocormia)
Disproportionate antecollis (Chin on chest, neck can only be passively and forcibly extended to its normal position with difficulty; despite severe chronic neck flexion, flexion elsewhere is minor).
Abnormal respiration (including inspiratory stridor and classically involuntary deep inspiratory sighs and gasps which are seen frequently in MSA. Sleep apnoea (arrest of breathing for >10 s), and snoring increased from premorbid level.
Intermittent loss of muscle atonia and appearance of elaborate motor activity (striking out with arms in sleep often with talking or shouting) associated with dreaming
Cold hands or feet (Coldness and colour change (to purple or blue) of extremities not caused by drugs Although may be exacerbated by ergot derived agents), blanching on pressure and poor circulatory return)
Emotional incontinence (i.e. inappropriate crying without sadness or laughing without mirth)

Table 1:1 Red flags to aid the diagnosis of MSA [Adapted from Wenning et al 2004]

Investigations

As outlined above, the diagnosis of MSA using the Consensus guidelines is based on clinical history and neurological examination. These guidelines do suggest that additional investigations can be used to further support the diagnosis, although they play no part in classifying into possible or probable MSA mainly because the results of investigations into MSA are not well known at the early stage of presentation when a diagnosis needs to be made. Autonomic function tests play an important role not only in supporting the diagnosis but also monitoring the progression of such features as orthostatic hypotension, which is a treatable symptom. In a similar way sleep studies and video fluoroscopic swallow testing help monitor the progress of respiratory and swallowing impairments, which may be at least modifiable factors.

Cardiovascular Autonomic Function Testing

Orthostatic hypotension (OH) is one of the most striking cardiovascular abnormalities that can develop in MSA but may be clinically silent, and is not an uncommon finding in healthy subjects over 50 years of age. OH is usually assessed with tilt table testing is part of standard autonomic function testing. In our autonomic function laboratory subjects are investigated after a stable haemodynamic baseline had been obtained after 20 minutes supine. The subject is then secured to the tilt table test and tilted head-up to 60^{o} for 10 minutes (or less time if not tolerated) before being returned to supine. BP and HR monitoring is measured both with Intermittent brachial BP values every 3 minutes using an automated Dinamap (Critikon) sphygmomanometer on the left arm and continuous measurement of beat-to-beat BP is simultaneously obtained non-invasively throughout with the Portapres II device on the middle finger of the right hand. OH, defined as a drop in systolic blood pressure of 20 mm Hg or more (or 10 mm Hg or more in diastolic blood pressure), compared with baseline

is assessed for on upright tilt, although supine to seated to standing posture may also be used in addition to the tilt table testing [Gilman et al 1999], although mild OH is not an uncommon finding amongst otherwise healthy subjects in a similar age range. In addition to OH, MSA subjects may also have a reduction in the expected increase in HR on upright posture.

The Valsalva manoeuvre allows assessment of the baroreflex pathways. The seated subject blows with an open glottis into a disposable syringe connected to the mercury column of a sphygmomanometer, attempting to maintain a forced expiratory pressure of 40 mmHg for 10 seconds. This normally causes a rise in intrathoracic pressure, leading to reduction in venous return and BP with increase in HR whilst the pressure is maintained. In healthy subjects this action induces an increase in sympathetic activity, resulting in a brief BP overshoot compared to baseline when the pressure is released. Normally this is rapidly compensated by a reduction in HR caused by activation of the baroreflex response to the BP overshoot. In sympathetic cardiovascular failure the initial sympathetic activation is impaired, thus when the pressure is released the BP remains low and only slowly returns to baseline. If there is parasympathetic impairment the HR does not markedly increase during the Valsalva manoeuvre itself. The Valsalva manoeuvre occurs over such a short time frame that beat-to beat assessment with devices such as the Portapres are essential in its analysis. Various derived ratios may be obtained from this test. One of the commonest derived is the Valsalva ratio which is the maximum rise in HR during the manoeuvre (phase II) divided by the minimum HR in the first 30 seconds after the pressure is released (phase IV). This ratio is usually 1 or less in autonomic failure. There are problems with this test however, with not all subjects able to perform it adequately, especially with the motor complications often found in MSA. In addition the test simultaneously measures facets of both parasympathetic and sympathetic activity. It therefore has a limited role in analysis of AF. A clearer test of parasympathetic cardiovascular function is probably the measure of HR variability on deep breathing. Age adjusted values for healthy subjects are available for comparison [Mathias

and Bannister 1999] However questions had been raised about the ability of abnormal cardiovascular autonomic function tests to differentiate conditions such as MSA and PD [Riley and Chelimsky 2003].

Pressor tests provide testing of the sympathetic nervous system and elicit a transient increase in systolic and diastolic blood pressure (SBP and DBP) and increased heart rate (HR) [Mathias 1999; Mathias 2004]. There are 3 standard pressor stimuli: Isometric Exercise (IE) evoked by sustained handgrip; Mental Arithmetic (MA) evoked by the subject performing serial subtractions; Cold Pressor (CP) evoked with application of an icepack to the hand. All three stimuli lead to pressor effects by activation of the sympathetic nervous system, but the pathways involved differ. CP evokes a pain response involving of Aδ and C afferent fibres and subsequent activation of brainstem structures leading to increased sympathetic outflow [Hilz et al 2002; Petrovic et al 2004]. With MA, by contrast, there is initial activation of central areas such as the right insula and right anterior cingulate, resulting in increased sympathetic outflow [Critchley et al 2000]. IE has important peripheral and central input to the medullary vasomotor centres, although there have been contrasting views as to the relative importance of these inputs in the generation of the IE pressor effect it is currently felt that both play a significant role [Alam and Smirk 1937; Victor et al 1989; Winchester et al 2000; Critchley et al 2000].

Urogenital Dysfunction-Bladder ultrasound and Urodynamics

Urogenital dysfunction is common in MSA. Erectile dysfunction is one of the earliest symptoms in Male MSA subjects, being almost universal [Kirchhof et al 2003]. Neurogenic bladder dysfunction may actually be commoner in early stages of MSA than OH [Sakakibara et al 1999]. Both incontinence and retention can be early problems. Whilst the incontinence can severely affect quality of life, and may predispose to reduced fluid intake in an attempt

to control symptoms, retention can result in urinary tract infection with occasionally serious sequelae. Detrusser failure appears to be a major mechanism [Ito et al 2006]. Whatever the underlying cause, uro-dynamic and static (such as bladder ultrasound) studies allow assessment of important variables such as post-void residual volume. At much over 100mls the risk of recurrent urinary tract infections is high and measures to reduce the volume such as clean intermittent self-catheterization (CISC) may be beneficial to reduce this risk. Unlike in PD, MSA subjects tend to show stress incontinence from an early stage, with urodynamic studies showing a particular pattern of progression. Detrusser hyper-reflexia, with abnormal urethral sphincter function (causing frequency and urge incontinence) occurs early in MSA. With advancing disease the bladder may become atonic, leading to increased post-void residual volumes [Kirby et al 1986].

Sphincter Electromyogram (EMG)

Due to the frequent early involvement of Onuf's nucleus in MSA, denervation of the external anal and urethral sphincters occurs. As with the denervation that occurs in motor neurone disease, the resultant spontaneous muscle activity and polyphasia that results may be detected with needle EMG. Although the technique can be challenging both for the operator and patient, some 80% of MSA cases show these findings [Palace et al 1997]. The prevalence of abnormalities in early stages of MSA is unknown. Although fairly sensitive, sphincter EMG has problems of specificity, especially in multiparous women (vaginal deliveries) where obstetric complications may similarly cause denervation changes in the sphincters [Colosimo et al 2000]. Similar changes have also been found in PD and PSP [Valldeoriola et al 1995; Giladi et al 2000]. Despite these limitations, sphincter EMG in experienced hands seems to a useful tool in helping to establish a likely diagnosis in early cases when other confounding factors have been excluded [Vodusek 2001].

Other Investigations

Polysomnography: REM-sleep behaviour disorder may precede other clinical features of MSA, sometimes by many years [Plazzi et al 1997]. This is likely to related to both marked striatal monoaminergic deficit and subcortical and brainstem disease in MSA with damage to the sleep atonia cells of the pons. In addition respiratory signs may be unmasked during polysomnography. Nocturnal or diurnal inspiratory stridor and classically involuntary deep inspiratory sighs and gasps are seen frequently in MSA. Sleep apnoea (arrest of breathing for >10 s), and snoring increased from premorbid level, or newly arisen, are important, not merely from a diagnostic perspective but also have therapeutic relevance with CPAP treatment sometimes indicated.

Video fluoroscopy: Dysphagia is a serious complication of MSA, and is closely related to overall prognosis [Muller et al 2001]. Both video fluoroscopy and manometry may be used to investigate dysphagia. As with idiopathic Parkinson's disease, the oral phase of swallowing was most commonly involved, with a delay in bolus transport from the oral cavity to the pharynx being the commonest dysfunction, found in over two-thirds of MSA. In nearly half of cases bolus holding in the oral cavity was also disturbed, with slowing of the upward relocation of the larynx was also found in over a third [Higo et al 2003]. The bolus transport phase was affected to a greater extent when cerebellar symptoms were prominent, presumably relating to poor tongue coordination impairing control of the food bolus [Ryuzaburo et al 2005]. These authors also raise a very important point, namely that aspiration can still occur in MSA even if it is not detected on standard video fluoroscopy. This may reflect the fact that video fluoroscopy is performed under optimum conditions, not always reflecting the conditions of everyday swallowing. Testing the cough reflex may increase the sensitivity of aspiration risk assessment [Addington et al 1999].

Pupillograhy: Pupil abnormalities may be found in MSA, although not universally. Iris atrophy and anisocoria were reported in both of the original cases of Shy and Drager's patients [Shy and Drager 1960]. Since then the finding of anisocoria has been replicated, although some patients with MSA may have in fact had had unilateral Horner syndrome. Up to 70 % of MSA cases appear to have normal pupils, despite extensive autonomic dysfunction elsewhere [Thomas et al 1970].

Blood Tests: Blood testing usually has a limited role in MSA management. Catecholamine levels and growth hormone levels following clonidine administration have been used to help in the diagnosis of MSA. Supine plasma noradrenaline levels are within the normal range, reflecting the preserved postganglionic sympathetic efferent activity, with Noradrenaline (NA) spill over from nerve terminals into the blood [Mathias 1999]. On upright tilt however, increases in NA are reduced relative to normal, reflecting the lack of sympathetic activation because of central sympathetic outflow obstruction in MSA [Mathias 1999]. Intravenous infusion of the selective α_2-adrenoceptor agonist clonidine has been used in the investigation of Parkinsonian syndromes, as clonidine-induced release of growth hormone (GH) is impaired in MSA but spared in pure autonomic failure (PAF) and PD where the lesion site is postganglionic [Thomaides et al 1992]. Similarly, both circadian release, and release secondary to postural challenge, of the hormone ADH from the posterior pituitary is impaired in MSA [Benarroch et al 2006], presumably because of hypothalamic involvement. Other roles of blood sampling include the occasional need to exclude celiac disease or SCA (spinocerebellar atrophy) genetic mutation in certain atypical presentations. Many MSA subjects are prescribed fludrocortisone to treat postural hypotension. As a mineralocorticoid, the possibility of hypokalaemia needs to be borne in mind and periodic potassium measurements may be indicated.

Cerebrospinal fluid (CSF) Analysis: Increased CSF levels of neurofilament light chain and tau and decreased levels of 3-methoxy-4 hydroxyphenylethyleneglycol have been found to relatively specific in differentiating the cerebellar subtype of multiple-system atrophy from idiopathic late-onset cerebellar ataxia. CSF potentially could provide a useful marker, as it is contiguous with the extracellular fluid of brain structures. Neurofilament light chain (NFL) protein appeared to have the greatest specificity. NFL proteins appear to have an important role in the axonal microenvironment, being involved in cytoskeletal plasticity. [Lycke et al 1998]. CSF examination however is not standard practice in the work-up of MSA, not least because it requires an invasive procedure (lumbar puncture) and NFL protein estimations are not widely available.

Imaging

MRI

MRI scanning of the brain has been investigated as a tool both to strengthen the diagnosis of MSA and to monitor its progression. This later aspect is an important baseline to establish now that trials of neuroprotective agents in MSA have begun [Geser et al 2005]. It has been known for some time that MRI may potentially show characteristic abnormalities in the striatum, brainstem, and cerebellum in patients with MSA. This has been used to aid differentiation of early MSA-P from PD in some research studies. Striatal abnormalities include putaminal abnormalities such as atrophy, T2 hypointensity and ‘‘slit-like’ marginal hyperintensity (Fig. 1.1). Only half of MSA-P subjects have putaminal slit changes on T2 within 3 years after symptom onset, increasing to 86% after 6 years [Horimoto et al., 2002]. Of putaminal changes, the atrophy appears to be most specific for MSA, whilst hyper-or hypointensities may also be seen in idiopathic PD [Bhattacharya et al.2002; Seppi 2005]. Exact sensitivities are difficult to calculate as MRI strength (in Teslas), slice thickness and

use of conventional or fast spin echo images will affect the image quality. The slit-like hyperintensity sign is likely to reflect gliosis [Schwarz et al 1996]. Brainstem changes include atrophy (especially of the pons and lower brainstem, middle cerebellar peduncles, and cerebellum). These areas may also demonstrate T2 weighted hyperintensity changes including the classical "hot cross bun" sign when the pontocerebellar degeneration takes a cruciform appearance (fig 1.1) [Bürk et al 2001; Bhattacharya et al., 2002; Seppi et al 2005]. This sign is felt to be due to degeneration of pontocerebellar fibres leading to hyperintensities in the pons and middle cerebellar peduncles, but is not unique to MSA [Muqit et al., 2001]. Cerebellar involvement primarily involves atrophy, which is often but not always associated with cerebellar dysfunction.

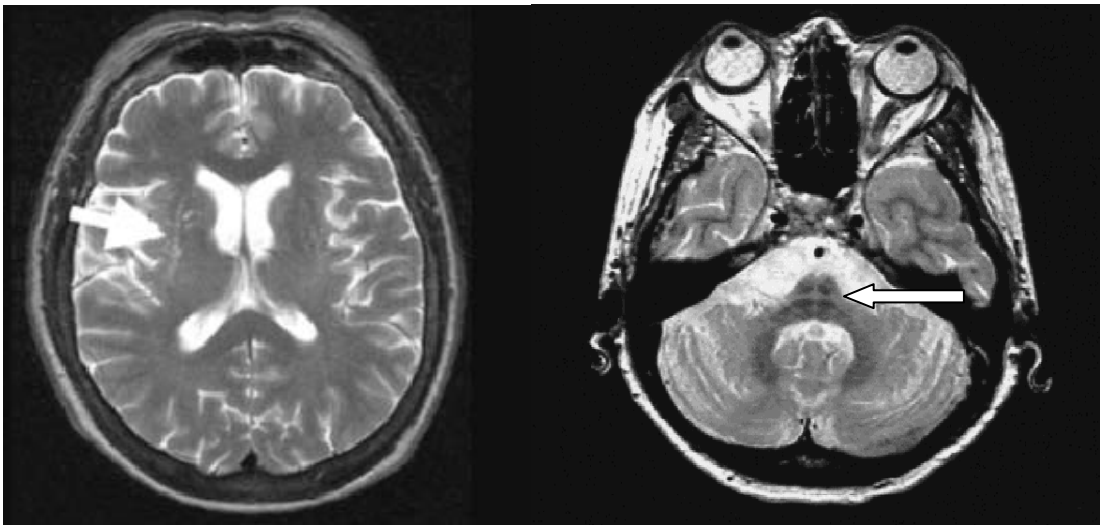

Figure 1:1: Slit-like putaminal hypointensity (left image-arrowed) [Seppi et al 2005] and hot cross bun sign (right image-arrowed) with extensive pontine, middle cerebellar peduncle and cerebellar atrophy [Bürk et al 2001]

Advanced MRI related Techniques

Whilst conventional MRI focuses primarily on structural findings, Proton magnetic resonance spectroscopy (1H-MRS) studies brain metabolism. 1H-MRS detects several metabolite signs, including N-acetylaspartate (NAA), choline (Cho) and creatine /phosphocreatine (Cr). NAA reflects the integrity of neurons, Cho the integrity of membranes and glial activity, with Cr being a marker for general metabolism. Reduced NAA/Cr ratios in the lentiform nucleus and the pons have been reported in MSA [Davie et al 1995; Watanabe et al. 2004]. There is evidence to suggest that the increased resolution provided by increased Telsa strength MRIs may further enhance the usefulness of 1H-MRS in diagnosing MSA at an early stage.

Diffusion-weighted imaging (DWI) is often included in standard MRI sequences in clinical use and utilizes the diffusion of water, classically used to diagnose acute ischaemia. It utilizes the fact that, whilst intact nerve tracts and other cellular architecture closely restrict the ability of water to diffuse, damage to these structures increases the ability of water molecules to diffuse in random directions. Quantification of diffusion is performed by applying differing field gradients, from which the apparent diffusion coefficient (ADC) is derived. The trace of diffusion tensor is then calculated from the sum of ADCs measured in three orthogonal directions allowing assessment of brain structures damaged by either acute or ongoing insults. DWI may be more accurate than IBZM SPECT in the differential diagnosis of MSA-P versus PD [Seppi et al. 2004]. Magnetization transfer imaging (MTI) sequences provide further details which are based on the application of irradiation selectively saturating the energy level of bound protons, inducing an exchange of magnetization between bound and free protons, thus reducing the signal intensity of bound protons. Magnetization transfer ratios (MTRs) are then calculated, correlating with the degree of myelinisation and with axonal density [Seppi et al 2005].

Atrophy may be assessed accurately by use of MRI-based volumetry. By focusing on the volume reduction in specific areas such as the striatum and brainstem it has been possible to fairly accurately distinguish MSA from PD and PSP although there was some overlap of cases [Schulz et al. 1999]. Comparison of brain FDG PET and MR volumetry has suggested near equal ability at diagnosing MSA, with no further gain in accuracy if the techniques were combined [Ghaemi et al. 2002)]. The use of voxel-based morphometry has highlighted more distant atrophy in MSA in the cortex, presumably reflecting striatal projections [Ghaemi et al.2002].

MRI may also be used to measure the progression of MSA. At present this is primarily a research tool but gaining clinical relevance in the era of trials of neuroprotective agents in MSA. Measurement of MSA progression by MRI and is also helpful in evaluating the sequence of changes as MSA progresses. Such studies have suggested that putaminal DWI parameters may be correlated with motor severity in patients with MSA, thus providing a marker for disease progression [Schocke et al., 2002, 2004].

MRI thus provides various techniques which may be used to potentially increase the diagnosis of early MSA, and to follow the progression of changes as the disease advances, both to provide a means to estimate disease progression, and to better understand the underlying pathological changes. Most of these sequences are used only for research projects and the diagnosis of MSA is still a clinical or neuropathological one based on consensus criteria [Gilman 1998;1999]. Of all the MRI techniques, that of DWI seems to show most promise for a wider clinical application in the future. DWI images are widely available on most MRI scanners, only take minutes to acquire, and show promise in the early detection of MSA. It is possible, if the early studies are confirmed with larger cohorts, that in the future MRI changes may be added into the formal MSA diagnosis criteria, as has been seen with the changes in multiple sclerosis diagnosis criteria in 2001 [McDonald et al 2001].

Functional imaging

Whilst MRI mainly focuses on structural changes, such as volume loss and effects on diffusion, functional imaging provides a means to examine the dynamic ability of the brain to function in terms of metabolism (usually based on glucose) or receptor binding. As the striatal receptors have been well studied in Parkinson patients, it is the dopaminergic system, which is often targeted in functional imaging of MSA, especially where there is an initial Parkinsonian presentation. Presynaptic nigrostriatal neurons can be imaged by assessment of dopa-decarboxylase activity and dopamine transporters. Postsynaptic dopaminergic neurone function is assessed by use of dopamine D2-receptor ligands. For example, PET studies ligands such as ^{18}F fluorodeoxyglucose [Perani et al 1995] have been able to distinguish the striatal of MSA-P and PD patients, with the differentiation aided by combination with dopamine D2-receptor functional imaging (receptor density being lower in MSA than in PD [Ghaemi et al 2002]

The dopamine transporter system has been studied with single-photon-emission CT using ^{123}I-2β-carboxymethoxy-3β-4-iodophenyl-tropane (β CIT). This system is affected in MSA, PD, and PSP and so is only useful in the reliable differentiation of these conditions from healthy controls [Kim et al 2002]. Extracranial sites have also proved useful in functional imaging studies of MSA, with single-photon-emission CT and PET ligands allowing study of cardiac sympathetic innervation. Iodobenzamide and metaiodobenzylguanidine single-photon-emission CT and fluorodeoxyglucose PET may be helpful in detecting early MSA. Scintography with ^{123}I-metaiodobenzylguanidine has shown loss of binding in patients with even early PD reflecting postganglionic sympathetic denervation, whilst MSA shows preserved binding, presumably reflecting the central, preganglionic lesion sites. This technique has been shown in provisional studies to have a high sensitivity (90%) and

specificity (95%) in distinguishing PD and MSA [Braune 2001]. As might be expected PAF, with its postganglionic sympathetic involvement, has evidence of cardiac sympathetic denervation as evidenced by 18F-dopa PET, compared to MSA where sympathetic innervation was again preserved [Goldstein et al 1997].

Pathology

The initial presentation of MSA can be reflected in the site of lesion burden. In MSA-P, the striatonigral system is the main site of pathology, reflecting the clinical phenotype, but degeneration normally includes the olivopontocerebellar system [Kume et al1993;Wenning et al 1996]. The putamen is shrunken with grey-green discolouration (figure 1.2) mainly effecting caudal and dorsolateral regions [Kume et al 1993]. The substantia nigra pars compacta also show degeneration, although cells of the pars reticulata are reported as normal [Wenning et al 2004]. Striatal cell loss or impaired function of D1 and D2 receptors in the striatum explains the lack of L-Dopa responsiveness in many cases of MSA.

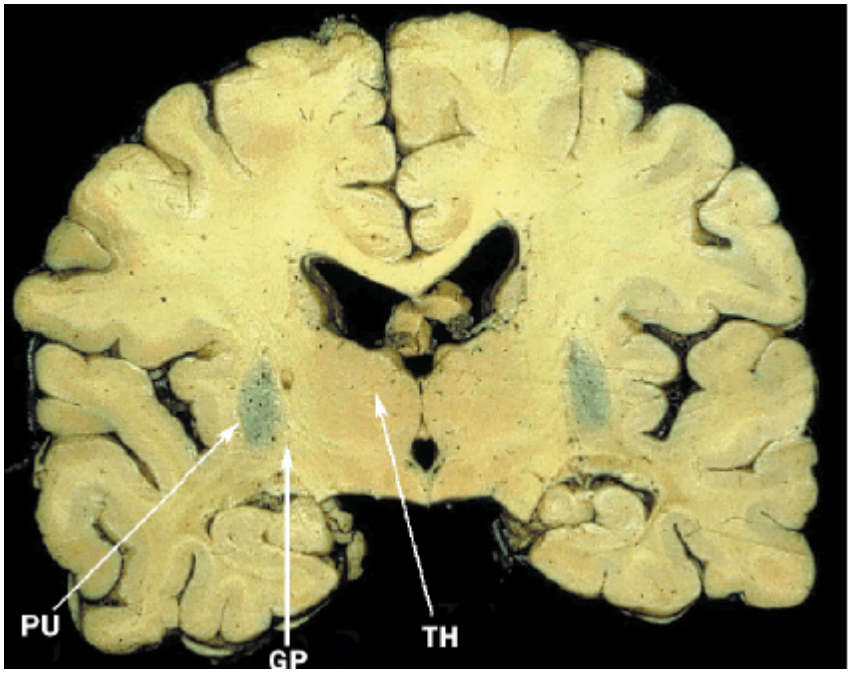

Fig 1:2 Putaminal (PU) and Globus palidus (GP) atrophy with grey-green discolouration of putamin. TH= thalamus [Wenning et al 2004].

With a MSA-C presentation, the reverse is true with the burden of lesion sites involving the olivopontocerebellar system, such as atrophy of the basis pontis and middle cerebellar peduncles, but the striatum and substantia nigra are also involved. Cell loss in the inferior olives, pontine nuclei and cerebellum explain the cerebellar ataxia, including nystagmus.

Despite these apparent differences in subtype of MSA, there is no difference between the frequency of neuronal cytoplasmic inclusions (NCI) in the putamen, pontine nuclei, and inferior olivary nucleus between MSA-P and MSA-C clinical subtypes, and one recent large post-mortem study failed to find any cases of pure SND (MSA P) or OPCA (MSA-C) from pathological studies [Ozawa et al 2004]. GCIs are consistently found in MSA despite the presentation [Papp et al 1989].

Autonomic involvement is evident as the disease advances no matter what the presentation. The dorsal motor nucleus of the vagus nerve, catecholaminergic neurons of ventrolateral medulla and locus coeruleus all show marked involvement [Sung et al 1979; Wenning et al

1997; Benarroch et al 1998]. Whilst spinal cord degeneration of sympathetic preganglionic neurons in the intermediolateral column has been postulated to result in OH, the degree of atrophy is not clearly correlated with degree of OH [Daniel 1999]. Erectile and bladder dysfunction appear to relate both to spinal involvement (parasympathetic preganglionic nuclei), and supraspinal involvement of the pontomedullary reticular formation [Papp et al 1994] . The spinal involvement localises to S2-4, including Onuf's nucleus [Konno et al 1986]. This preganglionic cell loss in the spinal cord (intermediolateral cell columns), leads to detrusor hyperreflexia by virtue of loss of inhibitory input of the pontine micturition centre, whilst Detrusser atony leads to retention.

Additional sites of gross pathological damage in MSA include the cerebral hemisphere, of interest given recent reports of cognitive impairment in MSA, and anterior-horn cells in the spinal cord [Konno et al 1986; Sima et al 1993]. Severe cell loss in the nucleus ambiguus, or resultant atrophy of posterior cricoarytenoid muscles help explain the laryngeal stridor which can be a serious complication in late MSA, and rarely the presenting feature [Hayashi et al 1997; Glass et al 2006.]. Loss of neurones in the ventrolateral region of the nucleus ambiguus includes loss of preganglionic autonomic outflow to the heart, and its dysfunction appears to contribute to the cardiovagal impairment in MSA [Hopkins et al 1996].

Subcellular Neuropathological damage in MSA

Wherever the gross pathological changes of MSA are found, the characteristic subcellular finding is that of the glial cytoplasmic inclusions (GCI), first found in 1989 [Papp et al 1989]. The fact that these inclusions are predominantly glial rather than neuronal in location sets MSA apart from other neurodegenerative conditions where the characteristic lesions are focused within the neurones themselves. GCIs are argyrophilic and half-moon, oval, or

conical in shape. GCIs distribution largely reflects the gross neuropathological locations such as basal ganglia, supplementary and primary motor cortex, reticular formation, and pontocerebellar system [Papp et al 1994]. Whilst the inclusions contain ubiquitin and tau, α-synuclein appears to be the major component of both GCIs and neuronal cytoplasmic inclusions (NCIs) in MSA [Cairns et al 1997;Wakabayashi et al 1998]. Although the exact function of α-synuclein is unknown, it is known to be a presynaptic protein which may be important in vesicle induced signalling. Whilst other disease processes such as Parkinson's disease, Alzheimer's disease, Lewy body disease, Amyotrophic Lateral Sclerosis and even PAF are all known to show inclusions with α-synuclein (Synucleinopathies), the inclusions in these other disease processes tend to involve neurones not glial and involve deposition of α-synuclein filaments which are insoluble in buffered saline, unlike in MSA [Jellinger 2003]. What is unclear is whether the α-synuclein contributes to the pathogenesis of MSA, or is a by-product of the underlying aetiology. The burden of GCI does reflect the severity of MSA in both olivopontocerebellar and striatonigral systems [Inoue et al 1997]. GCIs appear to occur before established neuronal damage in MSA, with myelin loss seemingly taking place earlier than axonal damage, at least in the pons [Wakabayashi et al 2005]. Similarly, early MSA cases have shown functional dysfunction in the brainstem (hypometabolism), and this may reflect the early autonomic failure sometimes seen in early MSA despite limited pathological evidence of neuronal damage [Wenning et al 1994; Taniwaki et al 2002].

Uncommon familial cases of PD have been associated with point mutations in the α-synuclein gene [Spillantini et al 1997]. Although cases of α-synuclein gene triplication have been reported to be associated with GCI like pathology, Lewy bodies are also present and the phenotype is that of Parkinson's disease [Gwinn-Hardy et al 2000]. However, no similar mutations have yet been identified in MSA, and oligodendrocytes (glial cells) may not even express α-synuclein mRNA at all [Ozawa et al 2001]. The possibility of glial cells taking up α-synuclein from damaged neurones using aberrantly expressed Rab5 endocytosis regulatory proteins found on MSA oligodendrocytes and then being unable to digest the protein leading

to inclusions is certainly possible [Nakamura et al 2000]. Evidence that may support this includes the fact that that α-synuclein is found in CSF from healthy subjects, suggesting release from neurones [Borghi et al 2000]. Whether or not the aggregations are related to ectopic accumulation of α synuclein or due to low level expression with subsequent faulty processing (including hyperphosphorylation) within the oligodendrocytes, the GCIs not only provide a key pathological feature of MSA but are likely to hold some of the secrets of the underlying aetiology. Work in this field is continuing rapidly and hopefully will soon yield important new information.

Management

Unfortunately to date there is no effective treatment for the underlying neurodegenerative process in MSA, although studies of neuroprotective therapies are now being undertaken [Wenning et al 2005]. In the mean time there is still much that can be done in terms of supportive management for MSA patients and their carers. Non-pharmacological management, including input from speech, language and continence advisors together occupational/physiotherapists, is extremely important as the disease progresses, and sometimes provides a more relaxed forum for specific concerns to be raised than in the busy doctor's clinic. OH is not only a common problem in MSA, but can usually be controlled with adequate treatment. The non-pharmacological and pharmacological management of OH has already been discussed in some detail earlier in this thesis (1.1: The Autonomic Nervous System in Health and Disease) and so will not be dealt with here. Cerebellar dysfunction is extremely difficult to treat pharmacologically at present. As with other neurodegenerative conditions, if tremor or myoclonus are marked, clonazepam or antiepileptics such as valproate or even isoniazid or baclofen are sometimes beneficial.

For the Parkinsonian features, although less effective than in PD, L-dopa replacement may still be beneficial especially in the early stages. Motor improvement in up to 79% of MSA cases with L-Dopa replacement has been reported in cases defined by the Consensus criteria [Testa et al 2001]. The benefits of L-Dopa replacement may last for up to 4 years [Wenning et al 2005]. Dyskinesias, especially those affecting the orofacial area and characteristically unilateral, are unfortunately often induced by L-Dopa replacement even when there is no apparent motor benefit [Boesch et al 2002]. Dystonias, either arriving primarily from the underlying disease or from drug side-effects, may respond to injection of Botulinum toxin injections into the relevant muscles, although the injections may need to be repeated after a few months. Furthermore L-Dopa can unmask or worsen OH in MSA. From the limited studies so far performed, dopamine agonists appear to be ineffective in most cases. Amantidine, a non-selective NMDA antagonist, is also usually ineffective with a few notable exceptions. Anticholinergics, such as trihexyphenidyl, whilst not usually effective for Parkinsonian features, may be useful for dystonia, rigidity and sialorrhea [Wenning et al. 2005]. Alternative options for sialorrhea include injecting the major salivary glands with botulinum toxin, which has shown considerable promise as a therapy [Mancini et al 2003].

Urological dysfunction is often managed by non-pharmacological approaches, such as adequate fluid intake and clean intermittent self-catheterization (CISC) when post-void residual bladder volumes are elevated. Indeed, adequate fluid intake and laxatives also help prevent constipation, which is not only common in MSA but can itself cause urinary retention. CISC is not always possible however when either Parkinsonian or cerebellar dysfunction significantly impair insertion. In these circumstances a carer may perform catheterization, or a permanent indwelling catheter may be chosen. In such cases a suprapubic catheter is a better long-term option in terms of reduction in complications. Pharmacological treatment is based on peripherally acting anticholinergic agents such as oxybutynin that

improve detrusor hyperreflexia and sphincter–detrusor dyssynergy. Unfortunately by virtue of their action these agents may actually induce urinary retention and drying of the saliva. Newer agents such as Trospium chloride, a nonselective quaternary ammonium compound, are more selective for the bladder as opposed to the salivary glands [Wenning et al 2005]. Desmopressin may provide relief from nocturnal polyuria, and also indirectly improve postural tolerance during the day [Mathias and Young 2003]. Erectile dysfunction may be overlooked but can represent one of the most distressing symptoms. Use of phosphodiesterase inhibitors such as sildenafil citrate may help in some cases but its vasodilating action on blood vessels may exacerbate OH [Hussain et al 2001]. Yohimbine or intracavernosal injection of papaverine or even penile implants may also be beneficial in selected cases [Beck et al 1994].

Dysphagia is always a concern in MSA because of the risk of aspiration pneumonia. Frequent assessments with the aid of speech and language therapy are an important component of management. At first mild dietary modifications such as thickened fluids and double – swallow techniques may be sufficient. Ultimately however nasogastric feeding or feeding via gastrostomy tubes may be required. Similarly respiratory function is an important factor to consider on every visit; sleep apnoea may benefit from continuous positive airway pressure therapy whilst stridorous patients may require tracheotomies, although this is rarely indicated [Iranzo et al 2000]. Ultimately MSA unfortunately remains a fatal illness: end-of life decisions should ideally be carefully considered on an individual basis, and psychological support provided if needed for the patient and their carers. There is hope of additional therapies in the near future. Studies into the possible neuroprotective roles of minocycline and riluzole are nearing completion with stem cell and neural trophic factor intervention possibilities further afield.

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1.3: Pure Autonomic Failure (PAF)

Introduction:

Pure Autonomic Failure (PAF) was first described some 80 years ago and initially named Bradbury-Eggleston syndrome [Bradbury and Eggleston 1927].It is only since the more rigorous classification and diagnostic criteria for MSA that we can be now sure about the diagnosis of PAF as earlier cases may have been confused with early MSA. It is clear that PAF is a rare disease. Despite being able to live up to decades after the diagnosis is made, we have less than 40 clearly diagnosed cases on our recent casebook of PAF subjects at The National Hospital for Neurology and Neurosurgery, the National tertiary referral centre for autonomic dysfunction. It is possible that the true prevalence is higher as patients may put up with their symptoms for many years before diagnosis. Indeed, in our experience it is sometimes ironically the supine hypertension which can finally bring the OH to the attention of a neurologist, as this is sometimes treated with antihypertensives without a standing BP being taken, and the resulting exacerbation of OH leading to a referral or admission.

Clinical Features of PAF: Like MSA, OH is the major symptom in many cases at presentation although symptoms related to sudomotor dysfunction such as hypohidrosis are also common. OH is often more severe than in MSA, although the orthostatic tolerance which subjects can develop is astonishing- I have been able to hold a rational conversation with one subject whilst their standing SBP had dropped to 45 mmHg (symptoms were just starting to develop at this time point)! Unlike MSA Parkinsonian or cerebellar symptoms are absent. Hypohidrosis has been shown to be a common symptom in PAF at presentation and to occur significantly earlier in the disease course than in MSA [Mabuchi et al 2005]. Sweating

abnormalities are usually more pronounced than in MSA with prominent anhidrosis common although this will usually only be a major problem for subjects in hot climates where excessive body temperatures can develop. Exercise would theoretically also be dangerous for this reason but in reality most subjects avoid vigorous exercise because this further exacerbates OH. Exceptions can occur-one of our younger subjects has climbed Ben Nevis since his diagnosis, although he required very frequent stops to sit or lie down. As with MSA erectile dysfunction is very common in male subjects, although urological disorders are less commonly seen than in MSA, with only 3 out of 8 PAF subjects reporting urinary urgency, urinary frequency, or incontinence in the first five years of symptoms [Mabuchi et al 2005]. With longer duration of symptoms the difference in respiratory symptoms becomes striking. Up to 80% of MSA subjects will have developed respiratory complications in the first decade of symptoms whilst no PAF subjects did so in 30 years of follow-up [Mabuchi et al 2005].

In the first few years care has to be taken in making a diagnosis of PAF as occasional cases of apparently 'pure' autonomic failure will declare as MSA after even 3 or 4 years. For this reason all the PAF cases used in this study had had their symptoms for at least 5 years (& in most cases over 10 years), as well as having their diagnosis confirmed using current criteria, and further backed up with additional testing such as plasma noradrenaline levels.

Clinical diagnostic criteria:

Pure autonomic failure is an idiopathic sporadic disorder characterized by OH usually with evidence of more widespread autonomic failure. No other neurological features are present. Reduced supine plasma norepinephrine levels are characteristic of PAF. [Anonymous 1998]. Older terms such as idiopathic orthostatic hypotension have been superseded by PAF as it has increasingly been realised that initial presentation with apparently 'pure' autonomic failure may merely be the presenting features of other conditions such as MSA or Parkinson's disease with autonomic failure. In view of this it is generally recognised that a minimum

period of 5 years is needed from symptom onset without additional features developing for the diagnosis of PAF to be made with confidence [Freeman 2004; Mabuchi et al 2005].

Investigations

Autonomic Function Testing:

Orthostatic hypotension (OH) is one of the most striking cardiovascular abnormalities that can develop in PAF, and although it is usually not clinically silent, patients often instinctively adapt their day to day activities and posture so that its effects may not be immediately obvious even with striking falls in BP on upright posture. As with MSA OH is usually assessed with tilt table testing is part of standard autonomic function testing as described in the discussion on MSA (1.1 Multiple system atrophy (MSA)). In our autonomic function laboratory subjects are investigated after a stable haemodynamic baseline had been obtained after 20 minutes supine. The subject is then secured to the tilt table test and tilted head-up to 60^{o} for 10 minutes (or a shorter time if not tolerated) before being returned to supine. BP and HR monitoring is measured both with intermittent brachial BP values every 3 minutes using an automated Dinamap (Critikon) sphygmomanometer on the left arm. Continuous measurement of beat-to-beat BP is simultaneously obtained non-invasively throughout with the Portapres II device on the middle finger of the right hand. OH, defined as a drop in systolic blood pressure of 20 mm Hg or more (or 10 mm Hg or more in diastolic blood pressure), compared with baseline is assessed for on upright tilt. Supine to seated to standing posture may also be used in addition to the tilt table testing [Gilman et al 1999], although mild OH is not an uncommon finding amongst otherwise healthy subjects in a similar age range. As with MSA, PAF subjects

usually show marked OH. In addition the expected HR rise on upright tilt is even more attenuated than in MSA. As with MSA the Valsalva manoeuvre, heart rate variability on deep breathing and pressor responses are also assessed and typically are markedly abnormal, these tests having been described in more detail in the previous chapter. Accurate comparisons of response to these standard autonomic function tests had not previously been reported, and are examined in this thesis (3.2: Validating Standardized Pressor Responses in MSA and PAF).

Urodynamics:

Although bladder involvement in PAF is a relatively late feature (mean time to onset has been quoted at 9 years after presenting complaint), eventually it is present in most PAF subjects [Mabuchi et al 2005]. In a study of 6 symptomatic PAF subjects, urodynamics revealed mixed results [Sakakibara et al 2000]. Post-micturition residuals were present in two subjects, small bladder capacities in two, detrusor hyperreflexia in four, and denervation supersensitivity of the bladder in two.

Polysomnography:

REM sleep behavioural disorder (RBD) appears to be an almost universal feature of synucleinopathies such as MSA, PD and diffuse Lewy Body Disease (DLBD) [Boeve et al 2004]. It is present in up to 90% of MSA subjects, often occurring years before more disabling features develop [Plazzi et al 1997]. In the late 1990s a study followed 10 patients on initial presentation of primary autonomic failure [Plazzi et al 1998]. These subjects had videopolysomnography performed and were followed up for approximately 5 years, after which time the diagnosis of MSA had been made in 4 with the remaining 6 being labelled PAF. Interestingly all 6 PAF subjects had had normal polysomnography whilst all 4 MSA

subjects showed signs of RBD with the histories suggesting onset years before. A later study of Videopolysomnography in 3 PAF subjects however revealed typical features of RBD with intermittent absence of muscle atonia during REM sleep in all patients. These episodes were accompanied by simple and complex motor behaviours like twitching and beating [Weyer et al 2006]. The discrepancy between the results of these two studies may reflect the likely greater duration of PAF in Weyer et al's group. Thus it appears PAF may exhibit RBD (possibly only later in the disease process) in keeping with the other synucleinopathies. Unlike MSA, severe sleep apnoea is not a noted feature, although mild apnoea was found in one PAF subject [Weyer et al 2006].

Pupillograhy:

Pupil abnormalities may be found in PAF [Shy and Drager 1960; Bremner and Smith 2006]. In the largest recorded study 67% of 33 PAF subjects had some form of pupillary abnormality. Almost 50% had bilateral Horner's syndrome (the bilateral ptosis being quite striking in some cases). Pupillotonia or reduced light/accommodation reflexes (reflecting predominant parasympathetic dysfunction) is less common [Bremner and Smith 2006]. The bilateral Horner's syndrome or pupillotonia were very specific in differentiating PAF from MSA although they did both occur in patients with autonomic failure caused by diabetes or amyloidosis. Interestingly in most patients the deficits had been detected by neither the patient nor their physician

Blood Tests:

Blood testing usually has a limited role in PAF management. Supine plasma noradrenaline levels are markedly reduced, reflecting the dysfunctional postganglionic sympathetic efferent

activity, with Noradrenaline (NA) spillover from nerve terminals into the blood reduced as a result [Mathias 2004]. On upright tilt, increases in NA are similarly reduced [Mathias 2004]. Recent suggestion of circulating antibodies binding to neuronal ganglionic acetylcholine receptors detected by a radioimmunoprecipitation assay requires further study to determine if PAF or autonomic neuropathy is the associated phenotype [Schroeder et al 2005].

Cerebrospinal fluid (CSF) Analysis:

CSF analysis has not usually been performed in PAF as it is not part of standard assessment, both because of the invasive nature of the procedure and the lack of any clear utility at present. One recent study looked specifically for antibodies reacting with structures of rat pons/medulla in patients with PAF as well as in MSA and controls [Imrich et al 2006]. No significant association was found.

Imaging

MRI:

Unlike in MSA, conventional MRI of PAF subjects fails to show significant abnormalities specific to the condition. Advanced MRI related techniques however have been used to show subtle distinctive features. Voxel-based morphometry in particular has suggested loss of grey matter density in cortical regions which have previously been shown to be involved in the generation and representation of bodily states of autonomic arousal [Critchley et al 2003]. During fear-conditioning, in PAF patients, the absence of peripheral arousal in response to a learnt threat-stimulus was associated with reduced activity in the amygdalar and insular regions which were activated in controls [Critchley et al., 2002]. These findings thus suggest

that the cingulate, insular, pontine, and amygdalar regions are involved in autonomic control, the afferent feedback loop of which is impaired in PAF. Voxel-based morphometry of 15 PAF subjects showed no global atrophy compared with controls, but a localised loss of grey matter density in the left anterior cingulate and right anterior insula/frontal operculum [Critchley et al 2003]. Only the degree of atrophy in the left cingulate gyrus clearly correlated with disease severity in the PAF subjects, possibly reflecting the relatively small size of this voxel-based morphometry study. In a PET study, increased right anterior cingulate activity was observed in PAF relative to control subjects during effortful tasks associated with increased sympathetic drive [Critchley & Mathias et al 2001]. Consolidating the results of these studies suggests that the left anterior cingulate may be involved in decreasing heart rate and blood pressures, and an earlier study had shown this area to be activated in healthy controls during volitional reduction in electrodermal activity [Critchley & Melmed, et al., 2001]. In such a system involvement of the left cingulate would exacerbate many of the symptoms in PAF, effectively leading to gradual disuse atrophy.

Critchley et al suggest that overall the observed localised grey matter atrophy seen in PAF reflected experience-dependent change resultant from loss of afferent input to brain regions involved in representation of autonomic states. Certainly increased stimulation has been suggested to result in increased grey matter density in taxi drivers in the posterior hippocampus that is implicated by functional neuroimaging in spatial navigation [Maguire et al., 2000]. Certainly this suggestion is of importance as PAF is considered essentially a peripheral and post-ganglionic disease in terms of major pathophysiology. The normal global grey/white/csf volumes as measured by this same study would add weight to the assumption that there is not widespread central dysfunction in PAF

.

Functional imaging:

As might be expected, PAF with its postganglionic sympathetic involvement has evidence of cardiac sympathetic denervation as evidenced by 18F-dopa PET, compared to MSA where sympathetic innervation is preserved [Goldstein et al 1997]. Functional brain imaging has been less clear cut, with a minority of cases studied suggesting possible striatonigral dysfunction [Brooks et al 1990; Compta et al 2006] in a few subjects.

Positron Emission Tomography (PET) scanning utilizing intravenous injection of 6-18F-fluorodopamine allows visualization of cardiac sympathetic innervation [Goldstein et al 1990]. In previous studies both PAF and PD with autonomic failure showed markedly reduced left ventricular myocardial concentrations of 6-18F-fluorodopamine–derived radioactivity [Goldstein et al 1997 & 2000]. Extracardiac post-ganglionic sympathetic denervation was suggested to involve decreased 6-18F-fluorodopamine–derived radioactivity in the thyroid gland and renal cortex in PD, with PAF subsequently being found to show a similar picture in the renal cortex but surprisingly showing preservation of sympathetic innervation to the thyroid [Li et al 2002; Tipre and Goldstein 2005]. Somewhat surprisingly given the marked sympathetic innervation of the nasopharyngeal region, neither PD nor PAF patients showed a reduction in sympathetic innervation on PET scanning. PAF has evidence of cardiac sympathetic denervation as evidenced by 18F-dopa PET, compared to MSA where sympathetic innervation was preserved [Goldstein et al 1997].

Pathology:

In the most complete autopsy of a PAF subject, performed 8 hours post-mortem, systemic autopsy findings were unremarkable except for mild cardiac hypertrophy, papillary muscle necrosis, moderate systemic atherosclerosis, moderate pulmonary emphysema and acute and chronic cystitis [Hague et al 1997]. Coronal sections of the brain revealed no gross

lesions. Inclusion bodies staining positive for α-synuclein have been identified in autonomic nerves in the epicardial fat, and capsular nerves of the adrenal and parasympathetic nerves in the bladder wall [Kaufmann et al 2001]. Inclusions were not encountered in the cerebral cortex or dorsal motor vagal nucleus. Postganglionic nerves were clearly involved with peripherally based lesion burden as expected for a postganglionic condition, with α-synuclein staining inclusions in the superior cervical ganglia sympathetic nerves (see Fig 1.3). However, in a single case report some Lewy bodies (not GCIs or NCIs) were seen in the substantia nigra pars compacta, locus ceruleus, sacral spinal cord (spinal tissue was difficult to assess due to severe ischaemic necrosis). One other autopsy case suggested moderate neuronal loss in the substantia nigra, locus ceruleus and intermediolateral cell columns of the spinal cord; however this case had previously been embalmed casting doubt on the relevance of a perceived moderate loss of neurons in these anatomical areas [Roessmann et al 1971]. Whether this was a chance finding reflecting age (63) of the patient or coincidental early disease process such as PD or Alzheimer's remains speculative. This same autopsy case had earlier been published. Although the only three earlier published cases of PAF autopsy had failed to note such central findings, technical factors may have reduced the yield in these cases [Bradbury and Eggleston 1927; Roessmann et al 1971; Ingelghem et al 1994]. Functional imaging has not significantly clarified the degree of striatal involvement, if at all, in PAF, with one study of fluorodopa F 18 positron emission tomography showing abnormalities of the nigrostriatal dopaminergic system in only 1 of 7 subjects, with a single case report suggesting straital involvement of the caudate and putamen with single-photon emission computed tomography with iodine 123 ioflupane [Brooks et al 1990; Compta et al 2006]. There remains the possibility that PAF is a "forme fruste" of PD in which the postganglionic autonomic lesions form the main clinical picture without progression [Oppenheimer 1980]. Whilst some doubt thus remains about the degree of central involvement in PAF, both pathological and physiological studies strongly support the

peripheral, postganglionic dysfunction of PAF [Bradbury and Eggleston 1927; Roessmann et al 1971; Ingelghem et al 1994; Mathias 2004].

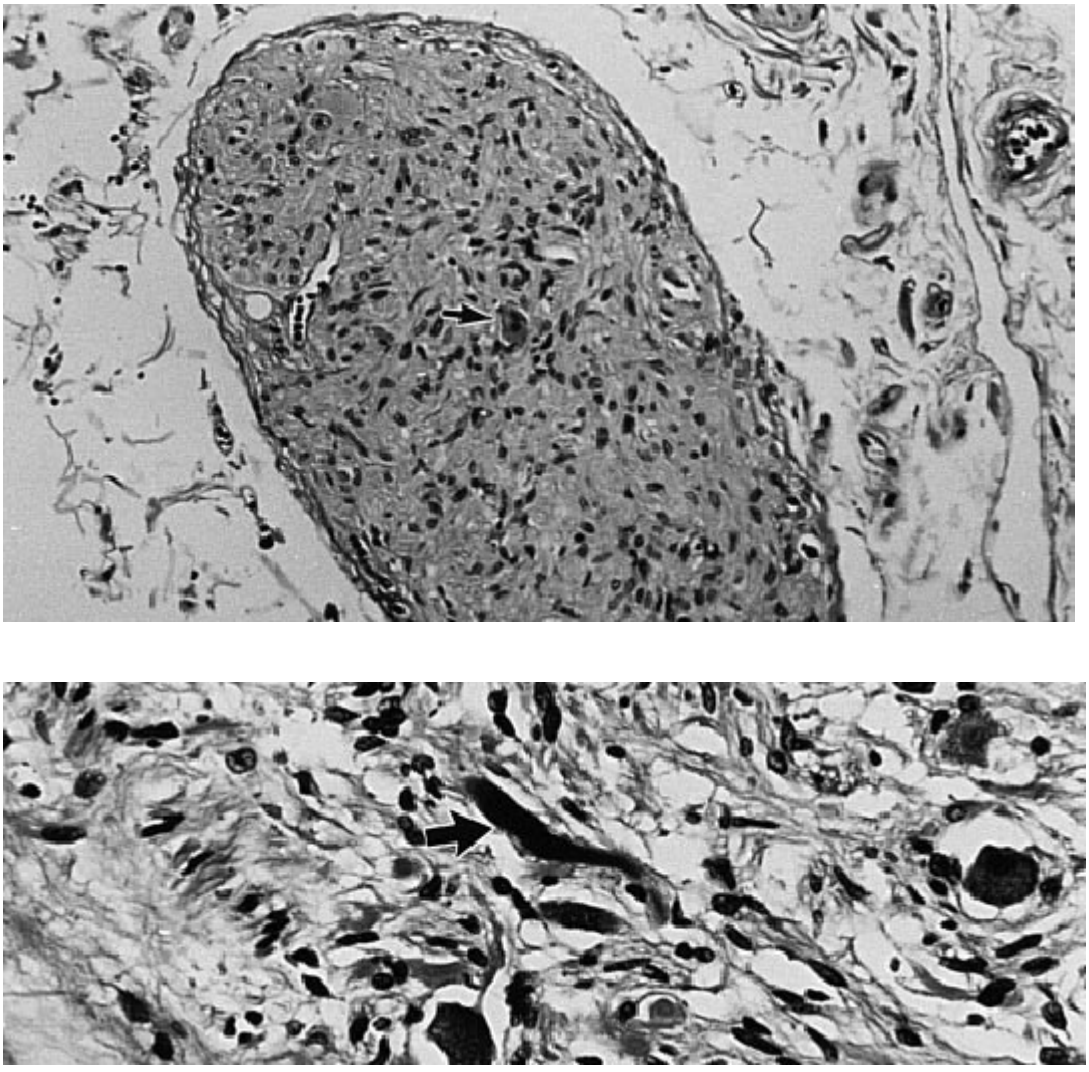

Fig 1:3 High power (X300) light microscope vie of H & E stained adrenal gland post ganglionic nerve (top picture) and sympathetic ganglion (lower picture) from PAF autopsy showing Lewy Bodies (arrowed). [Hague et al 1997]

Management:

Some of the management options discussed for MSA will be applicable to PAF, especially those concerning OH. The management of OH is considered in more detail in this thesis (1.1 The Autonomic Nervous System in Health and Disease). Given the possibility of survival for many decades post-diagnosis, concerns about the cumulative effects of supine hypertension in PAF are probably more relevant to PAF subjects in whom cardiac hypertrophy has been demonstrated [Vagaonescu 2000]. As such, a delicate balance of control of orthostatic hypotension should be employed, using measures such as head-up tilt at night and late-night meals where needed to minimize supine hypertension. It is important to realize that PAF, when well managed, is entirely compatible with a good quality of life. In one study activities of daily living were not significantly affected even many years after onset with survival up to 90 years of age reported [Mabuchi et al 2005].

Recently an exciting new approach to treatment has been suggested with immunotherapy. A case report of an apparent case of PAF with antibodies binding to neuronal ganglionic acetylcholine receptors [Schroeder et al 2005] demonstrated improvement of orthostatic hypotension following courses of plasma exchange. Whilst volume expansion from plasma exchange alone might be expected to improve orthostatic hypotension, the improvement in both the pressor responses and tests of parasympathetic function are not so easy to explain. An earlier case report of a subject who superficially resembled PAF with circulating antibodies to the ganglionic nicotinic AchR has been reported [Goldstein et al 2002]
In contrast to PAF however, the subject had normal left ventricular myocardial concentrations of 6-[18F] fluorodopamine-derived radioactivity and a marked increase in plasma NA on standing, suggesting that postganglionic sympathetic innervation was intact. Thus a label of autoimmune autonomic neuropathy maybe more appropriate than PAF. Whether or not some PAF subjects may have an autoimmune aetiology or not remains to be seen. For the present this approach raises possible hope of even better management of PAF in the future.

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1.4: Aims of Thesis

PAF primarily involves the post-ganglionic autonomic nervous system, whilst in MSA the pre-ganglionic structures are impaired. My central hypothesis is that this underlying neuropathological difference between MSA and PAF will lead to differing cardiovascular responses. By utililizing the non-invasive methods available in the autonomic laboratory the aim was to examine the differing cardiovascular responses in MSA and PAF to varying stimuli. In some cases (cold pressor, isometric exercise, inspiratory gasp) the stimuli were well established but the responses in MSA and PAF have not been. Other stimuli (such as water ingestion and inhaled CO_2) were more novel. It was hoped that such investigations would help further elucidate the underlying pathophysiology of these diseases. In the case of the water ingestion studies described in this thesis, a symptomatic benefit in orthostatic hypotension was demonstrated for the first time. By establishing differing responses in well-established cases of MSA and PAF it is hoped that future studies might usefully employ these methods to subjects first presenting with autonomic failure. At the initial presentation distinction between MSA and PAF is not always clear: better differentiation would be important for prognostication, management, and increasingly for better definition in selection of study populations for therapeutic trials.

2: Apparatus

1) Autonomic Laboratories:

All studies were performed in our dedicated autonomic laboratories at The National Hospital for Neurology & Neurosurgery Queen Square, London or at Imperial College at St.Mary's Hospital, London. These laboratories allowed the application of adequate experimental conditions including minimisation of ambient noise and controlled environmental conditions.

Temperature control was validated by a series of recordings based in the laboratories over a 4-month period in mid-year (April, May, June, July). Temperatures were taken from a wall-mounted mercury column thermometer based in the laboratory at mid-morning for each working day (Monday-Friday inclusive) on a randomly chosen week in each month.

Month	Temperature
April	24 °C
April	25 °C
April	24 °C
April	24 °C
April	23 °C
May	25 °C
May	22 °C
May	23 °C
May	23 °C
May	24 °C
June	25 °C
June	23 °C
June	26 °C
June	24 °C
June	23 °C
July	24 °C
July	25 °C
July	23 °C
July	25 °C
July	24 °C
MEAN TEMPERATURE:	24+/- 1 °C

Table 2:1 Autonomic Laboratory Temperature recordings over 3 months

The mean temperature of these recordings was 24+/-1^{0}C, the same value commonly quoted in published work by other authors for temperature references ranges in their autonomic laboratories. It is possible that temperatures in the laboratory would have been higher than this at the peak of summer, or lower in the midst of winter. Substantial variation would however seem unlikely given the consistency shown in Table 2.1 for each of the months tested. Furthermore, slightly higher temperatures in summer, and slightly lower temperatures in winter would probably have merely increased the standard deviation of the overall mean temperature rather than change the mean value itself.

2) Multi-Purpose Tilt Table (Akron):

For studies which included tilting in the protocol, an electronically operated, hydraulic tilt table was used. This was approximately 190cm in length and 70cm in width. The maximum recommended load was 180Kg, when distributed evenly. The table was fitted with lockable casters and stabilisers for safety in use. Additional security in upright positions provided by a footrest and body belts (2X narrow Belts, 1X Wide Belt), secured by means of Velcro strips threaded through the side chrome rails of the table. These straps were placed around the legs, waist and chest whilst the subject was still in the horizontal position. The body belts were placed to avoid excessive pressure on the subject, which might otherwise have compromised relaxed breathing. By the side of the tilt table, manually operated controls allowed raising or lowering of the table. In addition, these controls permitted tilting upright and back to horizontal. The degree of table tilt from the horizontal was gauged by means of a degree scale under the table, which was fixed, and a pointer permitted to move freely with the influence of gravity. Time to raise the bed from horizontal to 60^0 was 3 seconds, with similar time interval required to return the table to the horizontal from this inclination. Prior to each study a protective tissue cloth was placed on the table and changed at the end of each study. Between subjects the body straps were cleaned with disinfectant. Regular maintenance was carried out during the period of the studies.

Figure 2:1: Akron Multi Purpose Tilt Table ref:
http://www.akronproducts.co.uk/mutli%20tilt/index.htm

3) Dinamap automated oscillometric blood pressure recorder (Critikon)

A Dinamap automated oscillometric blood pressure recorder (including 1846SX model) was used throughout the studies of this thesis to obtain non-invasive BP & HR from over the brachial artery [Gorback et al 1991; Goonasekera & Dillon 1995]. The 1846SX (figure 2.2) has been shown by validation study to have a consistency of measurement compatible with research level requirements with accuracy of diastolic and MAP in particular comparable with intra-arterial measurements [Whincup et al 1992].

Figure 2:2: Dinamap on study subject

References:

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4) Portapres Model-II:

The Portapres (Model-II) is a non-invasive medical device used to monitor continuous finger arterial pressure waveform and to calculate estimates of SBP, DBP and HR. Subsequent Modelflow analysis can then be utilised to calculate additional cardiovascular indices such as CO, SV, TPR and EF. The Portapres (figure 2.3) is a lightweight device which fits around the wrist and is secured to the forearm with non-constricting Velcro straps. A hydrostatic height correction unit allows free movement of the arm in relation to heart level, and allows for measurements during tilting from the horizontal. Despite the fact Portapres can be used to record for up to 24 hours, and that no damage to fingers has resulted from the finger-cuff, patient discomfort from local congestion can arise with prolonged recordings. For this reason

on recordings in excess of 2 hours "finger-switching" is recommended. This method allows automatic switching between adjacent fingers fitted with appropriately sized cuffs after a set period, and thus allowing congestion in one finger to resolve whilst recording from the other finger. In practice finger switching was not required for the studies described in this thesis as the recording intervals were less than 2 hours. Thus the potential error of comparing arterial waveforms in different fingers was avoided. The Portapres calibrates itself at regular intervals during each recording (not less frequently than every 5 minutes) to prevent pressure drift. . The data from each study are stored on an in-built memory card, capable of storing more than 24 hours of finger arterial waveform data. The data were subsequently transferred to a PC (Dell Inspiron Laptop) via a serial link for beat-to-beat analysis with the Beatscope software.

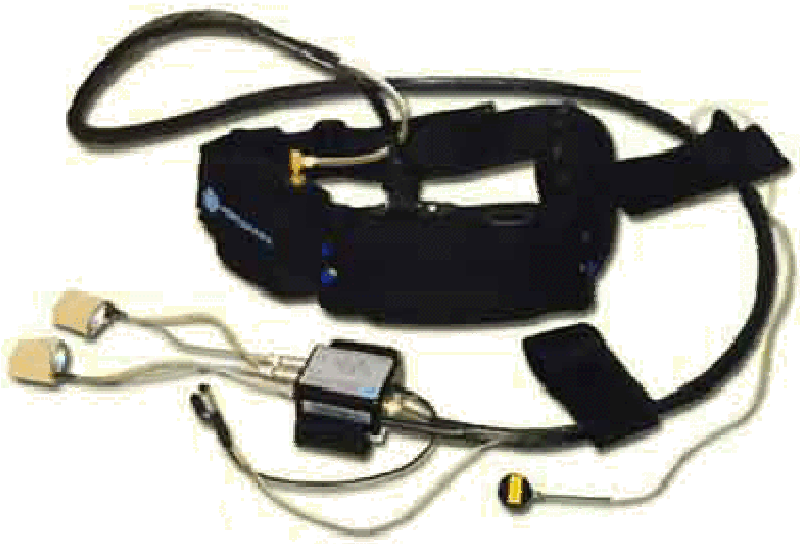

Figure 2:3 Portapres used for non-invasive beat-to-beat recording of cardiovascular indices. Two Velcro finger cuffs (bottom left) are shown connected to the fronted unit. Additional leads shown allow height correction to be performed prior to readings.

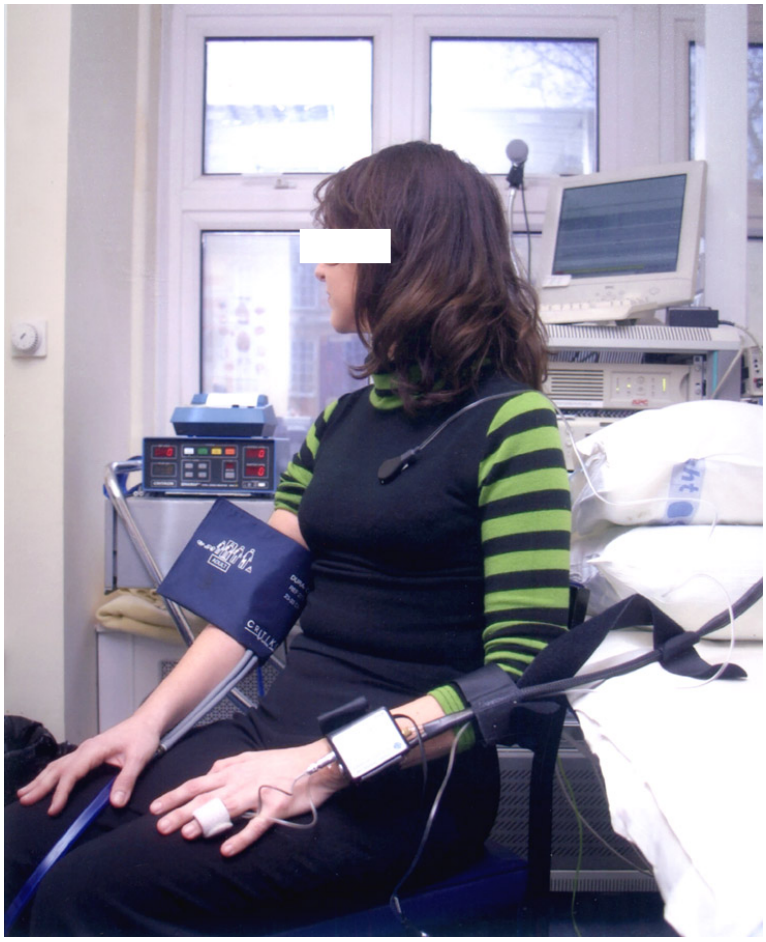

Figure 2:4 Portapres on left arm. Portapres height correction lead shown at heart level.

Power supply either from mains (using AC adaptor with a grounded 240 Volt 50/60Hz AC receptacle or batteries (Portapres non-rechargeable 13Ah Lithium batteries or Portapres rechargeable NiCd 2.4 Ah batteries). There is a low finger cuff LED current (<30mA), voltage (<1.8V) and power dissipation (<50mW) thus reducing risk of undue skin heating or electrical hazard. Any electrical short circuit in either finger cuff or main instrument, or an interruption in cable signal results in a cut off of cuff pressure within 1 second.

Fronted Unit

The fronted unit (see figure p21 Portapres2) contains the electronics for the infrared plethysmograph in the finger cuff and a switching valve to enable switching of finger cuffs if

required. In addition an air pressure control valve and pressure transducer is stored within the unit. The air pump unit is stored separately in Portapres waist belt (which also houses the battery if required, and the computer board of the Portapres, together with flash card for data storage). The pump unit, height correction unit, control unit and finger cuffs all attach to the fronted unit.

Finger cuff

Air pressure in the finger cuff is regulated to 380mmHg (0.5atm) by use of an electrically regulated air compressor in combination with a pressure transducer. In the event of internal computer malfunction, or cuff pressure of > 250mmHg sustained for 2.5 seconds, the cuff pressure is automatically released by means of a watch dog timer. During the initial start-up a pressure in excess of 295mmHg will result in cuff deflation. If fully occluded finger arteries are detected during the initial start-up the Portapres issues a warning display and shuts off the cuff pressure. Near full occlusion of finger arteries can increase the variability of readings. Such situations are highlighted on the Portapres display panel and in the data files by means of “?” or “!” signs in front of the displayed values.

The finger cuff consists of an air bladder and several layers of plastic, rubber material and Velcro. The cuff includes the electronic components such as Light Emitting Diode (LED) and photodiode of an infrared plethysmograph. The cuff is connected to the fronted unit by means of a cuff cable connector and air hose connector. The cuffs come in three sizes: small (white), medium (beige) and large (blue). These allow correct size fitting of cuffs to fingers so that the sides of the cuff bladder come into contact but do not overlap. The white cuff fits fingers with circumferences of 45-55mm; beige fits fingers with circumferences of 55-65mm; blue fits fingers with circumferences of 65-75mm). Once the correct size is chosen, the cuff is wrapped around the middle phalanx of a finger, ideally on the non-dominant hand to allow for activities using the dominant hand if required during a study. The orientation of the cuff is important with the LED and photodiode being placed symmetrically on the palmar surface of

the finger to allow maximal opposition with the two digital arteries in each finger. The cuff cable and air hose are then threaded between two adjacent fingers to the volar side of the hand to reach the fronted unit. The cuff cable and air hose may then be inserted into their respective sockets on the fronted unit. These steps are then repeated for a second adjacent finger if finger switching is required for the study. The finger cuff pressure means that the patient senses a slight pulsation in the cuffed finger synchronous to the heart beat.

Height Correction Unit

The Height correction unit (figure 2.4) is a liquid filled tube with a pressure transducer at one end. The transducer end is kept secured to the finger cuff (by means of Velcro attached to the Velcro of the cuff). The other end (reference end) contains a compliant plastic bag in the circular container which is kept at heart level. The height correction unit is attached to the Fronted unit by means of a height correction unit connector. Height correction is performed prior to each study by holding the both ends of the height correction unit at heart level and pressing the "Height" button on the Portapres control unit until height nulling has been confirmed on the display unit (see below). After confirming height nulling with the Start/Stop key the transducer end is attached to the finger cuff whilst the reference end of the height correction unit is taped at heart level.

Control Unit

The Control Unit has a 2 X 16 character Liquid Crystal Display (LCD) and a 6 key keypad (Figure 2.3 & 2.4). The control unit is connected to the Front Unit by means of a control unit cable connector. In addition a serial interface socket ("RS-232 to PC") can be used to download data from the Portapres flash card to the PC for analysis with Beatscope software.

Key	Main Function
Start/Stop	-Start a measurement -Stop a measurement -Confirm a query
Finger	-Set up finger switching mode -Arrow up key
Physio	-Switch on/off physiological calibrations
Event	-To mark events (exact event and time written down separately in log book) -Arrow left key
Height	-To perform initial height nulling allowing different hand and heart levels -Arrow down key
Output	-Perform analog output calibration. -Arrow right key

Table 2:2 Portapres Control Unit Keys

Control Unit Display:

When switched on but prior to a recording the display will show “Ready Mode”. The display shows time in hh:mm whenever event marker is pressed. During height nulling the display will signal when nulling has been successfully completed and subsequent confirmation by means of the Start/Stop key will return the display to ready mode. During recordings SBP and DBP are displayed (mmHg) as well as HR (bpm) as beat-to-beat values. Error signals appear on the display panel if the pump system fails to generate enough pressure, or if pressures are not stable enough. In addition, any problem with the Portapres circuitry or connections will also be displayed as an error.

Maintenance and calibration

The Portapres unit was regularly cleaned using a soft, slightly moistened cloth, avoiding the direct use of liquid to the unit. Calibration is carried out on a yearly basis by connecting a calibrated manometer to the air outlet of the fronted unit. Zero level is then checked and the arrow keys on the control unit used to increase the pressure in 50mmHg stages to 300mmHg. For each pressure level the accepted precision should be within 1% of the manometer reading

(for values outside these reference ranges TNO-TPD . These steps are performed for each of the two air outlets in the fronted unit.

5) Beatscope Software & Modelflow Measurement Principles

The analysis of the Portapres raw data is performed by Beatscope software supplied with the Portapres device. The Portapres measurements can be transferred to a PC for beat to beat analysis with BeatScope 1.1A Software to obtain brachial pressure waveforms and cardiac parameters using the ModelFlow cardiac output method. The Modelflow system unit is covered in some detail here as it is an important aspect of the beat-to-beat recordings employed in this thesis. The principle of haemodynamic measurement with the Portapres 2 is based on the volume-clamp method, first described by Jan Penaz [Penaz 1973] and the Physiocal system of [Wesseling 1995].The volume clamp method involves maintaining (clamping) the finger artery diameter, despite changing arterial BP, by means of a finger cuff with inflatable air bladder. Potential changes in arterial diameter are detected by the infrared photo-plethysmograph in the finger cuff which leads to appropriate changes in the finger cuff pressure by means of a fast pressure servo controller. Thus during systole the increase in BP results in increased light absorption from the LED and therefore a decrease in the signal detected by the plethysmograph. The Physiocal system is used to define and maintain the diameter at which the artery is clamped.

Volume-Clamp Method

Compliant arteries show greater changes with any given change in intra-arterial pressure than non-compliant ones. Arterial compliance is related to transmural pressure, which describes the difference between pressure inside the artery and that of the surrounding tissue. The pressure of the surrounding tissue is effectively regulated by the finger cuff. If this pressure is too high the arteries will almost be collapsed and diameter changes will be small for given

changes in intra-arterial pressure. Conversely, if the finger cuff pressures are too low the arteries will be distended and effectively of low compliance, with changes in intra-arterial pressure again having limited effects on arterial diameter. The optimum finger cuff pressure will maintain zero transmural pressure difference as arterial diameter changes in response to given changes in intra-arterial pressure will be maximal. Furthermore, a transmural pressure of zero means that the finger cuff pressure will provide an estimate of intra-arterial BP. At zero transmural pressure the artery is said to be 'unloaded'.

To maintain near-zero transmural pressure therefore, the finger cuff pressure must change, to oppose the changing intra-arterial pressure during each cardiac cycle. As outlined above, this is achieved by means of a negative feedback loop with the infrared photo-plethysmograph providing afferent signalling for the efferent servo controller to rapidly change finger cuff pressure. The finger artery is thus clamped a certain set point diameter.
The servo controller defines this set point and then detects changes from this diameter by means of the infrared photo-plethysmograph signal and responds by controlling a fast pneumatic proportional valve in the fronted unit. The proportional valve then modulates the air pressure generated by the air compressor, thus resulting in changes in finger cuff pressure to maintain finger artery diameter at the set point.

Physiocal System

.
When the Portapres cuff is deflated, the unloaded finger artery diameter corresponds with the pressure level at which amplitudes in the plethysmogram will be greatest. However, using the amplitudes alone is a relatively inaccurate way of determining blood pressure. The Physiocal algorithm [Wesseling 1995] utilises both the amplitude of pulsations and the shape of the plethysmogram when the finger cuff pressure is constant. The algorithm employs brief

periods at which the finger cuff pressure is fixed at several different pressure levels. These periods ("physiocals") are repeated at regular intervals during any recording. These data from two or more pressure levels can then be analysed to track the unloaded diameter of the finger artery (see Figure p17 Finger cuff manual). The example shown below illustrates this in practice.

1) When a fixed finger cuff pressure is deployed which is below the intra-arterial diastolic pressure, the arterial diameter will be relatively large so that the plethysmographic signal is low.
2) When the fixed cuff pressure is above the intra-arterial DBP, but below the MAP or SBP, then the plethysmographic signal will still be low, but will vary in diastole as the cuff pressure will be above DBP.
3) When the fixed cuff pressure is above MAP but below SBP the artery will remain collapsed for much of the cardiac cycle. The plethysmographic signal will still be high, only varying in systole.

The end-diastole period is analysed for each of the fixed cuff pressures. If the top of the plethysmographic signal at this time is a clear peak ("too sharp") then that fixed cuff pressure will be below MAP. If, however, the end-diastole pattern on the plethysmographic signal is a plateau ("too flat"), then the cuff pressure is too high. These characteristic shapes of the end-diastolic period on the plethysmograph will also depend on the vessel tone. For this reason the interpretation of "too sharp", or "too flat" is verified at different cuff pressures as described above. The Physiocal algorithm also ensures that the chosen unloaded artery diameter is not too close to the fully collapsed diameter of the finger artery and compares the calculated unloaded diameter with previous values obtained earlier in the recording. If a consistent value for the unloaded finger artery diameter is not obtained at the end of the above readings further fixed cuff pressures are employed automatically until a satisfactory diameter is obtained.

A similar situation is encountered at the start of each new recording; without previous diameter values for comparison the Physiocal algorithm deploys multiple steps of increasing fixed cuff pressures to obtain an initial value of finger artery diameter. After starting a new recording the frequency of Physiocals is initially set at every 10^{th} arterial pulsation. Only when a stable set point has been achieved (usually after 4 minutes of recording) will the frequency gradually be decreased down to a final frequency of one Physiocal for every 70 arterial pulsations. If at any point during the recording the variation in the set point diameter becomes too large (as might occur with extreme movement artefact), the Physiocal frequency will automatically increase again to maintain accuracy until stability returns.

During recordings the finger tip distal to the finger cuff usually appears slightly cyanosed, and the subject will often experience mild parasthesia (but not pain) in the area. The reason for this is that the arteries distal to the cuff are being held at zero transluminal pressure, and as such have about 60% of their original distended diameter. The blood that enters the finger tip during systole can only leave during diastole when the pressure in the fingertip vessels is greater than the cuff. This congestion leads to fractionally delayed venous return and thus greater deoxygenation of the venous blood. The net result is the slight colour change observed in the fingertips, whilst the congestion itself results in slight parasthesia. The Physiocal algorithm ensures that the finger artery is kept open during the recording and so arterial supply to the fingertip is not compromised. Thus, although finger cuff switching is recommended to avoid discomfort if continuous recordings exceed 2 hours, there have been no incidents of damage to the fingertip in over 20 years of use of finger cuffs for the Portapres/Finapres machines.

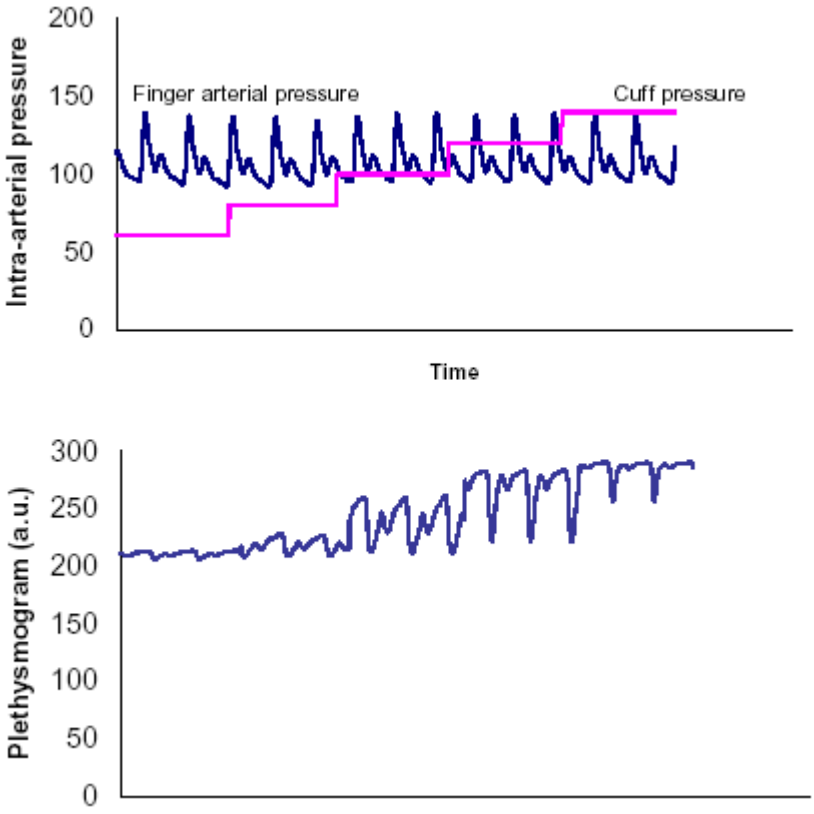

Figure 2:5 Finger plethysmogram at five cuff pressures [Taken from Portapres II user-manual TNO-TPD Biomedical Instrumentation]

In each systole the increase in aortic pressure causes inflow of blood into the arterial system. This inflow is, however, opposed by both aortic and peripheral systemic properties including arterial counter pressure and impedance. Modelflow simulates this according to the three-element Windkessel model of arterial input impedance [Burkhoff et al 1988; de Vaal et al 2005]. The three main components of this model are aortic characteristic impedance, (opposition of the aorta to pulsatile inflow), Windkessel compliance (representing the elastic storage capacity of the entire arterial system in response to increasing aortic pressure), and peripheral resistance. The aortic Windkessel compliance decreases substantially in a non-linear manner when aortic pressure increases. By contrast the aortic impedance increases moderately with increasing aortic pressure. Taking these non-linear relationships into account is important to allow appropriate corrections to the basic model for changing aortic pressure.

This has been done with post-mortem studies with results adjusted for age, sex, height and weight [Langewouters et al 1984].

VALIDATION STUDIES

The Portapres is considered sufficiently accurate to track *changes* in BP and correlates well with group data compared to the Dinamap 1846SX as part of a validation study for this thesis [Imholz *et al* 1998 & Figure 2.6]. Although estimation of SV using the Portapres with subsequent Modelflow analysis is considered accurate, even under orthostatic stress [Harms *et al* 1999], recent concern has been raised concerning the accuracy of CO [Remmen et al 2002; Azabji Kenfack *et al* 2004; Pitt *et al* 2004]. Earlier validation studies have suggested that Modelflow does provide reliable values for cardiac indices such as SV, which have been validated in a variety of settings [Harms et al 1999, Jellema, Imholz et al.1999 Langewouters et al.1998]. However, the reliability of the absolute values of CO have been questioned [Jellema et al 1999, Azabji Kenfack *et al* 2004; Pitt *et al* 2004], with a view that relative change of CO values in groups rather than individuals could still provide reliable data.

There has been some debate about the accuracy of the use of constants established from post-mortem aortic elastic characteristics in Modelflow analysis. These vessels differ from those of finger arteries and may therefore reduce the accuracy of the calculated value for CO. Comparison of CO values calculated by either Modelflow or by values obtained simultaneously by CO2 rebreathing suggested some limitations of the Modelflow absolute estimates, although the CO2 rebreathing method is not a gold-standard technique for CO estimate and only provides very intermittent values [Houtman et al 1999] Studies comparing intra arterial BP values with those obtained with the Portapres have been performed to investigate this further [Rongen et al 1995; Azabji Kenfack et al 2004]. The first of these studies actually found that the Finapres (the earlier version of the Portapres) absolute values

of MAP and DBP closely followed intrabrachial arterial values, although SBP was slightly underestimated by the Finapres [Rongen et al 1995]. Comparison of the Portapres with intraradial arterial BP measurements was made in a later study in which both were then subjected to Modelflow analysis to obtain CO estimates [Azabji Kenfack et al 2004]. In this study DBP, SBP and calculated CO were lower when obtained from the Portapres. However, several factors may have contributed to this discrepancy. There were fewer subjects in this second study (7 versus 15 from the Rongen et al study), and the subjects were much younger (mean age 24 years) compared with a mean age of 75.9 years in Rongen et al's group (much closer to the age of subjects used in my thesis). Furthermore the Modelflow used in the Beatscope software to estimate values for CO etc is based on intra-aortic pressures, not intra-radial. There are some differences between readings taken from these sites, and therefore it is conceivable that the intra-brachial techniques of Rongen et al may have been more accurate [Wesseling et al 1993]. Direct comparison with thermodilution again questioned the accuracy of Portapres/ Modelflow-derived CO for individual and absolute values. However, possible reasons for this discrepancy, not least of which is the inherent inaccuracy of thermodilution (+/- 15%) have been pointed out [Guyton et al 1973; Pitt et al 2004].

The Modelflow method actually determines stroke volume with CO subsequently calculated. The Portapres responds equally to increases and decreases in stroke volume, irrespective of the heart rate change [Pitt et al 2004]. Although there is therefore some debate about the accuracy of individual absolute values of cardiac indices such as CO obtained from Modelflow analysis of Portapres signals, there is consensus that the tracking of serial results of BP/HR/CO/SV/TPR from Portapres and subsequent Modelflow analysis, as performed throughout this thesis, is valid [Azabji Kenfack et al 2004; Pitt et al 2004]. Furthermore alternative non-invasive techniques to obtain beat-to-beat estimates of cardiac indices are fraught with difficulties. Impedance cardiography is limited by artefacts due to thoracic movements which also are likely to differ markedly between the study subjects of my thesis given the frequent respiratory system involvement in MSA [Kubicek]. Doppler

echocardiography requires the probe to remain fixed at a given position above the investigated artery which would be challenging during the postural change utilised in many of the studies in this thesis. As changes in values of groups of subjects were involved in all the major studies of this thesis it would therefore have been unethical to use invasive intra-arterial recordings in place of the Portapres recordings for the studies in this thesis.

We therefore feel justified in using the considerably less invasive Portapres/Modelflow methods in investigating the changes of cardiac indices in the studies of this thesis whilst acknowledging the greater importance to be given to trends of readings above the absolute derived values themselves.

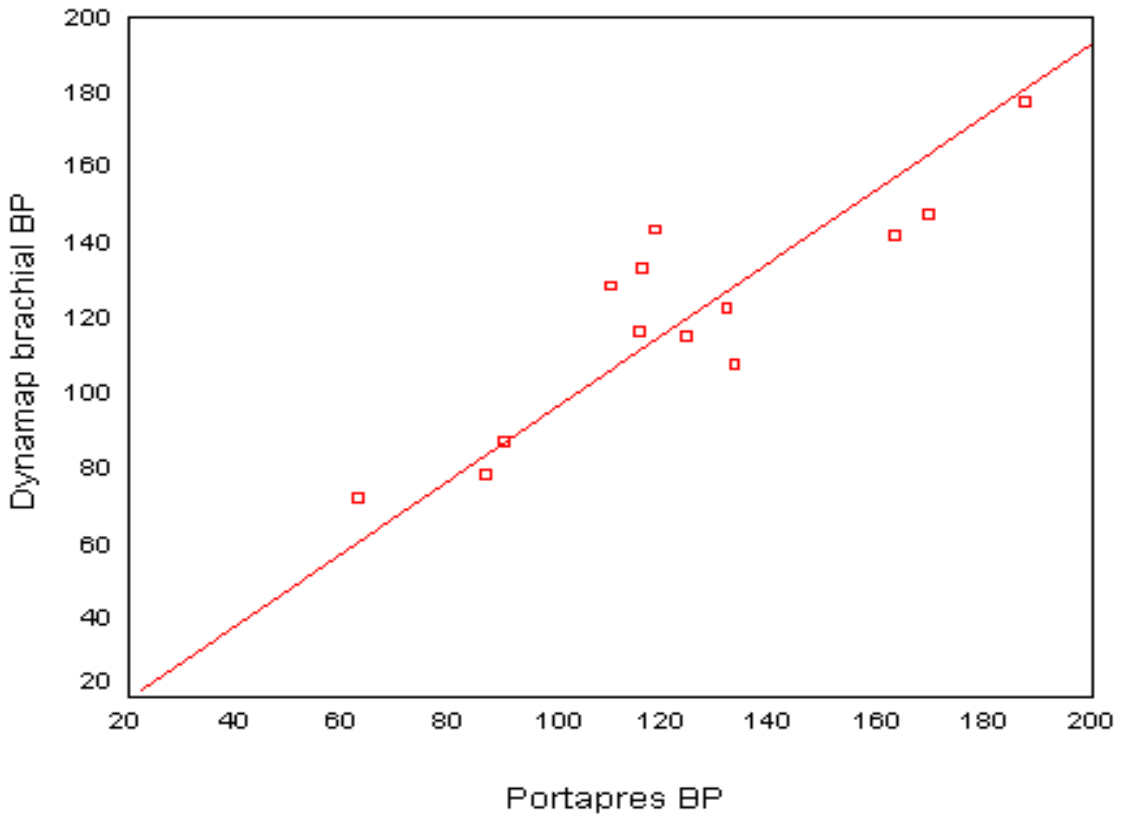

Figure 2:6 Correlation of seated SBP in 14 subjects with AF (7 MSA and 7 PAF) Obtained with automated brachial BP pressure cuff (Dinamap 1846SX) and Portapres on contralateral arm as part of this thesis.

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3.1: Introduction to Studies

The following studies were performed to enhance the differentiation of MSA and PAF based on the cardiovascular responses to various stimuli. A key concept to the studies is that of a comparison between a pre-ganglionic (MSA) and post-ganglionic (PAF) lesion model of autonomic dysfunction. Although there is some evidence that in PAF there maybe some additional pre-ganglionic disease burden, the post-ganglionic component is felt to predominate. This thesis will help to validate this assumption as the studies are based on predicted responses from theoretically pure models of pre- or post-ganglionic lesions.

The first study is essentially a large validation of existing practice by assessing the responses of MSA and PAF to our unit's standard cardiovascular autonomic function tests. This had not been previously been performed on such a large scale. Pressor tests are widely used autonomic function tests, yet the cutaneous vasomotor responses in pre- and post ganglionic models of autonomic failure had not previously been clearly determined. The use of laser Doppler technology has allowed this comparison to be performed in MSA and PAF subjects in this thesis.

The intravenous clonidine test is widely used to help distinguish MSA subjects. In these subjects the preganglionic lesion site includes hypothalamic involvement resulting in a suppression of the normal increase in plasma growth hormone (GH) response with the selective α_2-adrenoceptor agonist clonidine. The cardiovascular response to clonidine in MSA and PAF had not been well established however. The clonidine study in this thesis is the largest published series to focus on this aspect. Assuming the supine position immediately after upright posture may lead to a transient supine hypertension in MSA more than PAF subjects. Support for this observation was obtained from the studies on BP overshoot on tilt-reversal. Secretion of the posterior pituitary hormone ADH in response to upright tilt also

appears to be impaired in MSA but not PAF. As ADH is a pressor compound the possibility that it could be involved in the tilt-reversal supine hypertension in PAF was explored in these studies.

In addition to comparisons of cardiovascular responses in MSA and PAF to established autonomic function tests, newer pressor substances such as oral water and CO_2 inhalation were explored in MSA and PAF subjects, with possible diagnostic and therapeutic implications being explored. Although the water pressor effect had already been observed in autonomic failure, detailed haemodynamic responses to oral water with comparisons between MSA and PAF and symptom response had not previously been examined. Both systemic and regional cutaneous responses to CO_2 inhalation were studied in MSA and PAF subjects and the findings discussed in terms of possible novel means to aid differentiation of these conditions non-invasively.

3.2: Validating Standardised Pressor Responses in MSA and PAF

Abstract

Background & Aim: Haemodynamic responses to isometric exercise (IE: handgrip), mental arithmetic (MA) and cold pressor (CP) stimuli are frequently used in the assessment of autonomic failure. Multiple system atrophy (MSA) and pure autonomic failure (PAF) provide contrasting models of primary chronic autonomic failure. In MSA the lesion is central and pre-ganglionic, whilst in PAF the lesion site is peripheral and postganglionic. The value of pressor testing to aid differentiation of MSA and PAF has not been firmly established. We therefore evaluated the haemodynamic responses to standardised IE, MA, and CP stimuli in large groups of MSA and PAF subjects who had been tested in our autonomic laboratory.

Methods: We studied a total of 69 subjects (47 MSA and 22 PAF). Baseline supine systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were obtained with an automated sphygmomanometer (Dinamap). The subject then underwent standardised pressor stimuli with rest periods in between. These stimuli consisted of: IE (submaximal hand grip for 180 seconds); MA (serial subtraction calculations); CP test (contralateral hand placed between two ice packs for 90 seconds). The post-stimulus Dinamap recording was made at the end of each pressor test. Paired and un-paired t-tests were used to compare baseline and post-stimulus haemodynamics both within and between groups.

Results: There was no significant difference in baseline haemodynamics between the groups. Haemodynamic responses were attenuated in both groups to all pressor stimuli. In MSA HR increased following each pressor stimulus, whilst BP increased with IE and CP but not MA, which led to a fall in SBP and DBP. In PAF HR increased with CP and MA, but not with IE. There was no significant BP change with pressor stimuli. Comparing groups showed a greater

increase in HR following IE and CP in MSA than PAF and a greater increase in SBP in MSA following CP. SBP and DBP *decreased* to a greater extent in MSA following MA.

Conclusions: Our results suggest differences in pressor responsiveness in MSA and PAF. HR and BP increases tended to be preserved to a greater extent in MSA than in PAF. These differences may reflect the contrasting pathophysiology of these groups, and may aid their clinical differentiation.

Introduction

Standardised autonomic function testing includes the use of pressor testing to assess the autonomic control of the cardiovascular system [Mathias 2002]. Despite this the responses to pressor testing in pre- and post-ganglionic models of chronic autonomic failure have not been well delineated. This study therefore set out to assess the haemodynamics of pressor testing in multiple system atrophy (MSA) and pure autonomic failure (PAF) which provide models of pre- and post-ganglionic autonomic failure respectively [Mathias 2004].

In healthy subjects pressor tests elicit a transient increase in systolic and diastolic blood pressure (SBP and DBP) and increased heart rate (HR) [Mathias 2002]. There are 3 standard pressor stimuli: Isometric Exercise (IE) evoked by sustained handgrip; Mental Arithmetic (MA) evoked by the subject performing serial subtractions; Cold Pressor (CP) evoked with application of an icepack to the hand. All three stimuli lead to pressor effects by activation of the sympathetic nervous system, but the pathways involved differ. CP evokes a pain response involving of Aδ and C afferent fibres and subsequent activation of brainstem structures leading to increased sympathetic outflow [Hilz *et al* 2002; Petrovic *et al* 2004]. With MA, by contrast, there is initial activation of central areas such as the right insula and right anterior cingulate, resulting in increased sympathetic outflow [Critchley *et al* 2000]. IE has important peripheral and central input to the medullar vasomotor centres, although there have been contrasting views as to the relative importance of these inputs in the generation of the IE

pressor effect it is currently felt that both play a significant role [Alam and Smirk 1937; Victor *et al* 1989; Winchester *et al* 2000; Critchley *et al* 2000].

Because of the differing afferent pathways involved in the 3 pressor responses, the haemodynamic responses may vary in autonomic failure associated with specific lesion sites. Thus in subjects with complete high cervical spinal cord injury, MA will not elicit a pressor response whilst CP below the level of the lesion can produce an abnormal, enhanced pressor response termed autonomic dysreflexia [Mathias 2002; Mizushima *et al* 2003]. MSA and PAF provide contrasting models of chronic primary autonomic failure, with the lesion site being preganglionic and central in MSA but postganglionic in PAF [Daniels 2002; Matthews 2002]. We anticipated that the haemodynamic responses to standard pressor stimuli may vary between MSA and PAF because of the differing lesion sites. We therefore set out to assess the haemodynamic responses to IE, MA and CP in MSA and PAF subjects tested in our autonomic laboratories.

Subjects

We studied a total of 69 subjects with chronic autonomic failure who had been tested in our autonomic laboratories over the previous decade as part of their clinical assessment. In total 47 MSA (18 females and 29 males, mean age 58.7 ±10.0 years) and 22 PAF (11 females and 11 males, 63.3±8.6 years) were studied. MSA and PAF subjects had diagnosis made using existing criteria [Gilman et al 1998, Mathias 2004]. All MSA and PAF subjects had documented sympathetic and parasympathetic dysfunction with severe orthostatic hypotension. The mean disease duration for MSA was 4.6 +/-2.0 years; for PAF 12.9 +/- 62.7 years. There was no significant difference in baseline supine SBP or DBP between MSA and PAF (150.1 +/-26.1 / 84.5+/-15.5 versus 159.8 +/-29.3 / 89.0+/-16.6). HR was slightly higher in MSA than in PAF, but this did not reach significance (72.8 +/-10.1 vs. 69.3 +/- 12.3). Of the 22 PAF, 16 were on fludrocortisone, 4 were on ephedrine and 5 on midodrine. Of the 47

MSA, 21 were on fludrocortisone, 12 on ephedrine, 5 on midodrine and 12 on dopaminergic medication for Parkinsonism. Vasoactive medication was withdrawn from the night prior to the study. Local ethics approval from The National Hospital for Neurology and Neurosurgery London was obtained to use the results of these pressor tests.

Methods

All studies were performed in a dedicated, temperature controlled (24+/-1^{0}C), autonomic laboratory. Each subject had brachial SBP, DBP and HR recorded with a Dinamap (Critikon) automated sphygmomanometer before and immediately after each pressor test. Once a stable baseline was obtained, the subject first underwent pressor testing as follows:

1) IE test: The subject gripped a partially inflated sphygmomanometer cuff at maximum intensity for 2 seconds and then ceased gripping. The target grip intensity was calculated as follows:

$$\text{Target Grip intensity} = \frac{\text{Maximum grip} - \text{initial cuff pressure}}{3}$$

The subject then sustained handgrip to above target grip intensity for 3 minutes. A Dinamap reading was taken at end of this period and compared with baseline.

2) MA test: The subject underwent 1 minute of performing serial subtractions starting with 7 from 100 being requested to perform the calculations as fast as possible. Answers were verbalised and more complex subtractions requested if judged that serial 7's were not sufficiently challenging for the individual subject.

3) CP test: Ice cold packs 0-4^{0}C were placed on dorsum of the hand for 90 seconds.

After each stimulus, SBF was allowed to return to stable baseline levels before commencing the next stimulus.

Statistics Dinamap recordings of SBP, DBP and HR were compared within each group before and after each pressor test (using paired t-test). Between group comparisons were made by calculating the change in BP or HR following each pressor stimulus, and then comparing the mean change for each group using non-paired t-test. Statistical significance was taken as $p<0.05$.

Results

There was no significant difference in baseline haemodynamics between the groups. Haemodynamic responses were attenuated in both MSA and PAF to all pressor stimuli as compared with values obtained for age-matched healthy controls [Mathias 2002].

MSA showed a significant increase in SBP and DBP following CP (ΔSBP +11.2 +/- 13.3; ΔDBP +3.6 +/- 5.8 mmHg), but only showed a smaller rise following IE (ΔSBP +2.6 +/- 10.3; ΔDBP +2.1 +/- 8.0 mmHg). Following MA there was a small *reduction* in SBP and DBP (ΔSBP -2.1 +/- 10.3; ΔDBP -1.6 +/- 6.6 mmHg). In MSA HR increased following each pressor stimulus (Figure 3.2: ΔHR following IE: +5.9 +/-5.7 bpm; following MA +4.9 +/-5.4 bpm; following CP +4.8+/-6.4 bpm).

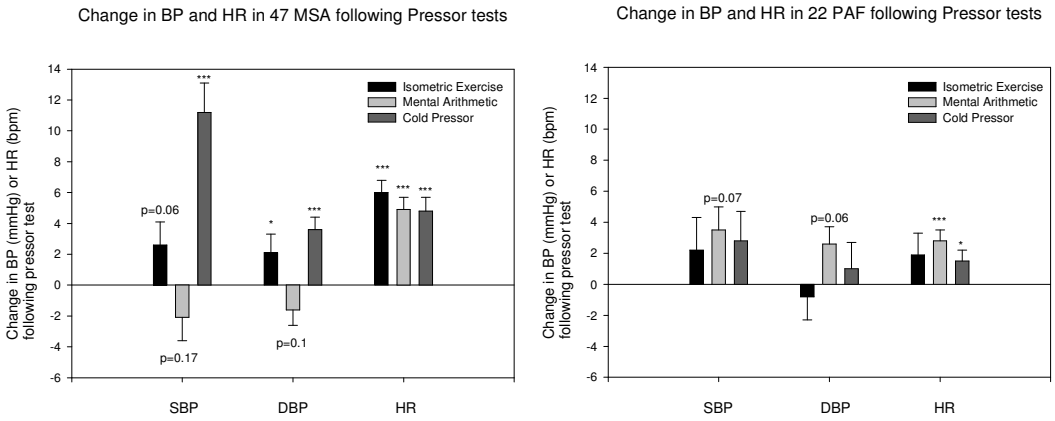

Figure 3.2.1:ΔSBP, ΔDBP and ΔHR following pressor tests in MSA and PAF (error bar = SE).*=p<0.05; **=p<0.01; **=p<0.001 comparing results before and after test (paired t-test)

PAF showed no significant changes in SBP or DBP following any pressor test. There was a small increase in HR in PAF following MA and CP (Figure 3.2.1; ΔHR following MA +2.8 +/-3.3 bpm; following CP +1.5+/-3.3 bpm) but there was no significant change in HR following IE. The reduction in SBP and DBP seen in MSA was significantly greater than with PAF (Figure 3.2.2). HR increase following IE and CP was significantly greater in MSA than PAF (Figure 3.2.2) and there was a significantly greater increase in SBP following CP in MSA compared with PAF.

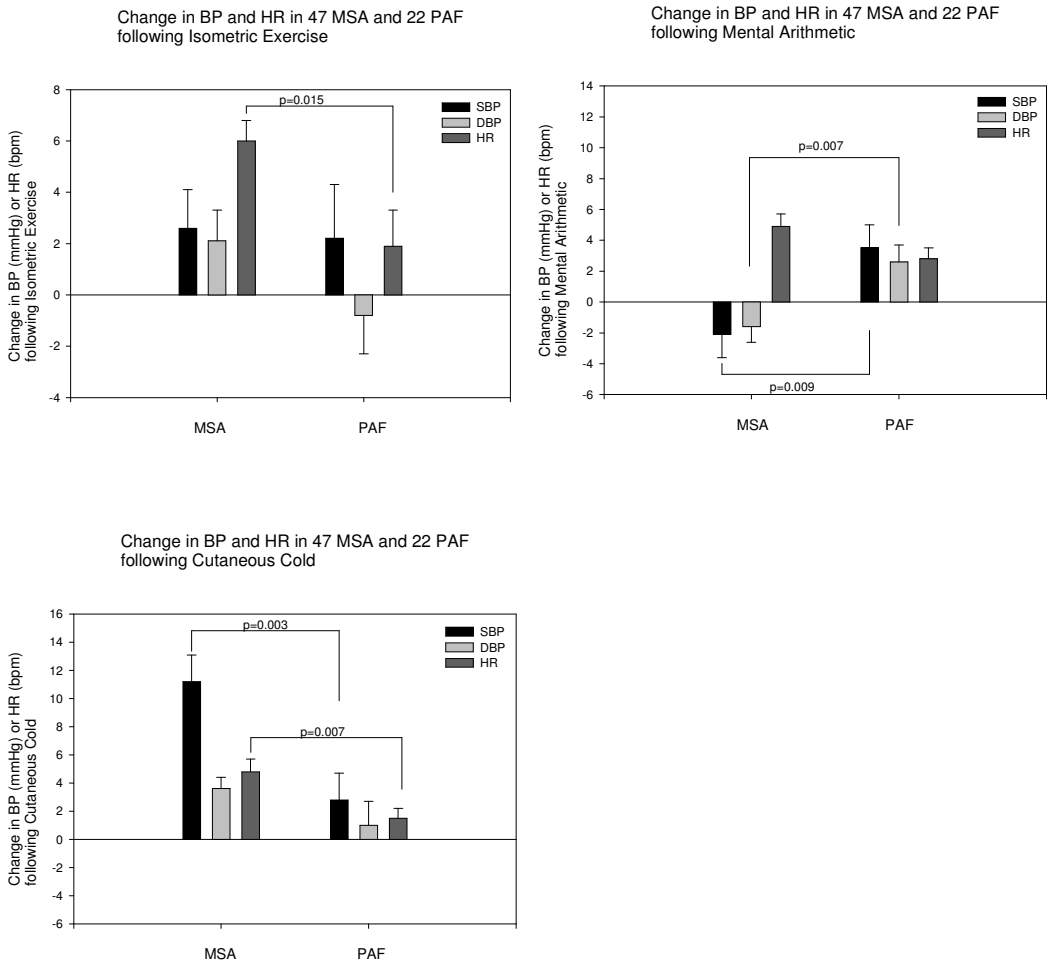

Figure 3.2.2: ΔSBP, ΔDBP and ΔHR following pressor tests; MSA and PAF (error bar = SE). *=p<0.05; **=p<0.01; **=p<0.001 comparing MSA with PAF (t-test)

Discussion

We have found differences in the response to pressor stimuli between MSA and PAF despite similar baseline haemodynamics. Both groups showed attenuation of pressor response following standard IE, MA and CP testing, as would be expected given the sympathetic impairment in MSA and PAF. However, responses varied with different pressor stimuli. SBP was increased to a greater extent in MSA following CP, whilst IE and CP produced a greater

increase in HR in MSA than in PAF. Intriguingly, following MA SBP and DBP actually decreased in MSA, this reduction being greater than seen with PAF.

Previous reports on the haemodynamic effects of pressor testing in autonomic failure have been limited by small numbers or by the use of MSA but not PAF subjects. Two such studies in MSA reported attenuation of pressor response to CP [De Marinis *et al* 2000; Kimber *et al* 2000], IE and MA [Kimber *et al* 2000] without specific comment as to any differences between pressor responses. The one previous study to compare the pressor responses in MSA and PAF showed no clear differences in pressor responsiveness, but did not report on pressor response to MA [Shannon *et al* 2000]. Thus the differential effects of standard pressor testing in MSA and PAF had not been well established previously.

In PAF there is profound post-ganglionic fibre and parasympathetic impairment. As these are believed to be integral to the pressor responses to IE, MA and CP, it would be expected that there would be a similar attenuation of BP and HR response in PAF to all 3 stimuli, as indeed we found. However, the pressor responses in MSA, where the post-ganglionic nerves are still functional, varied according to the stimulus. CP produced a significant increase of SBP in MSA, which was significantly greater than seen in PAF. How can this partial preservation of pressor response to CP in MSA be explained? In controls CP elicits nociceptive afferent signals from Aδ and C nerve fibres in the periphery, which are then transmitted via the spinothalamic tract to cortical and limbic structures [Lovallo 1975].The brainstem and hypothalamus seem to be especially important in integrating the pressor response to the initial noiceptive stimulus [Petrovic *et al* 2004]. It has been suggested that the periaqueductal grey (PAG), locus coeruleus and C1 cells of the rostral ventrolateral reticular nucleus are important components of this integration between early nociception and pressor response [Faneslow 1982; Reis *et al* 1989 Aston-Jones *et al* 1999; Becerra *et al* 2001; Daniel 2002] with subsequent increase in sympathetic outflow via the intermediolateral cell column (IML).

However, the locus coeruleus, C1 cells and IML are known to be pathologically involved in MSA [Wenning *et al* 1997; Benarroch 2002].

Given the central pathological involvement in MSA of structures felt important in generating the CP response it seems somewhat surprising that there is still such preservation of the SBP pressor response to CP. Alternative pathways may also be important in generating the CP response and these may be preserved to a greater extent allowing some preservation of pressor response. There has been some suggestion of spinal cord level reflexes being able to generate a pressor response to CP. High level complete spinal cord lesion patients, like MSA, have intact post-ganglionic nerves but here the central integration of nociceptive stimuli and autonomic response is dissociated from the peripheral afferent stimulus. It is known that in stable, complete cervical cord lesion patients, CP below the level of the lesion results in a heightened pressor response [Mizushima *et al* 2003]. However, unlike in MSA the ventromediolateral column is intact in SCI and dissociated from supraspinal control. This results in autonomic dysreflexia not only to CP stimulus, but even minor stimuli such as bladder tapping, a situation not seen in MSA. Also, because of the complete dissociation of afferent signal and central vasomotor control centre, there is no change in HR following CP in these patients. HR increases elicited following CP may be more important in generating a pressor response than vasoconstriction [Yamamoto *et al* 1992]. Some preservation of vagal withdrawal may still occur in MSA resulting in the observed increase in HR in MSA and resultant pressor response.

Following IE MSA showed only slight increase in SBP and DBP. MSA showed an increase in HR, which was significantly greater to that seen in PAF. In PAF there was no significant change in BP or HR following IE. The situation with IE is however more complex than with CP. In normal subjects central command pathways, resulting in voluntary contraction of muscle, cause activation of the sympathetic outflow [Victor *et al* 1989; Winchester *et al* 2000; Critchley *et al* 2000]. In addition, peripheral input from the muscles and local vessels results

in both a pressor effect [Alan and Smirk 1937; Mitchell *et al* 1977; Victor *et al* 1989] despite vasodilatation of the skeletal muscle vascular bed [Corcondilas *et al* 1964; Joyner *et al* 1999]. The mechanism involved in this vasodilatation has been much debated in the past century, but it appears most likely to be a non-neural effect involving local NO release [Joyner and Halliwell 2000]. NO release from the endothelium of muscle bed vessels may be produced both by circulating adrenaline, also indirectly by local metabolites in contracting muscles which result in vasodilatation which in turn increases endothelial NO by a flow-dependant means [Gilligan 1994; Joyner and Proctor 1999; Reed *et al* 2000]. Skeletal muscle vasodilatation appears to be preserved in autonomic failure as well as in subjects following stellate ganglion blockade or α-adrenoceptor blockade [Dietz *et al* 1997; Reed *et al* 2000]. Thus in MSA or PAF where efferent vasoconstriction is known to be impaired, the preservation of exercise-induced vasodilatation will tend to further attenuate BP rise. Finally, MSA subjects may not be able to generate as great as an intensity of IE as PAF or normal subjects because of features such as parkinsonism or cerebellar dysfunction which are often also present in MSA. Thus the haemodynamic responses to IE seen in MSA and PAF seem likely to reflect various aspects of the underlying condition.

The afferent origins to MA are clearly central in origin. In addition to the pressor response induced by MA, vasodilatation in certain regional beds is known to occur in healthy controls [Blair 1959, Lindqvist *et al* 1996]. Initially it was felt that sympathetic activation resulted in vasodilatation via cholinergic vasodilator nerves, as seen in animals and in human non-acral skin [Roddie *et al* 1957], especially as the vasodilatation was attenuated in sympathectomised arms or with prior atropine [Blair 1959]. However, it is currently felt that the major mechanism of muscle bed vasodilatation in response to mental stress in humans is via adrenaline, which acts partly through release of local nitric oxide [Dietz *et al* 1994; Eisenach *et al* 2002]. It is known that, in normal subjects, whilst sympathetic activation by CP will result in raised noradrenaline and adrenaline, it is adrenaline which is predominantly released with MA [LeBlanc *et al* 1979]. In addition a greater increase in HR was seen following MA

than seen with CP. The increase in HR was correlated with increase in adrenaline whilst increase in BP was correlated with increase in noradrenaline. If the NA action is blocked with phentolamine infusion in normal controls, a vasodilatation in the forearm is seen to CP or IE similar to that normally seen with MA. A similar vasodilatory response to IE is seen in normal controls after axillary blockade [Lindqvist *et al* 1996]. In MSA we observed a decrease in SBP and DBP after MA. Unlike in PAF, in MSA NA and adrenaline are still produced to similar levels as in normal controls. The relative increase in A and NA following MA is not known in MSA, but MSA are known to have impaired increase in NA in response to head-up tilt [Mathias 2002]. A relatively greater impairment of NA compared to a release following MA may therefore help explain the slight depressor effect that we noted in MSA.

We have clarified the responses to standardised pressor testing in MSA and PAF. Differences have thus been highlighted between these groups which may relate to the contrasting lesion sites in these conditions. These differences have clinical significance. As standardised pressor tests are routinely used in the autonomic investigations of subjects with autonomic failure, these differences may aid the separation of MSA and PAF. A further aspect occurs in relation to orthostatic hypotension, a condition common to both MSA and PAF. Whilst the pressor responses in PAF were uniformly attenuated, an increase in SBP was retained in MSA following CP. It may therefore be that, in the event of presyncope in MSA a painful stimulus may elevate SBP enough to retain consciousness whilst the subject is trying to lie down. However, mental concentration may worsen SBP and DBP in MSA, a factor which may warrant consideration in subjects engaged in stressful situations, such as public speaking, whilst upright.

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3.3: Cardiovascular Responses to Clonidine in MSA and PAF

ABSTRACT

Objective: To assess the effects of clonidine on blood pressure (BP) and heart rate (HR) in multiple system atrophy (MSA), where the lesion site is preganglionic, and in pure autonomic failure (PAF) where it is postganglionic.

Background: In normal subjects intravenous infusion of the selective α_2-adrenoceptor agonist clonidine reduces BP and plasma noradrenaline (NA) levels via central α_2-adrenoceptor [α_2] action, as well as inducing growth hormone (GH) release. Clonidine-induced GH release is impaired in MSA, but spared in PAF. However, the haemodynamic effects of clonidine have not been extensively studied in these disorders.

Methods: We examined intravenous clonidine test results (performed in our autonomic laboratories using the London Autonomic Units protocol [Thomaides *et al* 1992]) in 58 patients, 39 with probable MSA and 19 with PAF. Systolic (S) and diastolic (D) BP and HR and plasma noradrenaline (NA) levels were measured supine at baseline and for up to 60 min post-clonidine.

Results: Clonidine resulted in a significant BP fall in MSA, which occurred earlier (within 15 minutes of clonidine), and to a greater extent, than seen in PAF. Both MSA and PAF showed HR reduction after clonidine, although this was significantly greater in MSA than PAF. NA levels decreased significantly after clonidine in both groups. Although basal NA levels were lower in PAF than MSA, there was no difference in NA reduction relative to baseline between groups. MSA showed significant negative correlation between basal NA levels and BP response to clonidine.

Conclusions: Clonidine infusion reduces BP and HR in both MSA and PAF, but to a greater extent in MSA. The greater vasodepressor action of clonidine in MSA suggests that there is partial preservation of brainstem sympathetic outflow pathways in MSA, and may reflect its action at sites in the brainstem and spinal cord that were in part functionally preserved in

MSA. Despite similar degrees of NA reduction after clonidine, the vasodepressor effect of clonidine was attenuated in PAF compared with MSA. This attenuation in PAF may reflect greater peripheral α_2-adrenoceptor denervation supersensitivity due to the postganglionic lesion site. These BP differences may thus reflect the underlying lesion site in MSA and PAF, and the haemodynamic data following clonidine infusion may help differentiate these conditions.

Introduction

Intravenous infusion of the selective α_2-adrenoceptor agonist clonidine has been used in the investigation of parkinsonian syndromes, as clonidine-induced release of growth hormone (GH) is impaired in multiple system atrophy (MSA) where the lesion site is preganglionic, but spared in pure autonomic failure (PAF) where the lesion site is postganglionic [Thomaides *et al* 1992; Zoukos et al 1993]. Clonidine also has significant cardiovascular effects, and reduces blood pressure (BP) in normal and hypertensive subjects [Warren *et al* 1989, Kooner *et al* 1991, Zoukos *et al.* 1992; Kimber *et al* 2001] by central sympatholytic action. The haemodynamic response to clonidine may vary therefore in MSA and PAF because of their contrasting lesion sites. However, the haemodynamic effects of clonidine in autonomic failure are not well understood, and previous studies have only reported results from small numbers of patients.

Previous assessments of clonidine effects in small numbers of MSA have shown a reduction in SBP and DBP similar to that seen in healthy controls [da Costa *et al* 1984; Thomaides *et al* 1992]. The haemodynamic effects of clonidine in PAF have not been clearly established. Previous results in PAF vary, from no effect on BP [da Costa *et al* 1984], a pressor action [Robertson *et al* 1986] and a slight vasodepressor action [Thomaides *et al* 1992]. Clonidine formulation also differed in these studies with oral [Robertson *et al* 1986] and intravenous

forms [da Costa *et al* 1984; Thomaides *et al* 1992] being used. There is therefore uncertainty in the existing literature as to the haemodynamic response of PAF to clonidine, and this may be related in part to the relatively small numbers of subjects studied in previous studies. We set out to assess the haemodynamic responses to intravenous clonidine (which is now the established means of clonidine-GH testing) in a large group of patients with MSA and PAF.

Subjects and Methods

We retrospectively examined data from the clonidine-GH test [Kimber et al 1997] performed in our Units over the last decade. A total of 58 subjects with chronic autonomic failure were studied in whom the diagnosis had been made using established criteria [Gilman et al 1998, Mathias 2004] (39 MSA, 19 PAF). PAF subjects were carefully selected for certainty of diagnosis, having isolated autonomic symptoms for a minimum of 10 years and being age-matched with MSA. In addition only subjects able to omit medication prior to the study were included. The mean age of MSA (23 males and 16 females) was 59 +/- 11.8 and PAF (11 males and 8 females) 63.9 +/- 8.8 years with no significant difference between the two groups. Mean disease duration in MSA was 4.6 +/- 2 years. A total of 14 PAF and 20 MSA subjects were on vasoactive medication prior to the study. Of these subjects 15 MSA and 14 PAF were on fludrocortisone, 4 MSA and 3 PAF were on ephedrine, 4 MSA and 5 PAF were on midodrine, and 5 MSA were on L-Dopa medication. All subjects had documented sympathetic and parasympathetic dysfunction with severe orthostatic hypotension. Vasoactive medication was omitted from the evening before the study. After an overnight fast the subject was rested supine in a dedicated autonomic laboratory for 30 minutes. With the subject supine, brachial BP and HR readings were measured intermittently using the automatic sphygmomanometer (Dinamap, Critikon, UK). Venous access was obtained with cannulation of an antecubital fossa vein to allow infusion of clonidine. Clonidine was infused intravenously over a 10 minute period with a total dose of 2μg/Kg body weight in 20ml of normal saline. Further BP and heart rate (HR) recordings were taken 15, 30, 45 and 60

minutes post-clonidine and compared with baseline. The plasma levels of noradrenaline (NA) at baseline and 60 min post-clonidine were analysed. Electrochemical detection for NA concentration was subsequently performed using high-performance liquid chromatography [Smedes *et al* 1982; May *et al* 1988; Mathias *et al* 1990].

Statistics

Mann-Whitney U test was used to analyse the difference in parametric parameters between the MSA and PAF patients. The difference before and after clonidine infusion in each group was assessed with Wilcoxon's signed rank test. Correlation between BP response to clonidine and plasma NA concentration was assessed with Spearman's Correlation Coefficient.

Results

There were no significant differences in supine SBP and DBP pre-clonidine between MSA and PAF. SBP and DBP were significantly reduced after clonidine infusion compared with baseline in both MSA and PAF (Table 3.3.1). However, the vasodepressor response to clonidine occurred sooner in MSA than PAF, with significantly greater reduction in BP seen in MSA at 15, 30 and 45 minutes post-clonidine. The reduction in SBP and DBP were significantly greater in MSA than in PAF at 15, 30 and 45 minutes post-clonidine (Figure 3.3.1).

Baseline HR was significantly higher in MSA than PAF ($p<0.05$). Both groups showed a significant reduction in HR after clonidine infusion (Table 3.3.1).The reduction in HR was significantly greater in MSA than in PAF at 15 and 30 minutes post-clonidine (Figure 3.3.1).

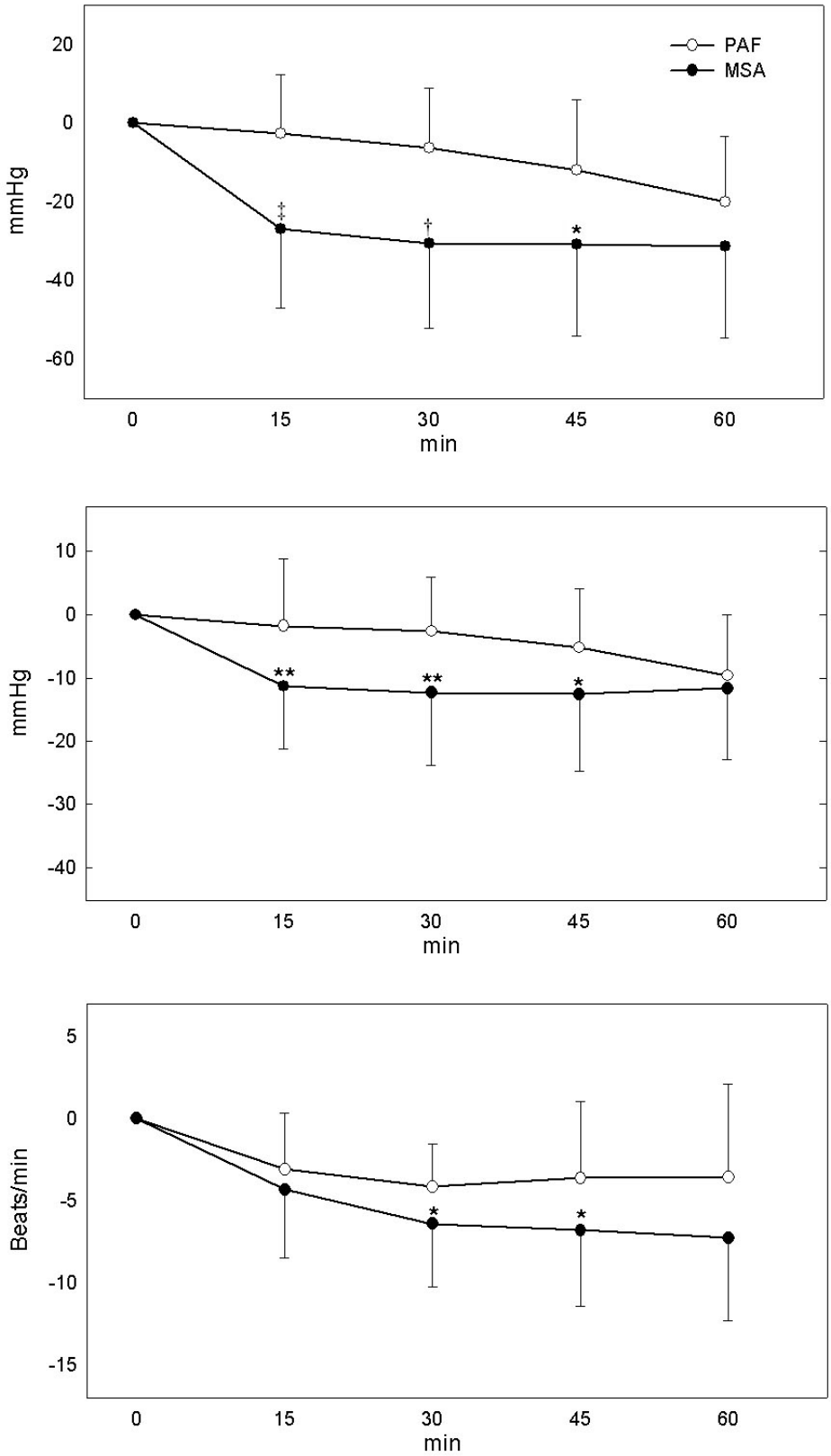

Figure 3.3.1: Changes in systolic BP (upper panel), diastolic BP (middle panel) and heart rate (lower panel) in PAF versus MSA following Clonidine at minute 0. Comparison with Mann-Whitney U test baseline level. *p<0.05, **p<0.005, †p<0.0001, ‡p<0.00005.

	Baseline	15 min	30 min	45 min	60 min
SBP (mmHg)					
MSA	146+/-31	119+/-29	116+/-26	115+/-25	115+/-26
p value		p<0.0000005	p<0.0000001	p<0.0000001	p<0.0000001
PAF	166+/-21	154+/-25	150+/-21	145+/-28	136+/-26
p value				p<0.01	p<0.0005
DBP (mmHg)					
MSA	83+/-15	71+/-13	70+/-13	70+/-13.6	67+/-28
p value		p<0.000001	p<0.000001	p<0.0000005	p<0.0000001
PAF	86+/-13	86+/-15	84+/-15	82+/-14	77+/-13
p value					p<0.0005
HR (beats/min)					
MSA	72+/-10	67+/-9	65+/-9	65+/-9	64+/-9
p value		p<0.00001	p<0.0000005	p<0.0000005	p<0.0000001
PAF	66+/-8	63+/-9	62+/-9	62+/-8	62+/-8
p value		p<0.005	p<0.0005	P<0.005	p<0.005

Table 3.3.1: BP and HR pre- and post clonidine in 39 MSA and 19 PAF. p value = comparison with the baseline in each group

The baseline level of plasma NA was significantly lower in PAF (116.4 +/- 52pg/ml) than MSA (276 +/- 111.7 pg/ml (p<0.00000005) (Figure 2). Levels of NA significantly decreased in both MSA (-67%) and PAF (-70%) 60 min after clonidine infusion (Figure 3.3.2). In MSA but not PAF there was a significant negative correlation between ΔSBP 30 minutes post-clonidine and basal NA levels (Figure 3.3.3).

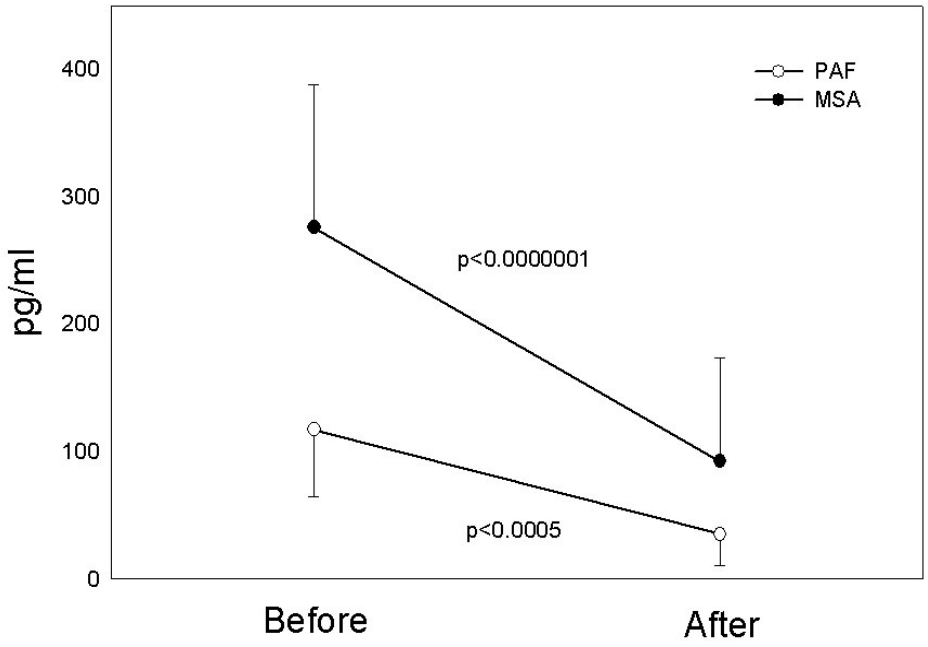

Figure 3.3.2: The plasma levels of noradrenaline concentration before and 60 minutes after clonidine infusion. The difference between means of NA concentration before and after clonidine infusion in each group was assessed with Wilcoxon's signed rank test.

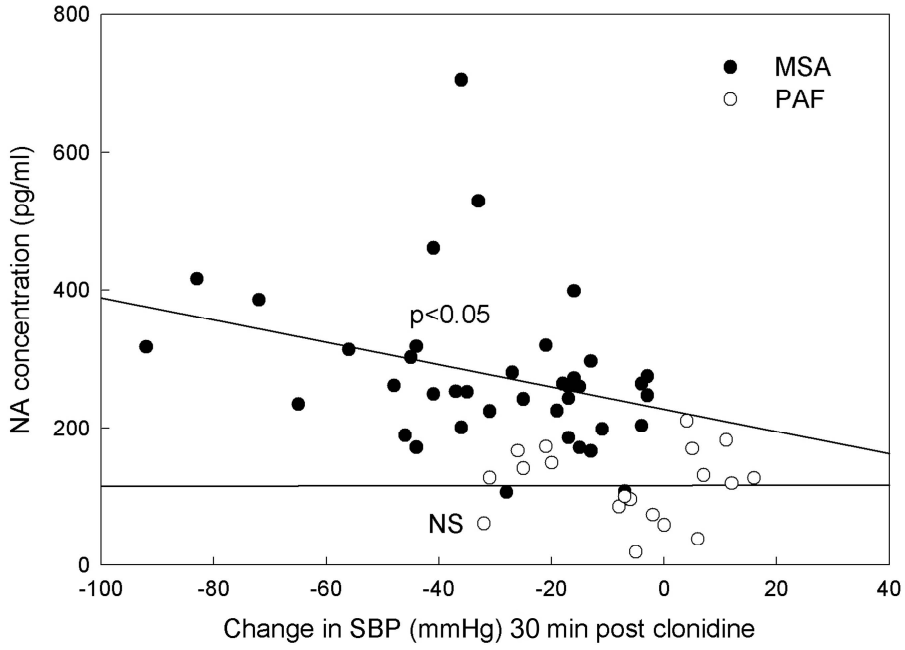

Figure 3.3.3: Correlation between baseline resting supine Noradrenaline (NA) levels and ΔSBP at 30 minutes post-clonidine in MSA (upper trace) and PAF (lower trace) p<0.05 denotes significant correlation in MSA between NA levels and ΔSBP

Discussion

Our study has shown clear differences in BP responsiveness between MSA and PAF after intravenous administration of the selective α_2-adrenoceptor agonist clonidine. We found that clonidine reduces SBP and DBP in both MSA and PAF following clonidine. The BP fall occurred sooner in MSA than PAF. BP reduction was significantly greater in MSA than PAF, being similar in magnitude to the reduction previously seen in healthy controls [Warren *et al* 1989; Kooner *et al* 1991; Thomaides *et al* 1992; Zoukos *et al.* 1992; Kimber 2003]. There was a significant reduction in HR following clonidine in both groups, being greater in MSA than PAF. NA levels were significantly reduced in both groups to a similar extent.

An earlier report of the pressor effects of clonidine in autonomic failure [Robertson et al 1986] utilised a variable dose (0.1-0.8mg) of oral clonidine in a heterogeneous group of 12 subjects, including 4 patients with MSA and 8 patients with idiopathic orthostatic hypotension (probable PAF). In another study, with data pooled from 2 PAF and 4 MSA, a low dose (1.5 μg/kg) of intravenous clonidine resulted in no significant change in BP [da Costa et al 1984]. A small reduction in BP was shown with 2μg/kg intravenous clonidine in 10 PAF [Thomaides et al 1992]. As the haemodynamics of clonidine are dependent on the concentration of clonidine in the blood [Davies *et al* 1977, Kroin *et al* 1996], with greater pressor effects seen at higher doses, it may be difficult to compare results between intravenous fixed dose clonidine and variable-dose oral clonidine. In our study we used the now accepted protocol for clonidine-GH testing based on subject body weight (2μg/kg) [Thomaides *et al* 1992], and we used substantially larger numbers in each group than previous studies had done.

Clonidine has been reported to reduce HR in normal subjects [Warren *et al* 1989, Kooner *et al* 1991, Thomaides *et al* 1992; Nurnberger *et al* 2003]. In our study a HR reduction was seen in both groups, being significantly greater in MSA than PAF. Previous reports of the HR effects of clonidine in MSA have shown a non-significant reduction following clonidine. Of note

these studies involved smaller numbers of MSA than in our present study [da Costa *et al* 1984; Thomaides *et al* 1992; Zoukos 1992]. In PAF the HR response following clonidine has not been firmly established, with a few subjects showing a marked reduction in response to oral clonidine [Robertson *et al*1986], but other studies suggesting no change in heart rate [Zoukos *et al* 1992; Thomaides 1992]. No previous direct comparisons of HR change in MSA and PAF have been reported. The HR effect of clonidine in PAF or MSA therefore had not previously been established, partly as a result of the small numbers previously studied

Postulated preganglionic cerebral sites of clonidine action have included the nucleus tractus solitarii and anterior hypothalamus [Isaac 1981]. The central effects of hypotension and bradycardia appear to be mainly mediated by α2 adrenoceptor agonist action in pressor area of ventrolateral medulla, resulting in increased inhibition of sympathetic outflow [Punnen *et al* 1987; Wang *et al* 2003]. This central sympatholytic action would explain the reduction in BP and HR seen in normal subjects. Clonidine is also known to have agonist actions at imidazoline-1 receptors, and activation of these receptors is known to produce a hypotensive effect [Prichard and Graham 1997]. Imidazoline-1 receptors, like α2 adrenoceptors, are well localised in the rostral ventrolateral medulla in man and other species [Bricca *et al* 1989]. It has been postulated that the agonist action of clonidine on imidazoline-1 receptors may play a role in the hypotensive and bradycardic effects of clonidine. However, moxonidine, a selective imidazoline-1 agonist at the rostral ventrolateral medulla cardiovascular regulatory centres, results in reduced BP and NA levels but does not cause bradycardia in humans [Haxui *et al* 1994; Prichard and Graham 1997]. Although intact vagi seem to be important in the bradycardia induced by clonidine, an additional reduction in heart rate may result from a direct action on the heart [Tsai and Lin 1987; Mukaddam-Daher *et al* 1997]. This later point remains an intriguing possibility to help explain the clonidine-induced reduction in HR we observed in both MSA and PAF, given the extensive parasympathetic impairment seen in these subjects.

Patients with high cervical, complete, spinal cord injury do not show a decrease in BP following oral or intravenous clonidine [Reid *et al* 1977; Kooner *et al* 1991]. Thus intact central sympathetic outflow appears important for clonidine to result in a vasodepressor effect. With intravenous clonidine 2μg/Kg body weight, high cervical cord lesion patients show an initial pressor response to clonidine. This fact, taken together with the low basal NA levels seen in high cervical cord injury patients has lead to the suggestion that the pressor effect of clonidine in these subjects may be related to chronic sympathetic denervation supersensitivity. The pressor effect is likely to be mediated by vasoconstriction resulting from activation of postsynaptic peripheral α2 adrenoceptors rather than α1 adrenoceptors [Kiowskiw *et al* 1983]. Presynaptic α2 adrenoceptors are present on the postganglionic nerves and when activated tend to inhibit neurotransmission. However, clonidine produces a pressor rather than vasodepressor action in high cervical cord injury, and so post-synaptic activation appears to be the major effect. α2 adrenoceptors are present in large numbers in the intermediolateral cell column of the spinal cord, and have been suggested to play a role in the haemodynamic effects of clonidine [Sinha *et al* 1973; Reid *et al* 1977; Unnerstall et al 1984]. In high cervical cord lesion patients clonidine reduced the pressor response to bladder stimulation, thus providing evidence to suggest that additional preganglionic spinal sites are involved in the sympatholytic action of clonidine [Mathias *et al* 1979].

The prominent BP, HR and NA level reduction in MSA following clonidine infusion suggests a degree of functional preservation of brainstem pathways such as the nucleus tractus solitarius and its associated baroreflex pathways, despite being areas known to be involved pathologically in MSA. The preservation of vasodepressor effects of clonidine suggests that there is at least partial preservation of baseline vasomotor sympathetic outflow in MSA. Clonidine reduces plasma NA levels in normal subjects [Reid *et al* 1977, Warren *et al* 1989, Zoukos *et al.* 1992] and in MSA and PAF [Zoukos *et al* 1992; Thomaides 1992]. Our study showed similar relative reductions in NA levels to those previously seen in normal subjects and in MSA and PAF. We found a similar relative reduction in NA levels in MSA (67%) and

PAF (70%) following clonidine. This suggests that the central α2 adrenoceptor sympatholytic effects of clonidine may occur to a similar extent in MSA and PAF. Post-synaptic α2 adrenoceptors are also present on the smooth muscle of peripheral vasculature, and their activation results in vasoconstriction. In pithed rabbits clonidine results in a pressor effect mediated predominantly by this post-synaptic α2 adrenoceptor activation at low doses, and by post-synaptic α1 adrenoceptor activation only at higher doses [Pompermayer *et al* 1999]. The relative importance of post-synaptic α2 adrenoceptor agonist action of clonidine in a single patient with autonomic failure has been suggested previously with the pressor effect of oral clonidine being markedly attenuated by pre-treatment with yohimbine (α2 adrenoceptor antagonist), but not by pre-treatment with prazocin (α1 adrenoceptor antagonist) [Onrot *et al* 1987]. Chronic denervation of post-ganglionic sympathetic nerves, as seen in PAF, induces supersensitivity of peripheral α-adrenoceptors to a greater extent than in MSA where the lesion is preganglionic [Polinsky, (2002)]. We found lower basal NA levels in PAF, which are known to be associated with peripheral adrenoceptor sensitivity [Senard *et al* (1991), Polinsky, (2002)]. Animal studies have also suggested that non-acute sympathetic denervation abolishes the vasodepressor effect of clonidine, which produces a pressor action [Petty *et al* 1976]. The attenuation of blood pressure fall following clonidine in PAF may therefore result from additional peripheral agonist action on supersensitive α2 adrenoceptors counteracting the central vasodepressor action.

There may be a negative correlation between NA concentrations and pressor response to clonidine. In a previous study [Robertson *et al* 1986], where responsiveness to *oral* clonidine (0.1-0.8mg) was assessed in a heterogeneous group of 12 subjects with differing forms of AF, there was a significant negative correlation in the group as a whole between BP and basal NA levels. In our study, if pre- and postganglionic autonomic failure patients are considered together, there is also a significant negative correlation between basal NA levels and pressor response to clonidine. However, the correlation is only seen in MSA when MSA and PAF cases are considered separately (Figure 3.3.3). The disparity between these findings reflects

the problems inherent in considering two fundamentally different groups as a homogenous entity. We found a negative correlation between SBP after clonidine and baseline NA levels in MSA. PAF showed no such correlation. At first sight these results may suggest that MSA and not PAF demonstrate pressor supersensitivity in response to low NA levels. Alternatively, in PAF, where postganglionic impairment is extensive, supersensitivity may have been present to a similar degree in all subjects regardless of absolute NA levels. With MSA, if some subjects demonstrated a degree of supersensitivity whilst others did not, then a correlation such as we found may be explained.

There are additional clinical implications to our observations. This study demonstrated a differing BP response to clonidine, with a vasodepressor effect occurring earlier and to a greater extent, in MSA than in PAF. This occurs despite a similar relative reduction in NA levels in both groups following clonidine. The attenuation of BP drop in PAF compared with MSA may reflect a denervation supersensitivity to clonidine in PAF, resulting in vasoconstriction via peripheral α2 adrenoceptors. A major role for the intravenous clonidine test in clinical practice is to add in the differentiation and diagnosis of central and peripheral autonomic conditions with GH responses providing important information. Our results have confirmed that BP responses to clonidine also differ significantly between MSA and PAF, and that comparing these difference responses following an intravenous, standard dose of clonidine may be a further aid in separating these conditions.

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3.4: Cardiovascular Responses to Water Ingestion in MSA and PAF

Introduction

The ingestion of water increases seated blood pressure (BP) in chronic autonomic failure [Jordan et al 1999]. This pressor effect first occurs between 5 and 15 minutes after water ingestion, reaches a peak effect at about 30-35 minutes, and lasts for just under an hour. The volume of water ingested influences the pressor response; 480ml producing greater effect than 240ml, but temperature (9^{o}C and 24^{o}C) of ingested water does not seem to be an important factor [Jordan *et al* 2000]. Although the underlying mechanisms of the pressor action of water in AF had not previously been determined, possible mechanisms include partial residual sympathetic activity, baroreflex dysfunction, gastric distension or fluid redistribution. Multiple system atrophy (MSA) and Pure Autonomic Failure (PAF) provide contrasting models of chronic autonomic failure. In MSA the underlying lesion site is central and pre-ganglionic, whilst in PAF it is distal and post-ganglionic [Polinsky et al 2002]. The detailed haemodynamic response to water ingestion while seated has been studied in detail in PAF [Cariga & Mathias 2000], but there have been no comparisons with MSA. We postulated that differences in pressor response to water between the two groups would relate to the different lesion site in PAF and MSA, and may provide information on the mechanism of the pressor effect. The results of these studies have been published [Young & Mathias 2004a; Young & Mathias 2004b].

Remarkably for such a fundamental process as water drinking, the pressor effects of water ingestion have only been described recently. The first suggestion that water ingestion could increase blood pressure in autonomic failure was reported in 1983 with improvement of orthostatic symptoms in a subject with autonomic failure who drank large quantities of

seawater. [Frewin & Bartholomeusz 1983], although a pressor effect in autonomic failure was only confirmed at the end of the 1990s. Jordan et al first reported the pressor effect of drinking 480ml of tap water in 1999. They observed that in seated young healthy subjects there was no change in blood pressure (BP) following water ingestion. Older healthy subjects showed an 11mm Hg rise in systolic BP following ingestion of the same volume of water. A more substantial rise in Systolic BP was observed in seated subjects with chronic autonomic failure. In some the increase was in excess of 30mm Hg. The pressor effects described above began a few minutes after ingestion of water and peaked around 20 minutes. As tap water contains small quantities of cations, which could conceivably be a factor in pressor responses, the effects of similar volumes of distilled water were studied in patients with severe sympathetic denervation due to pure autonomic failure (PAF) [Cariga & Mathias 2001] and in multiple system atrophy (MSA) [Young & Mathias 2004a]. Distilled water also resulted in a pressor effect of similar magnitude and time course to that observed with tap water. Whilst the pressor effects of oral water are likely to last for no more than about an hour, the potential of water ingestion as a therapy for orthostatic hypotension has been suggested (Mathias 2000). Suggestions of a smaller rise in BP have been made in spinal cord injury patients following water ingestion (Tank et al 2003). Interestingly possible improvements in orthostatic tolerance in intermittent autonomic dysfunction such as vasovagal syncope and in normal subjects exposed to negative lower body pressure in upright posture or blood donation (Lu et al 2003; Schroeder et al 2002; Hanson & France 2004).

Orthostatic hypotension (OH), common to both PAF and MSA, may cause severe symptoms, and often is inadequately controlled by medication. We determined if water ingestion would improve standing BP and OH symptoms in these subjects. Previous work suggested that OH in chronic autonomic failure improved 35 minutes after drinking 480ml of water, although the effects on symptoms were not known [Jordan *et al* 2000]. As water increases seated BP 5-15 minutes after ingestion, OH may be improved at this time also. We therefore measured standing BP 15 and 35 minutes after water ingestion in our subjects.

It has recently been suggested that the effects of swallowing itself might also be an important factor [Endo *et al* 2002] in the water pressor effect. The only comparison of the haemodynamics of oral water with those of water directly instilled into the stomach was performed in young control subjects in whom water does not produce a sustained pressor effect. As a follow-up study we therefore set out to record the effects of instilling 480ml of distilled water via a gastrostomy tube into a PAF subject to compare the haemodynamic response with those found in previous studies of oral water ingestion in PAF [Cariga & Mathias 2000].

Physiology of water ingestion water ingestion

Water drinking is a fundamental aspect of animal life. In man typically 2-3 litres of fluid is ingested each day, either in response to thirst stimuli or as a result of direct volition. The volume of the typical stomach is typically only 50ml when empty but can expand to a capacity of approximately 1 litre after a meal [Sherwood L 2004] although this varies considerably with age and body size. The stomach is distensible to a point, although ingested volumes much in excess of 500ml typically start to lead to sensations of fullness or discomfort [Penagini et al 1998], usually giving rise to a sensation of satiety inhibiting further intake until the contents is reduced by gastric emptying. Usually fluid is taken in the form of hypotonic solutions (tap water, tea, carbonated beverages etc), often in association with eating. The mix of ingested fluid and food is termed chyme. The fluid/food ratio of chyme and its volume can significantly influence gastric emptying. This is important because the tight junctions of the gastric mucosa markedly limit the absorption of water whilst the chyme is still within the stomach. Once the chyme clears the pyloric junction and enters the vast surface area of the permeable small intestine water absorption from the gut lumen to the extracellular and then intracellular spaces is rapid. The marked differences in gastric

emptying between water and mixed meal ingestion is illustrated in Figure 3.4.1 in normal subjects, using scintography, which has now largely superseded other radiological methods [Lawaetz et al 1989; Lin et al 2005]. The situation is slightly more complex for mixed meal/fluid ingestion as the different components tend to be released from the stomach to the small intestine at different rates with fluids often preceding the solid components [Lin et al 2005]. Furthermore the volume and tonicity of chyme may be significantly altered by secretions and absorption, additional components in determining gastric emptying.

There is a basic peristaltic reflex in the gut whereby local luminal dilation results in an ascending muscular excitation pathway and descending inhibition. The higher co-ordination of the movement of chyme from the stomach to small intestine is provided by a vagally mediated reflex originating in the brainstem, whilst additional control is provided by a prevertebral reflex arc responding to stomach visceral afferents [Thompson DG 2003]. Normally a vagally mediated reflex on ingestion inhibits the periodic Migrating Motor Complex, which periodically propagates distally from the stomach in the fasted state. For water ingestion in normal subjects the net result is a fairly rapid gastric emptying as shown in the upper graph of Figure 3.4.1. Greater stomach volumes are emptied more rapidly [Noakes et al 1991; Doran et al 1998].

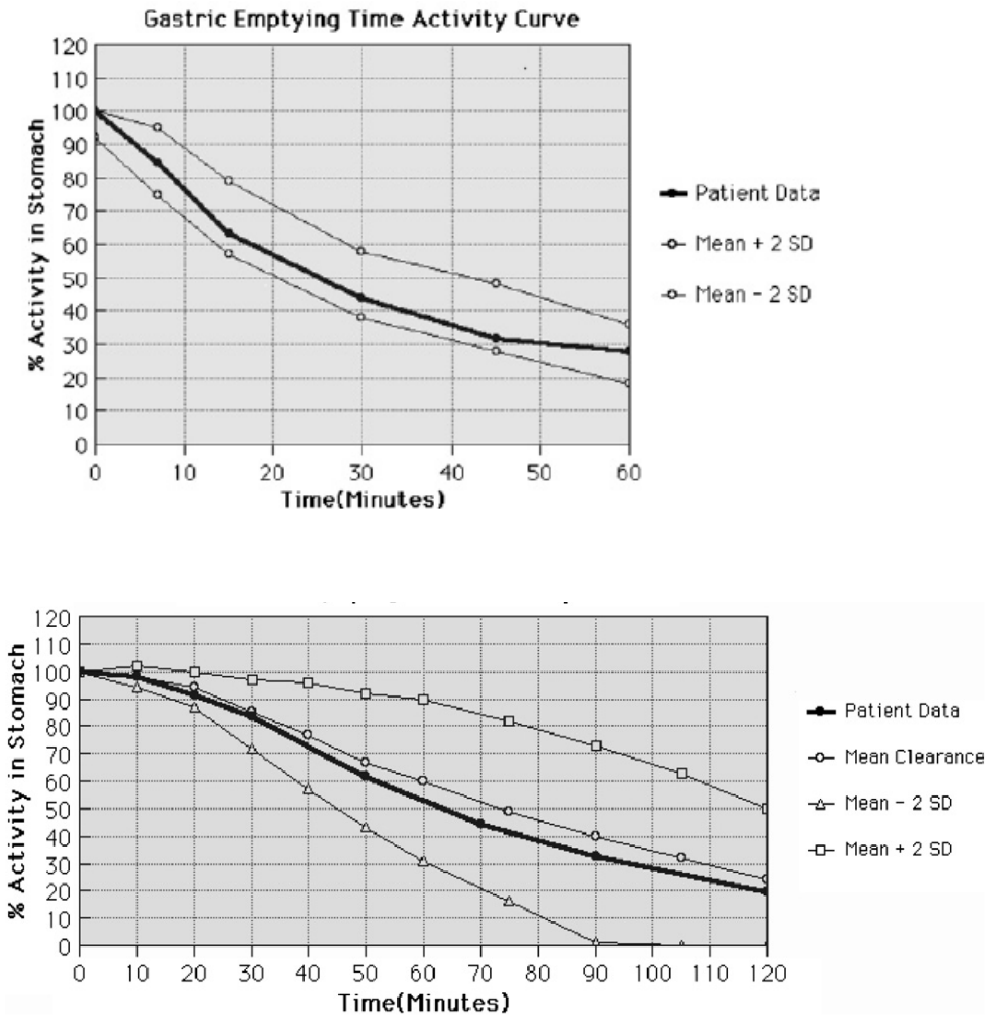

Figure 3.4.1: Measurement of gastric emptying in normal subjects following ingestion of 300 ml water (above) or a similar volume of mixed meal (120ml water, 2 large scrambled eggs, a piece of toast and a teaspoon of butter) (below) at time '0' by use of by Gamma Scintigraphy (Ref Lin et al 2005).

Following gastric emptying, chyme enters the duodenum (the portion of the intestine from the pyloric sphincter to the ligament of Treitz). This part of the gut is highly permeable with evidence suggesting that pure water is absorbed at least as fast as isotonic 6% carbohydrate-electrolyte drink, possibly because the duodenum tends to bring the chyme towards isotonicity by means of secretions/absorption [Lambert et al 1997]. These results suggest that the rate of tracer accumulation in the blood after ingestion of different volumes of test drinks

is not a reliable indication of the availability of the ingested fluid, but gives at least a qualitative measure of the sum of the effects of gastric emptying and intestinal water absorption [Lambert et al 1997].

Although the physiology of gastric emptying has been extensively studied in normal subjects, the situation in autonomic failure is less clear, with initial evidence suggestive of accelerated gastric emptying in PAF [Mathias and Bannister 2001]. However, the possibility of slowing of gastric emptying in primary autonomic failure has recently been reported [Maule et al 2002]. These authors used ^{13}CO2-octanoid acid breath test (OBT) and electrogastrogram (EGG) to assess gastric emptying following a standard test meal of one scrambled egg, two slices of bread, 50 g ham and 10 g butter in 3 PAF and 9 MSA subjects. The egg yolk was labeled with 0.1 ml of ^{13}CO2-octanoid acid. 13C-octanoid acid is rapidly absorbed in the duodenum, but not stomach, and metabolized in the liver; following oxidation, the resulting ^{13}CO2 is excreted in the breath. Breath samples were collected at baseline and at 15-min intervals analyzed by isotope-selective nondispersive infrared spectrometry. Half time gastric emptying (T1/2) was thus calculated with T1/2 ≤144 minutes considered normal. Gastric emptying was found to be delayed in 5/9 MSA and 1/3 PAF. In contrast 2/3 PAF had relatively fast gastric emptying with gastric emptying t ½ of 97 and 80 minutes. EGG found normogastria in 6/9 MSA and bradigastria in 3/9. In PAF 2/3 showed bradigastria and 1/3 (same subject with gastric emptying t ½ of 80 minutes) showed tachigastria. Thus the gastric emptying rates in MSA and PAF appears to be abnormal in many cases, but whether this is consistently delayed or accelerated is not clear at present. In particular it should be noted that the study by Maule et al utilised oral food and the gastric emptying with water may well be different as with normal subjects.

Water ingestion thus has cardiovascular effects in both normal and diseased man. The mechanisms of the pressor response remain unclear. Better understanding of the aetiology of

the water pressor effect would be likely to aid both understanding of the pathophysiology of autonomic failure, and the treatment of orthostatic hypotension which can be so debilitating in these patients. As alluded to above, patients with other causes of orthostatic intolerance such as PoTS and vasovagal syncope and hypotension related to exercise or food may also benefit by oral ingestion of water, although more confirmatory studies are needed in this regard. Finally it should be remembered that the importance of the cardiovascular effects of water drinking potentially extends well beyond the field of autonomic medicine. Even the small increases in blood pressure noted in older "healthy" subjects after water drinking could be significant in the long term in the western world where hypertension is endemic- certainly the very limited studies available so far would suggest a transient effect greater than that seen with the addition of table salt to food which has been extensively targeted as a cause of hypertension in the last few decades. In addition early phase drug trials incorporate the ingestion of water with study medication as if it were inert, yet subsequently monitor cardiovascular responses including heart rate and postural hypotension. That water ingestion has very real cardiovascular effects has now been established; to attempt to unravel the mechanisms by which water exerts these effects is now therefore the next very important step ahead.

Initial study: Effects of oral water on seated and standing haemodynamics in MSA and PAF

Subjects and Methods

A total of 14 patients with chronic autonomic failure were studied (7 MSA, 7 PAF). The mean age of MSA was 62 +/- 9.5 (4 males, 3 females) and PAF 59 +/- 10 (3 males and 4 females). MSA and PAF were diagnosed using existing criteria [Gilman et al 1998, Mathias 2004]. All patients had documented sympathetic and parasympathetic dysfunction with severe orthostatic hypotension (Table 3.4.1). PAF had greater supine BP than MSA. There were no differences in the vasoactive medication between the 2 groups; 3 in each were on fludrocortisone, 2 in each on ephedrine, 1 PAF took midodrine and 2 subjects in each group were on no medication. No subjects were on anti-parkinsonian medication. Vasoactive medication was withdrawn the night prior to the study. All patients gave informed consent to participate in the study. The study had ethical approval from the National Hospital for Neurology & Neurosurgery.

		MSA	PAF	Normal range
Supine SBP		139.4 (24.0)	157.4 (25.0)	
Standing SBP		99.4 (16.9)	96.9 (33.3)	
Change in SBP	Standing	-40.0 (28.0)	-60.1 (25.0)	< -20
	Hand grip	6.2 (5.2)	7.0 (5.9)	≥17
	Cold Pressor	8.7 (19.0)	2.6 (6.9)	≥15
Supine DBP		82.0 (11.2)	86.9 (9.7)	
Standing DBP		60.6 (12.7)	52.7 (11.3)	
Change in DBP	Standing	-22.3 (16.1)	-33.4 (8.5)	<-10
	Hand grip	4.2 (7.1)	0.7 (6.4)	≥11
	Cold Pressor	5.3 (10.1)	-0.7 (2.7)	≥10
Sinus Arrhythmia Ratio		1.11 (0.05)	1.06 (0.06)	>1.2
Valsalva Ratio		1.27 (0.18)	1.19 (0.24)	>1.4

Table 3.4.1: Autonomic Function testing on study patients (7 MSA, 7PAF); SBP=Systolic Blood Pressure mmHg (SD); DBP=Diastolic Blood Pressure mmHg (SD)

Study design

On the morning of the study the patients fasted after a light breakfast at 0800 hours. The study took place in a dedicated autonomic laboratory between 1000 and 1300. After emptying the urinary bladder, the patient was seated and BP was recorded both intermittently and continuously. Intermittent brachial BP values were obtained every 3 minutes using an automated Dinamap (Critikon) sphygmomanometer on the left arm. Continuous measurement of beat-to-beat BP was obtained non-invasively throughout the study with the Portapres II device (Figures 3.4.2 & 3.4.3) on the middle finger of the right hand. Subsequent calculation of cardiac output (CO), total peripheral resistance (TPR), and stroke volume (SV) using Model flow analysis was made. This has previously been validated at rest and in relation to stimuli such as head up tilt [Harms et al 1999, Jellema et al 1999a, Jellema et al 1999b, Langewouters et al 1998, Voogel et al 1997]. After 30 minutes of baseline recordings, the patient stood for 5 minutes (stand 0), and brachial BPs were recorded 3 and 5 minutes after standing up. The patient then returned to the seated position. Following standing the patient was questioned on the presence and severity of orthostatic symptoms. After baseline BP had been re-established, the patient drank 480ml of distilled water at room temperature within a target time of 5 minutes (mean time taken in MSA was 3 minutes 47 seconds, for PAF 3minutes 4 seconds with no significant difference between groups). On completion of water ingestion the patient remained seated for a further 15 minutes. The patient then stood in an identical manner to Stand 0; this was termed Stand 1, the first after water ingestion. On completion of Stand 1, the patient returned to the seated position for a further 15 minutes. After this time (now 35 minutes after water ingestion) the patient stood for 5 minutes (Stand 2) in an identical manner to the previous stands. During each episode of standing hypotensive symptoms were assessed [Mathias et al 1999]

Data Analysis

Dinamap values for systolic and diastolic BP (SBP, DBP) were compared at 3 and 5 minutes into each stand before and after water. Patients acted as their own controls with BPs for stands 1 and 2 being compared with their own stand 0 values. Paired Student's t-test was used with statistical significance being taken as $p<0.05$. Mean beat-to-beat data for each minute post water ingestion was calculated and the results compared using repeated measures of ANOVA with Bonferroni correction for multiple testing, utilising the same statistical approach as had been performed in the only previous beat-to-beat study of water ingestion in PAF [Cariga & Mathias 2001].

Results

Pooled Dinamap data for all 14 patients, before and during standing:

The 14 AF subjects showed a significant fall in SBP and DBP (Dinamap data) on standing compared to seated values. There was no significant change in the drop on standing after water ingestion, but, as seated SBP and DBP rose significantly over this time, the net result was a significant increase in standing SBP and DBP at both 15 and 35 minutes after water ingestion as compared with baseline. These results are shown in Table 3.4.2.

Pooled Beat-to-beat data for all 14 patients, before and during standing:

Mean seated baseline values for all 14 subjects were compared with mean values for the 5-minute stands before and after water. Pre-stand values of 103.3 (SD 35.5) / 60.9 (SD 19.3) showed a significant drop on Stand 0 to 79.4 (SD 30.0) / 48.5 (SD 19.5) (Table 3.4.3). As with the Dinamap data, a significant increase in standing SBP and DBP compared to Stand 0 was seen during both periods of standing after water. Standing SBP for Stand 1 was 99.0 (SD 25.0) / 61.0 (SD 16.9); for Stand 2: 103.3 (SD 29.8) / 64.9 (SD 19.5). As for the seated baseline, there was an increase in TPR in each stand after water, although this did not reach significance. In addition there was a smaller increase in CO, which also failed to reach significance.

		Stand 0 before water		Stand 1 15 min after water		Stand 2 35 min after water	
Seated value just prior to Stand	**SBP**	**110.6**	(25.1)	**122.9**	(29.0)	**133.9**	(25.5)
	DBP	**69.6**	(12.9)	**76.4**	(13.9)	**80.4**	(14.1)
	HR	**73.7**	(11.2)	**70.5**	(8.6)	**70.0**	(9.7)
3min of Stand	**SBP**	**79.5**	(21.5)	**101.0*****	(23.3)	**99.6*****	(24.0)
	DBP	**51.5**	(15.0)	**63.6****	(13.0)	**64.0****	(14.0)
	HR	**82.9**	(15.2)	**75.8****	(13.1)	**77.2****	(13.2)
5min of Stand	**SBP**	**77.4**	(25.6)	**95.3****	(23.0)	**95.4*****	(22.9)
	DBP	**49.6**	(16.3)	**63.4****	(16.1)	**60.4****	(16.1)
	HR	**81.9**	(16.2)	**76.6****	(14.6)	**78.9**	(14.1)

Table 3.4.2: Dinamap Blood Pressure (SBP and DBP) and Heart Rate (HR) in 14 patients with AF (7 MSA, 7 PAF) on standing, before and after ingestion of 480ml water. Values in mmHg +/- (SD) and beats per minute +/- (SD): Stand 1 and 2 compared with Stand 0. *= p< 0.05; **= p< 0.01; ***= p< 0.001.

Symptoms Experienced on standing

After standing on each occasion the subjects were questioned about the presence and severity of 3 common symptoms of OH: light-headedness, visual disturbance and "coat hanger" neck pain in the occipital and shoulder region [Mathias et al 1999, Bleasdale-Barr et al 1998]. Of the 14 subjects, 11 experienced one or more of these symptoms during Stand 0. All 11 of the subjects symptomatic on Stand 0 noted subjective improvement in their orthostatic symptoms during Stands 1 and 2. There were 3 asymptomatic subjects (2 MSA and 1 PAF) who remained without symptoms during both subsequent standing periods.

MSA						
	Seated	Stand 0	Seated	Stand 1	Seated	Stand 2
SBP	102.8 (28.9)	93.7 (32.2)	113.0 (28.5)	102.4* (24.6)	118.9 (28.2)	108.2* (33.7)
DBP	59.7 (17.4)	56.6 (21.4)	69.0 (19.5)	63.3* (17.6)	71.2 (19.1)	68.3* (22.4)
CO	5.2 (1.7)	4.4 (1.3)	4.7 (1.7)	4.4 (0.9)	4.8 (2.0)	3.5 (1.1)
TPR	1.0 (0.5)	1.1 (0.6)	1.3 (0.7)	1.1 (0.4)	1.1 (0.5)	1.4 (0.7)
SV	68.7 (20.4)	51.7 (17.1)	74.4 (8.6)	54.5 (8.7)	72.6 (10.5)	49.5 (11.1)
HR	76.1 (10.2)	87.2 (17.6)	63.0 (16.8)	81.3 (14.9)	65.2 (19.6)	82.0 (9.4)
PAF						
	Seated	Stand 0	Seated	Stand 1	Seated	Stand 2
SBP	102.8 (67.2)	67.2 (23.6)	130.3 (39.2)	95.6** (26.9)	131.6 (40.4)	98.4** (26.9)
DBP	62.1 (41.5)	41.5 (15.9)	77.3 (17.7)	58.6* (17.2)	80.0 (20.3)	61.5* (17.2)
CO	3.5 (1.5)	2.8 (0.9)	3.7 (1.9)	3.5 (1.5)	3.6 (2.2)	3.3 (1.5)
TPR	1.7 (1.1)	1.3 (0.9)	1.8 (0.8)	1.6 (0.8)	1.9 (1.2)	1.7 (1.0)
SV	47.9 (18.5)	38.4 (13.8)	67.8 (6.1)	49.1 (19.0)	67.3 (7.3)	45.6 (18.2)
HR	72.1 (11.2)	76.2 (13.3)	53.6 (24.5)	71.4 (8.0)	52.2 (27.6)	71.1 (9.4)
POOLED DATA FOR ALL 14 PATIENTS						
	Seated	Stand 0	Seated	Stand 1	Seated	Stand 2
SBP	103.3 (35.5)	79.4 (30.0)	121.7 (34.1)	99.0* (25.0)	125.2 (34.1)	103.3* (29.8)
DBP	60.9 (19.3)	48.5 (19.5)	73.1 (18.4)	61.0* (16.9)	75.4 (19.4)	64.9* (19.5)
CO	4.3 (1.8)	3.5 (1.3)	4.2 (1.8)	4.0 (1.3)	4.2 (2.1)	3.7 (1.4)
TPR	1.3 (0.9)	1.2 (0.8)	1.6 (0.8)	1.3 (0.7)	1.5 (1.0)	1.6 (0.8)
SV	58.3 (21.6)	44.6 (16.3)	71.1 (7.9)	51.8 (14.5)	69.9 (9.1)	47.6 (14.6)
HR	74.1 (10.5)	81.3 (15.8)	58.3 (20.8)	76.4* (12.6)	58.7 (24.0)	76.6* (12.6)

Table 3.4.3: Beat-to-beat haemodynamics in MSA and PAF on standing, before and after water. Values +/- (SD): SBP=systolic blood pressure mmHg; DBP=diastolic blood pressure mmHg; CO=cardiac output l/min; TPR=total peripheral resistance MU; SV=stroke volume ml; HR=heart rate bpm. Stand 1 and 2 compared with Stand 0. *= p< 0.05; **= p< 0.01; ***= p< 0.001.

Subset analysis (MSA and PAF data):

a) Dinamap data

Baseline seated BP did not differ significantly between MSA (117.3 +/-18.6 / 73.0 +/-13.8mmHg) and PAF (103.9 +/-30.1 / 66.3 +/-11.9), as shown in Tables 3.4.4 and 3.4.5. Stand 0 resulted in a significant drop in BP at 3 and 5 minutes in both MSA and PAF with no significant difference between MSA and PAF patients. Subgroup analysis showed a similar increase in standing BP after water ingestion in the 7 MSA and 7 PAF patients, with no significant difference between MSA and PAF.

		Stand 0 before water		Stand 1 15 min after water		Stand 2 35 min after water	
Seated value just prior to Stand	**SBP**	**103.9**	(30.1)	**126.4**	(32.9)	**137.9**	(30.5)
	DBP	**66.3**	(11.9)	**78.7**	(10.8)	**81.1**	(14.2)
	HR	**71.1**	(11.4)	**66.0**	(5.2)	**65.3**	(6.8)
3min of Stand	**SBP**	**68.4**	(18.5)	**97.3***	(25.4)	**96.9***	(26.2)
	DBP	**43.4**	(10.7)	**61.0***	(16.3)	**63.3****	(16.0)
	HR	**77.0**	(13.9)	**69.7***	(8.2)	**69.9***	(8.1)
5min of Stand	**SBP**	**64.4**	(20.8)	**88.9***	(22.5)	**92.1****	(25.1)
	DBP	**40.4**	(12.1)	**59.4***	(16.5)	**57.7***	(17.2)
	HR	**75.1**	(13.1)	**69.1**	(8.9)	**70.7**	(8.8)

Table 3.4.4: Dinamap Blood Pressure (SBP and DBP) and Heart Rate (HR) in 7 patients with PAF on standing, before and after ingestion of 480ml water. Values in mmHg +/- (SD) and beats per minute +/- (SD): Stand 1 and 2 compared with Stand 0.
*= p< 0.05; **= p< 0.01; ***= p< 0.001.

		Stand 0 before water		Stand 1 15 min after water		Stand 2 35 min after water	
Seated value just prior to Stand	**SBP**	**117.3**	(18.6)	**119.4**	(26.7)	**129.9**	(21.0)
	DBP	**73.0**	(13.8)	**74.1**	(17.0)	**79.7**	(15.1)
	HR	**76.7**	(11.1)	**75.7**	(9.3)	**75.0**	(10.4)
3min of Stand	**SBP**	**90.6**	(19.5)	**106.0****	(22.2)	**102.3***	(23.6)
	DBP	**59.6**	(14.8)	**66.3**	(10.4)	**64.7**	(13.0)
	HR	**89.8**	(14.6)	**82.8***	(14.8)	**85.8**	(14.7)
5min of Stand	**SBP**	**90.3**	(24.5)	**101.7***	(23.3)	**98.6**	(21.9)
	DBP	**58.9**	(15.2)	**67.3****	(15.9)	**63.0**	(15.9)
	HR	**89.8**	(16.8)	**85.3***	(15.7)	**88.5**	(13.5)

Table 3.4.5: Dinamap Blood Pressure (SBP and DBP) and Heart Rate (HR) in 7 patients with MSA on standing, before and after ingestion of 480ml water. Values in mmHg +/- (SD) and beats per minute +/- (SD): Stand 1 and 2 compared with Stand 0.
*** = p< 0.05; ** = p< 0.01; *** = p< 0.001.**

b) Beat-to-beat haemodynamic data:

Beat-to-beat data were assessed before and during the 15 minutes immediately following water ingestion, during which time the patients remained seated. In PAF an increase in seated SBP and DBP was recorded after water ingestion, which first reached significance 5 minutes post-water ingestion (Figure 3.4.4) and remained so until the end of the study. Seated TPR increased first reaching significance 5 minutes after water ingestion and remaining so until the end of the study. Seated CO, SV and EF did not significantly change during the study. There was a non-significant reduction in seated HR over the first 15 minutes post water ingestion in both subsets (PAF baseline HR +/- SD: 73.5 +/- 10.1; PAF 15 minutes post water: 67.8 +/- 6.1; for MSA: baseline 78.2 +/- 9.7; MSA 15 minutes post water: 74.4 +/-8.6 bpm). Beat-to-

beat data during each stand (Table 3.4.3) showed no significant difference between MSA and PAF.

In the MSA subjects an increase in seated SBP and DBP was recorded after water ingestion, which first reached significance 13 minutes post-water ingestion (Figure 3.4.5) and remained so until the end of the study. Seated TPR increased first reaching significance 13 minutes after water ingestion and remaining so until the end of the study. Seated HR showed a reduction, which failed to reach statistical significance. Seated CO, SV and EF did not significantly change during the study.

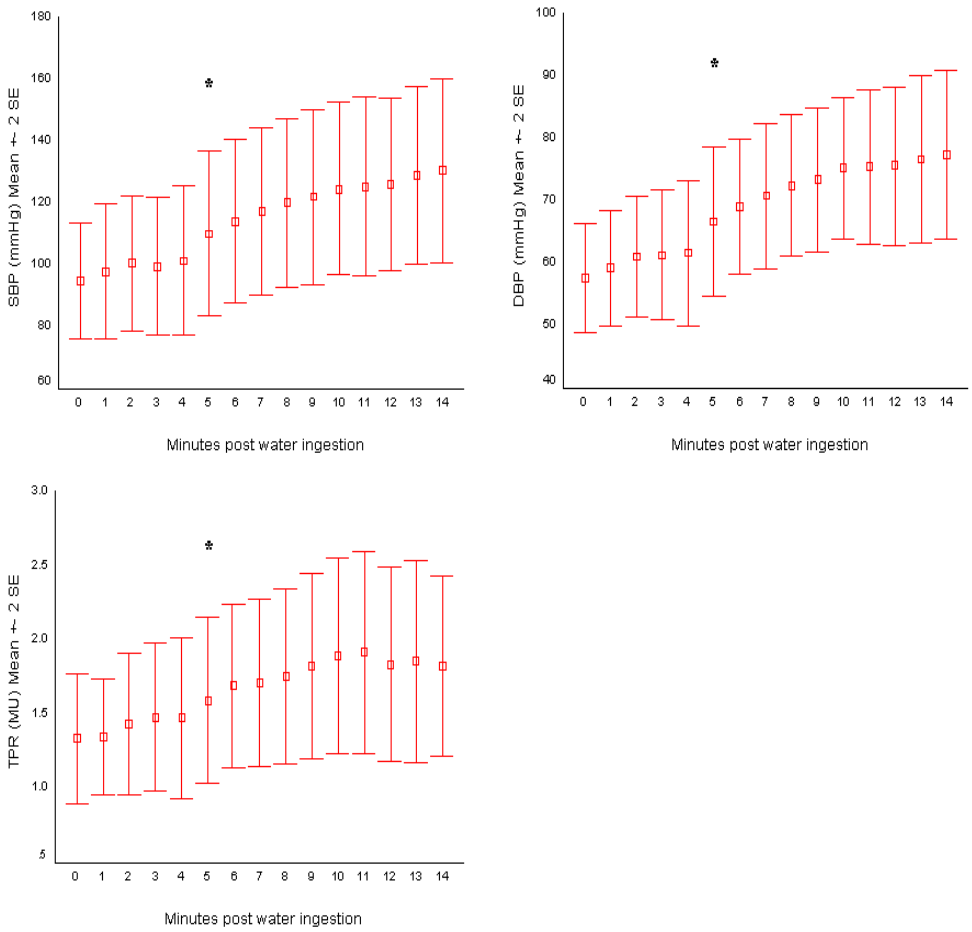

Figure 3.4.2 seated beat-to-beat haemodynamics in PAF during the first 15 minutes post-water ingestion. Upper left panel: Systolic BP (SBP); upper right panel: diastolic BP (DBP); lower panel: Total Peripheral Resistance (TPR). "0" minutes =Mean baseline seated value; *=First significant increase as compared with baseline

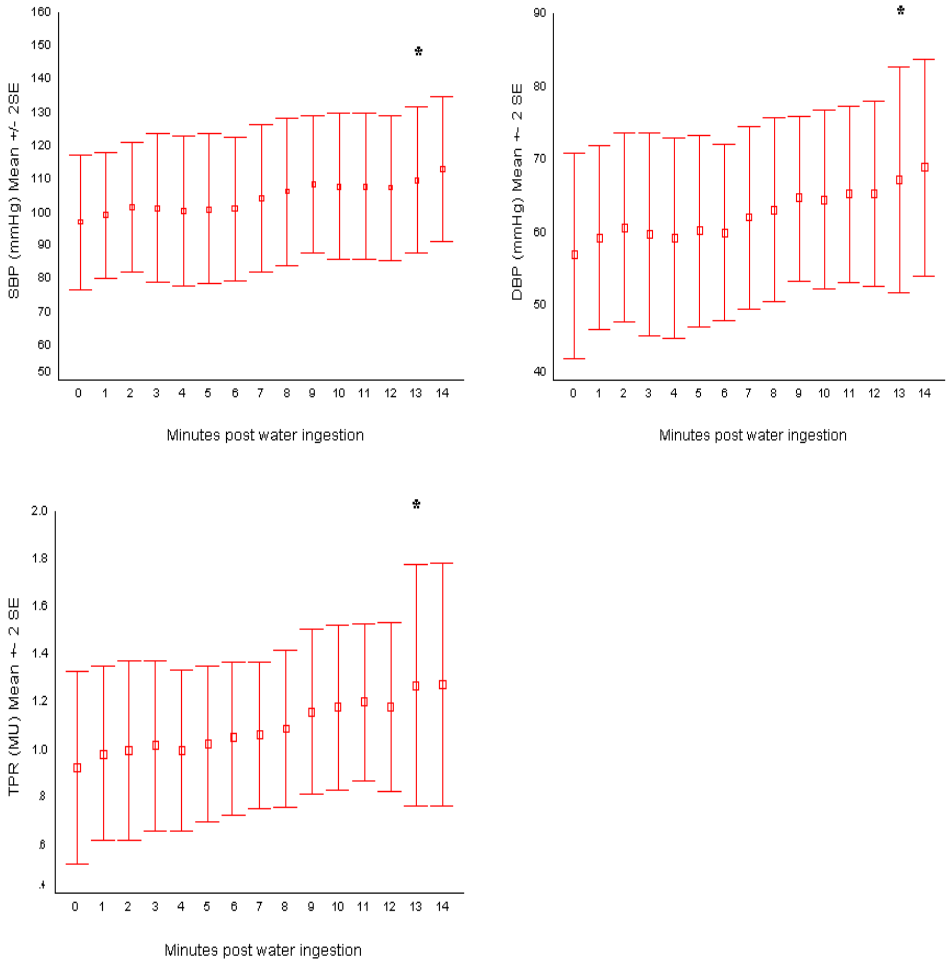

Figure 3.4.3 seated beat-to-beat haemodynamics in MSA during the first 15 minutes post-water ingestion. Upper left panel: Systolic BP (SBP); upper right panel: diastolic BP (DBP); lower panel: Total Peripheral Resistance (TPR). "0" minutes =Mean baseline seated value; *=First significant increase as compared with baseline

Follow up study: Haemodynamic effects of instilling 480ml water directly into stomach in PAF

Introduction

The exact mechanisms of the pressor action of water in AF are unclear, although possible mechanisms include partial residual sympathetic activity, baroreflex dysfunction, gastric distension or fluid redistribution. It has recently been suggested that the effects of swallowing itself might also be important .The only comparison of the haemodynamics of oral water with those of water directly instilled into the stomach was performed in young control subjects in whom water does not produce a sustained pressor effect factor [Endo *et al* 2002]. This study found a brief increase in BP *during* water drinking which lasted for 90 seconds after completion of water ingestion. In the first part of this study no increase in BP over baseline was found in the first 60 seconds after completion of drinking in 14 subjects with AF. Subjects with AF often demonstrate a brief fall in blood pressure on performing a valsalva manoeuvre. It is therefore possible that a partial valsalva manoeuvre during drinking may have masked any transient BP rise in these subjects during drinking. Thus the process of swallowing might still be important in the generation of the water pressor effect in AF. We therefore set out to record the effects of instilling 480ml of distilled water via a gastrostomy tube into a PAF subject to compare the haemodynamic response with those found in previous study of oral water ingestion in PAF [Cariga & Mathias 2001].

Subject and Methods

Our subject was a 69 year old female with a longstanding (>20 years) diagnosis of PAF confirmed with clinical and biochemical investigations using existing criteria [Mathias 2004]. She had had a gastrostomy tube placed in the previous year because of dysphagia, an

occasional complication of PAF. She had documented sympathetic and parasympathetic dysfunction with severe orthostatic hypotension (Table 3.4.6). Vasoactive medication was withdrawn the night prior to the study and informed consent obtained.

		Subject	Normal range
Supine SBP		131	
60° Head up tilt SBP		66	
Change in SBP	Head up tilt	-75	< -20
	Mental arithmetic	-7	> 13
	Hand grip	-13	≥ 17
	Cold Pressor	11	≥ 15
Supine DBP		80	
Standing DBP		47	
Change in DBP	Head up tilt	-33	< -10
	Mental arithmetic	8	> 9
	Hand grip	-9	≥ 11
	Cold Pressor	8	≥ 10
Sinus Arrhythmia		Not present	12 bpm

Table 3.4.6: Autonomic Function testing on study patient SBP=Systolic Blood Pressure mmHg; DBP=Diastolic Blood Pressure mmHg

Study Design

On the morning of the study the subject fasted after a light breakfast at 0800 hours. The study took place in our dedicated autonomic laboratory between 1000 and 1300. After emptying the urinary bladder, the subject was seated and BP was recorded both intermittently and continuously. Intermittent brachial BP values were obtained every 3 minutes using an automated Dinamap (Critikon) sphygmomanometer on the left arm. Continuous measurement of beat-to-beat BP was obtained throughout the study with the Portapres II device on the middle finger of the right hand. Subsequent calculation of cardiac output (CO), total peripheral resistance (TPR), and stroke volume (SV) using Model flow analysis was made. This has previously been validated at rest and in relation to stimuli such as head up tilt [Harms et al 1999, Jellema, Imholz et al 1999, Jellema et al 1999, Langewouters et al 1998, Voogel et al 1997]. After 20 minutes of stable baseline recordings, 480ml of distilled water at room temperature was instilled into her gastrostomy tube over a period of 5 minutes. The subject remained seated for a further 40 minutes with continued haemodynamic recordings as described above. Orthostatic symptoms were assessed before and after water. [Young and Mathias 2004b]

Results

Dinamap baseline seated BP was 67 (1.5) / 45 (1.2) with HR of 77 (1.2). Values during instillation of water were similar to those at baseline (Table 3.4.7). An increase in SBP and DBP was observed from 5-8 minutes post water and peaked between 20 and 35 minutes post water with mean values of 103.8 (2.6) / 68.8 (1.7). This rise in SBP and DBP was confirmed with the beat-to-beat data. There was no significant change in heart rate throughout the study. Analysis of the beat-to-beat data with Modelflow showed no change in cardiac output, stroke

volume or heart rate, but an increase in TPR, which mirrored the increase in BP (Figures 3.4.6 & 3.4.7). At no stage either during or after the water instillation did the subject experience any pain or discomfort. Prior to the water the subject experienced symptoms of orthostatic hypotension, light headedness and "coat hanger" distribution neck and shoulder pain [Bleasdale-Barr *et al* 1998] in the seated position. These symptoms were typical for this subject after sitting or standing in the morning. These symptoms fully resolved 10 minutes after the water was instilled and did not return for the duration of the study.

	Before water	During water instillation	2-5 minutes post water	5-8 minutes post water	20-35 minutes post water
Dinamap SBP	67.3 (1.5)	66.7 (1.5)	74.0 (7.7)	83.0 (5.7)	103.8 (2.6)
Dinamap DBP	44.5 (1.2)	42.3 (3.2)	51.0 (4.2)	57.0 (4.2)	68.8 (1.7)
Dinamap HR	71.3 (1.5)	71.0 (0.0)	71.0 (0.0)	75.0 (4.7)	70.0 (0.6)
Portapres SBP	65.4 (3.8)	74.6 (6.0)	84.1 (6.6)	98.2 (3.3)	129.0 (4.9)
Portapres DBP	48.0 (2.3)	54.1 (7.6)	59.4 (3.6)	66.4 (1.5)	83.4 (2.4)
Portapres TPR	1.9 (0.2)	2.2 (1.0)	2.3 (0.2)	2.4 (0.2)	3.3 (0.2)

Table 3.4.7: Haemodynamic changes before, during, and after gastric instillation of water in PAF subject. Values given as means +/- (SD). TPR=Total Peripheral resistance in MU.

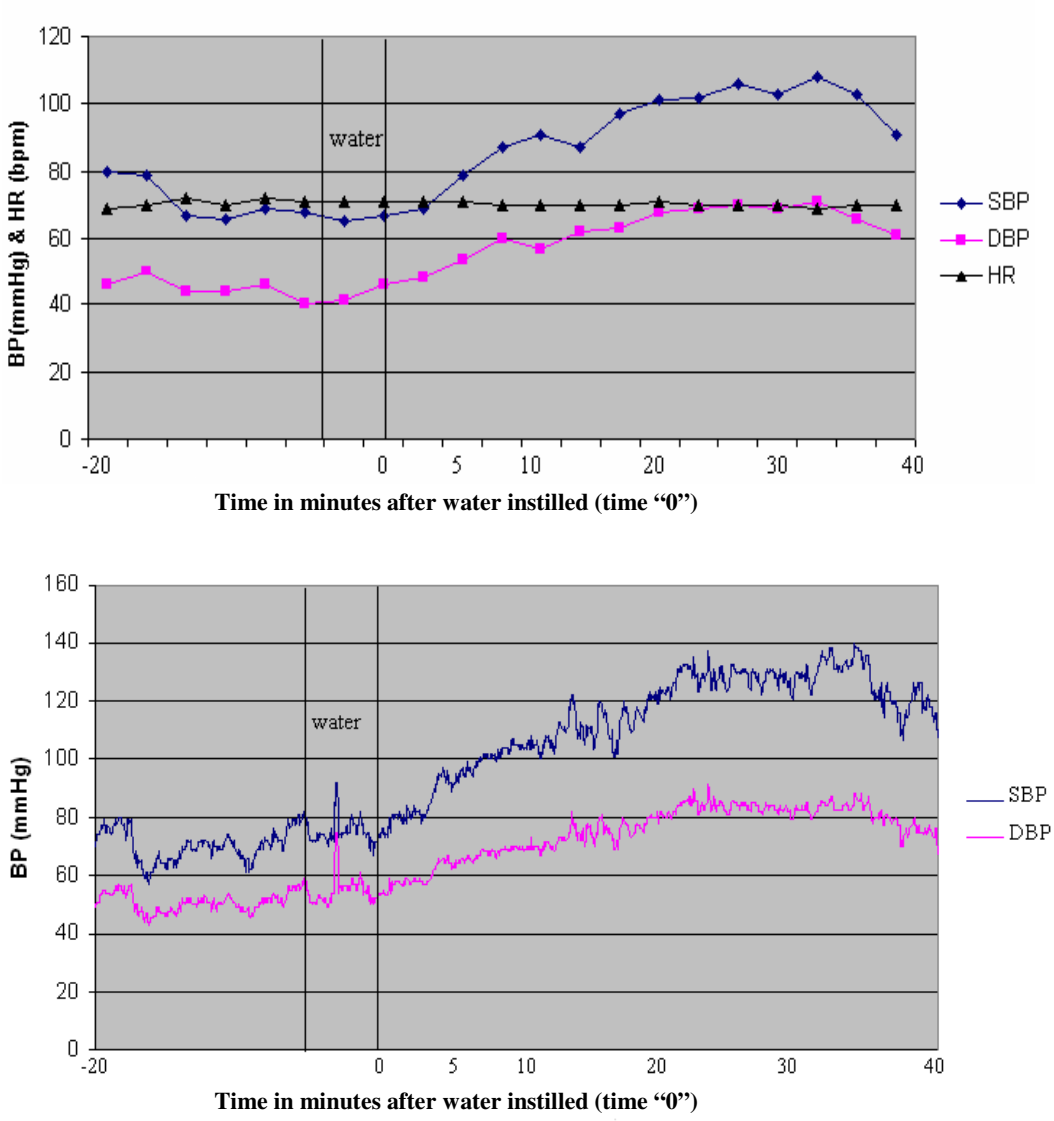

Figure 3.4.4: Seated haemodynamic responses before, during and after instillation of 480ml of distilled water through gastrostomy tube. Upper trace shows Dinamap brachial BP and HR responses. Lower trace shows beat-to-beat Portapres data over the same time period. water instillation finished at time "0".

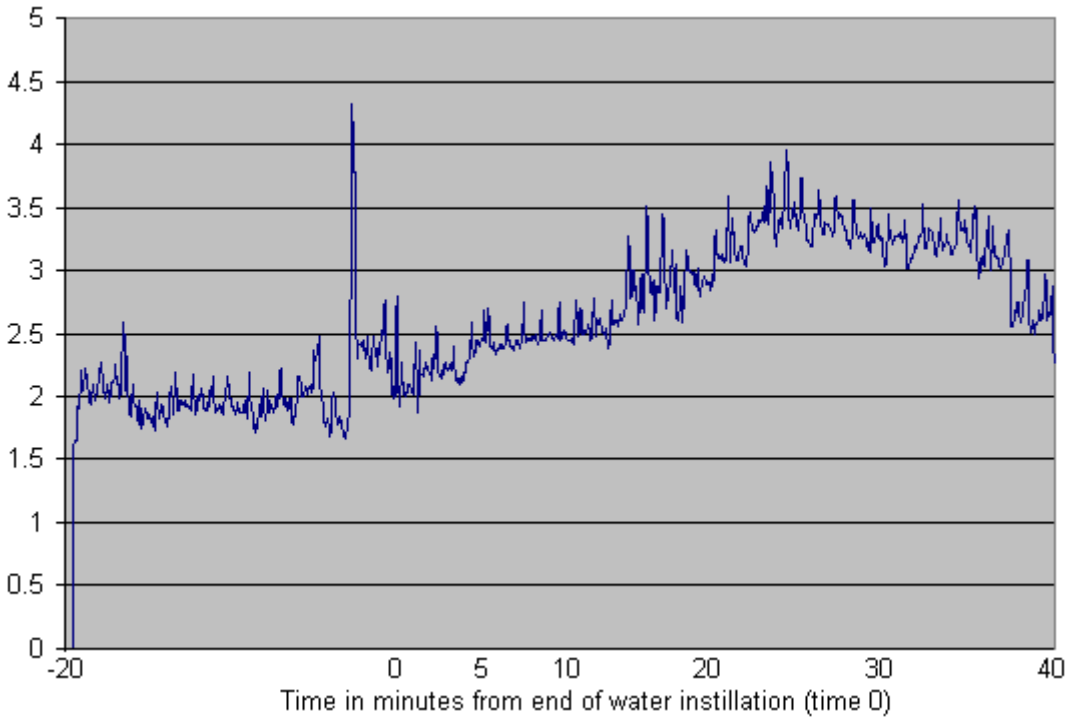

Figure 3.4.5: Total Peripheral Resistance in MU (Y-axis) as calculated from Portapres data with Model flow analysis. Water instillation finished at time "0".

Discussion

We have shown that water can induce a pressor effect in autonomic failure when instilled directly into the stomach. Furthermore, the magnitude and timescale of the rise in BP was similar to that seen in the first part of the study when oral water of the same volume was ingested in PAF. Thus with either oral or directly instilled water, seated BP in PAF seems to start to increase 5 minutes after water with an increase in TPR being a likely underlying factor. Apart from a transient increase in TPR (Figure 3.4.7), the instillation process itself did not correspond with clear haemodynamic changes. It is possible that movement artefact may have been important in producing the brief increase in TPR during water instillation.

The subject experienced typical orthostatic symptoms even in the seated position, and noted resolution of these symptoms after water. These findings were thus consistent with the symptomatic improvement noted in the 14 subjects in the first part of this study. A possible confounding factor might have been a pain induced pressor response due to instillation of 480ml of water with subsequent gastric distension. This is improbable for two reasons; firstly, pressor responses to painful stimuli such as the cold pressor test are reduced in PAF. Secondly this subject felt no discomfort either during or after the water instillation. Our study has therefore shown similar haemodynamic and symptomatic effects to those previously noted with oral ingestion of water in PAF. Although this study was limited to a single subject it provides evidence that the water pressor effect in chronic autonomic failure is related to gastric effects rather than oropharyngeal ones.

The results from the initial study have shown that oral ingestion of 480ml of distilled water results in subjective and objective improvements in OH on standing in both MSA and PAF subjects when measurements were made at 15 and 35 minutes after water. In addition the detailed analysis, utilising continuous haemodynamic measurements pre- and post-water,

indicate that there are temporal differences in the pressor response between MSA and PAF. In previous studies [Jordan *et al* 1999, Shannon *et al* 2002] there did not appear to be differences, and it may be that intermittent measurements every 3 or 5 minutes without continuous recordings may have made comparisons difficult.

The apparent haemodynamic differences between MSA and PAF while seated warrant discussion. In PAF the pressor effect occurred sooner (within 5minutes) unlike MSA where it occurred 13 minutes after ingestion. This may provide further light on the mechanisms responsible for the pressor response, as in PAF all the subjects had clear evidence of substantial peripheral sympathetic denervation; in MSA the predominant lesion was likely to be preganglionic. The pressor response to water is known to be dependant on the volume ingested, and may be the result of gastric distension increasing sympathetic nerve activity by reflex mechanisms, as has been described in normal subjects [Rossi et al 1998]. This response is likely to be dependant on residual sympathetic activity, and is therefore expected to be minimal in PAF, and greater in MSA. This would be consistent with the effects previously described of yohimbine, an α_2 agonist that is dependant on sympathetic activity and where the pressor response was related to the pressor response of water [Jordan *et al* 2000]. However, this was not the case in our studies as the response was as great in PAF with a more rapid onset than in MSA An alternative may be that the pressor response in PAF was related to denervation supersensitivity, which has been well documented in this group, and is greater than in MSA [Polinsky 2002]. It is possible that in PAF, even a small amount of NA released could have acted on supersensitive receptors, although this seems less plausible than the release of other vasoconstrictor substances, including factors such as endothelin, which may then exert the pressor response. These possibilities may explain the rise in peripheral resistance found in this study. A further substance to be considered is vasopressin, which is known to be released in PAF but not MSA in the head up position [Kaufmann *et al* 1992].

Another factor to be considered with water ingestion is the effect of fluid repletion, in patients who are known to be prone to salt and water loss, especially when they are recumbent [Mathias *et al* 1986]. It may be that in MSA there is greater disruption of vasopressin secretion, rendering them even more fluid depleted. It is conceivable that the longer time taken to reach a pressor response, in MSA, was a reflection of greater intra- and extra vascular fluid depletion. It is not clear how quickly after ingestion water is absorbed from the gut in MSA or PAF and in particular how much would have been absorbed in PAF within 5 minutes after ingestion, when the pressor effect of water first occurred.

Mechanisms of the pressor effect of water ingestion

The exact mechanism by which water ingestion results in a pressor response remains uncertain. Various factors are important in determining blood pressure; these include cardiac output, tone in resistance vessels (influenced by either circulating or locally produced constrictor or dilator substances), the state of capacitance vessels, intravascular volume and overall fluid status. The sympathetic nervous system acting on a beat-by-beat basis through the baroreceptor reflex to allow rapid adjustment of blood pressure. There have been various studies attempting to determine the precise mechanisms by which water exerts its pressor effect. The studies outlined above suggest the importance of increased total peripheral resistance in the generation of the water pressor effect as well as providing evidence on the latency of the effect; in PAF a prompt response within 5 minutes of ingestion in seen whilst in MSA a slower response was observed.

Effects of water temperature and volume

To determine if there was temperature and volume dependency, four subjects with autonomic failure were studied [Jordan et al 2000]. There was no difference in the pressor response when water at either 9^{o}C or 24^{o}C was administered. The pressor response was related to the volume ingested, as systolic blood pressure increased by up to 50mm Hg after 480 ml water, and by 30mm Hg after 240 ml when studied in 4 subjects with chronic autonomic failure, raising the possibility of reflex sympathetic activation from stomach distension in the generation of the pressor response. In an initial study in one MSA study I found that an even lower volume (around 200ml) was sufficient to generate a marked pressor response. This subject was relatively small and felt a sensation of fullness at this volume. Studies in healthy animals and humans have demonstrated that graded gastric balloon distension causes a rise in Muscle Sympathetic Nerve Activity MSNA and blood pressure [Rossi et al., 1998]. However, in normal subjects, oral water ingestion was shown to raise MSNA but not elevate blood pressure [Scott et al., 2001]. Conflicting results were later shown in another group of normal young subjects where oral water ingestion caused an initial transient rise in blood pressure and heart rate but a decrease in MSNA [Endo et al., 2002]. In that study the same volume of water administered by a stomach tube over 20 min, caused no change in either MSNA or blood pressure, raising the possibility that the initial pressor response resulted from the pharyngeal/oesophageal phase of water drinking. The degree of distension may be a factor in explaining this discrepancy-it is known that excessive dilatation the stomach may result in extreme bradycardia and even hypotension presumably via vasovagal mechanisms [Hmouda et al 1994]. The swallowing stage of drinking does not appear to be involved in generating the pressor effect in chronic autonomic failure, with a pressor effect to water having been demonstrated when water is instilled directly into the stomach of a patient with PAF [Young & Mathias 2004b] or MSA [Lipp et al 2005].

Recent comparisons between haemodynamic responses to gastric distension with a barostat have been made in 8 young and 8 older normal subjects [van Orshoven et al 2004]. In this study the older subjects tended to show a higher increase in mean arterial pressure, heart rate and total peripheral arterial resistance despite showing a significantly attenuated increase in MSNA compared to the young subjects. The older subjects reported less symptoms of stomach distension, although there was no significance in stomach compliance between the young and older subjects. Possible reasons for these results might include the known reduction in central arterial compliance with ageing suggesting that even the attenuated increase in MSNA seen in the older group could still result in a significant pressor effect. An alternative possibility would be that the attenuation of sympathetic outflow in response to gastric distension in older normal subjects and in autonomic failure alters the local effect of locally released vasoactive substances akin to the known vasopressor supersensitivity known to occur in PAF.

That volume alone is insufficient to produce a pressor effect is clear from the fact that food consumption is associated with post-prandial hypotension in chronic autonomic failure. Thus with the same PAF patient in whom a pressor effect was shown following instillation of water via a gastrostomy tube, instillation of food resulted in a reduction in blood pressure; the nocturnal instillation of food in this patient actually resulted in the reversal of her pre-existing supine hypertension at night. There are several possible factors in explaining the discrepancy in blood pressure response to ingestion of food or water. Food, especially carbohydrates, results in splanchnic vasodilatation in AF as a result of insulin release and subsequent Nitric Oxide release. Secondly the presence of food substantially slows gastric emptying, thus delaying the absorption of water from the small bowel (FIGURE 1). Final additional factors such as osmoceptor activation would be more likely with relatively hypotonic chyme following water ingestion alone.

Effects of water osmolality

The first publication suggesting that water ingestion may lead to pressor response in chronic autonomic failure described a patient who had noted that the ingestion of seawater improved their postural hypotension symptoms [Frewin & Bartholomeusz 1983]. Subsequent investigations confirmed that a pressor effect occurs in chronic autonomic failure after ingestion of either tap water [Jordan et al 1999] or distilled water [Cariga & Mathias 2001]. The studies detailed in this chapter utilised distilled water. In dogs data have suggested that gastric distension with saline results in a pressor response, but that the magnitude of BP increase is twice as great when distilled water is used [Haberich 1968]. In humans hypoosmolar water infused through a nasogastric tube induces greater sweating than isomolar saline, and it had been suggested that this might reflect greater sympathetic activation by hypo-osmolar solutions [Haberich 1968]. More recently the possible effects of osmolarity have been studied in MSA [Lipp et al 2005]. A total of 10 patients were studied in a semi supine position with 500ml of either normal saline (iso-osmotic) or distilled water (hypo-osmotic) infused into the stomach over 5 minutes through a nasogastric tube. There was fairly marked variability in response. However there was a significant difference in the BP response between the 2 groups, with distilled water but not saline producing a pressor effect between 10 and 40 minutes post-ingestion. Whilst varying osmolarity may effect the BP response to ingested water by osmoreceptor mechanisms, confirmatory studies of these provisional results would be helpful given the small size and variability in the studies so far.

Vasoactive neurohumoral substances

The pressor effect to water occurs after a latency of at least 5 minutes, and I have demonstrated an association with increased TPR in both MSA and PAF in the studies of this chapter. These observations raise the possibility of local or systemic vasoconstrictor

substances in its aetiology. The time course of the rise in blood pressure and total peripheral vascular resistance after water ingestion in PAF is slow (over minutes), with an even later rise in MSA. This is considerably slower than after activation of reflexes that are known to raise blood pressure. In tetraplegics with high spinal cord lesions, in whom central sympathetic outflow is interrupted but vagal efferents preserved, activation of viscera (such as the urinary bladder and large bowel), or induction of skeletal muscle spasms, results in autonomic dysreflexia with a rapid rise in blood pressure, often within a few seconds, as a result of activation of spinal sympathetic reflexes below the segmented level of lesion [Mathias *et al* 1976a]. The response to water ingestion recently has been reported in tetraplegics [Tank *et al* 2003]. The pressor response to water varied; mean supine finger blood pressure rose from 123±8/165±4 to 138±8/73±4 mmHg after 40 minutes, while heart rate fell from 64±2 to 60±2 bpm. In some there was no pressor response. The reasons for this are unclear. In this study, the patients were studied supine, with the upper body at 15°; in previous studies patients were seated. The pressor response to water, without a gravitational stimulus, would be expected to be as great or greater, if sympathetic activation was responsible, especially in tetraplegics who are known to response briskly to such stimuli. The latency of the response was unlike that of autonomic dysreflexia, and similar to the slower time course in autonomic failure, raising the probability that similar mechanisms accounted for the pressor response to water in the different groups. Thus reflex sympathetic activity seems unlikely to adequately explain the latency of the water pressor response. Could a neurohumoral response better fit the observed time course?

Systemic circulating hormones, such as vasopressin and angiotensin-II are powerful vasoconstrictors. However, in normal subjects, plasma vasopressin and renin activity did not change following water ingestion, and levels were not reported in autonomic failure except for plasma renin, which was reported unchanged in 2 subjects with autonomic failure [Jordan *et al* 2000]. Plasma noradrenaline levels increased after water ingestion in both young and older normal subjects [Jordan *et al* 2000 & Scott *et al* 2001], but there was no correlation

between the rise in plasma noradrenaline levels and the changes in blood pressure. Younger normal subjects do not change BP in response to oral water despite a rise in plasma noradrenaline [Scott *et al* 2001]. The increase in circulating plasma noradrenaline in these subjects following water ingestion is more likely to reflect the increase in sympathetic nerve activity suggested by increased MSNA than have direct vascular effects [Scott *et al* 2001]. The noradrenaline responses to water ingestion in autonomic failure are not known.

Locally released vasoactive substances also warrant discussion in the context of water ingestion. Inhibition of vasodilators or increased levels of vasoconstrictors such as endothelin could theoretically cause locally mediated pressor effects. It is known that distension of an isolated rat stomach results in release of somatostatin and reduced release of gastrin [Li 2003]. Both somatostatin and intrinsic cholinergic pathways were involved distention-induced inhibition of gastrin release. Although the effect of water ingestion on these hormones in man is not known, the combination of release of somatostatin and reduced release of gastrin is interesting as somatostatin causes splanchnic arteriolar vasoconstriction by PKC-dependent vasoconstrictors, possibly via SSTR2 receptors [Reynaert & Geerts 2003], and enhances endothelin-1-induced vasoconstriction [Huang et al 2002]. Gastrin on the other hand is a vasodilator. Gastrin is released by gastrin-releasing peptide, which has recently been shown to be a potent systemic vasodilator in healthy human subjects [Clive et al 2001]. The effects of gastrin on blood pressure are not as clear, although pentagastrin, a synthetic analogue, appears to increase BP and HR in man [Tavernor et al 2000]. The situation in chronic autonomic failure is complicated by the fact that basal gastrin and release following hypoglycaemia may be increased in PAF, but reduced in MSA [Polinsky et al 1988]

Pharmacological Studies

Pharmacological studies have been used at both ganglionic and pre-synaptic sympathetic levels. The possibility that residual sympathetic nerve activity may have been a factor in generating the pressor effect to water has been studied with trimethaphan ganglionic block [Jordan *et al* 2000]. Plasma noradrenaline was measured in two MSA patients, before and after ganglionic blockade. In both MSA subjects plasma noradrenaline levels fell, indicating suppression of residual sympathetic nerve activity. In neither was there a pressor response when water was administered after ganglionic blockade. The lack of a pressor response to water in MSA after ganglionic blockade raised further the possibility of activation of sympathetic neural mechanisms. Further evidence in normal subjects was considered to favour this, as the pressor response to water was also absent after ganglionic blockade [Jordan *et al* 2000]. However, this was performed only in younger subjects, who did not have a pressor response to water. Furthermore the pressor response is clearly seen in PAF subjects with extensive sympathetic denervation [Cariga & Mathias 2001].

Yohimbine acts on α-2 pre-synaptic receptors resulting in noradrenaline release and thus its effect is dependent on the integrity of sympathetic nerve terminals. In patients with primary autonomic failure there was a correlation between the pressor response to yohimbine and to water, suggesting that those with less sympathetic denervation had a greater pressor response [Jordan *et al* 2000]. This would be in keeping with a previous report where it was observed that patients with PAF with the greatest increase in blood pressure after water had a normal or even exaggerated pressor response [Jordan *et al* 1999]. This also implies the reverse, that patients with substantial sympathetic failure are less likely to have a pressor response to water. However, in later studies [Cariga & Mathias 2001] and in my studies outlined in this chapter, a marked pressor response to water ingestion also occurred in PAF with marked sympathetic dysfunction. Furthermore, in spinal cord injury tetraplegics, who have preserved sympathetic nerve terminals and have exaggerated pressor responses to vasoactive drugs and

a range of visceral and other stimuli [Mathias & Frankel 2002], the pressor response to water was similar or less than patients with autonomic failure. This suggests that other possibilities are needed to explain the pressor response. Finally it should be noted that Yohimbine is not a pure α-2 antagonist. It also displays partial agonist activity at cloned human 5-HT1A receptors, significant affinity for 5-HT1B and 5-HT1D receptors and antagonist properties at dopaminergic D2 receptors [Millan et al 2000]. Of these the D2 effect might be especially significant given the known action of other D2 antagonists such as domperidone and gastric motility and blood flow.

Correction of Fluid depletion

For a variety of reasons fluid depletion is more likely to occur in patients with autonomic failure. Tubular absorption of sodium and other solutes, is influenced by sympathetic nerve activity, and may account for the urinary sodium and water loss in such patients [DiBona & Wilcox 2002]. When supine patients with autonomic failure often develop raised blood pressure, sometimes to a marked extent. This in turn increases renal perfusion pressure and probably contributes to recumbency-induced polyuria [Schalekamp 1985, Kooner *et al* 1988]. Overnight, if lying flat their body weight can fall by 1-1.5 kgs, which worsens both the symptoms and degree of orthostatic hypotension in the morning [Mathias *et al* 1986]. Correction of this deficit is the basis for nocturnal use of the vasopressin-2 agonist desmopressin, which reduces water loss, overnight weight loss, improves morning orthostatic hypotension as well as reducing symptoms [Mathias *et al* 1986, Mathias & Young 2003]. It is possible that this might have been a factor in my studies as the protocol required that the subjects fasted after a light breakfast at 0800 on the day of the study. This was comparable with earlier studies and reduced the confounding variables of residual pressor effect from fluid consumption, or post-prandial hypotension from food. However, it also meant that early dehydration might have been present for the reasons outlined above.

An important consideration when considering possible correction of fluid depletion is the speed with which water is absorbed from the gut, given that the pressor effect is observed as rapidly as 5 minutes post-ingestion. As significant volumes of water are not absorbed into the portal circulation until water leaves the stomach to enter the duodenum, gastric emptying is a key concept. In normal subjects, the emptying half time of 800 ml of water is 21 minutes [Ploutz-Snyder et al 1999], as measured with magnetic resonance imaging. With smaller volumes of 500 ml this is likely to be different. In normal subjects, water alone increases portal blood flow, measure by Doppler sonography [Host *et al* 1996]. Extrapolating from studies in normal subjects, who are well hydrated, may be erroneous if compared to relatively dehydrated autonomic failure patients. In normal subjects, hydration is recognised as reducing orthostatic responses. Gastric emptying in autonomic failure may be more rapid than in normal subjects [Mathias & Bannister 2001], and absorption of even a small fluid volume could create a sufficient pressor effect in patients because of impaired baroreflex function. In studies on rehydration after fluid restriction in normal subjects, the importance of extravascular hydration was thought to be of greater importance than intravascular hydration, in determining orthostatic tolerance [Harrison *et al* 1986].

Water ingestion initially may raise plasma volume, but it is likely that redistribution then occurs with subsequent movement of fluid from intravascular into extravascular spaces. Saline has advantages over water alone, because it is stored extracellularly and causes greater explosion of both extra- and intravascular spaces (Harrison 1986). This explains the benefit of saline drinking in autonomic failure (Frewin & Bartholomeusz 1983), although ingestion of salt and water often causes side effects, including diarrhoea. Recent work on the effects of different osmolarities of water however suggests that hypo-osmolar water produces a greater pressor response on ingestion in MSA than isotonic saline [Lipp et al 2005]. The haematocrit, as a measure of plasma volume was measured in normal subjects before, 30 and 60 minutes after water ingestion [Jordan *et al* 2000]. No significant changes in the haematocrit were observed, seemingly excluding increases in intravascular volume as a factor. A variety of

factors can influence interpretation of haematocrit in this context, especially in subjects with denervation [DiBona & Wilcox 2002]. Furthermore, the pressor effect of water occurred after 5 minutes and peaked at 30 minutes, making it likely that water redistribution had occurred when the first measurements were made post-water ingestion. To measure the direct effects of plasma expansion, intravenously administered 5 % dextrose was infused into five PAF, but over a 60-minute period [Jordan *et al* 2000]. After infusion, plasma volume increased by 5.3% and blood pressure by 18mm Hg, compared to 52mm Hg with oral water. Patients with autonomic failure usually have a brisk pressor response to intravenous fluid replacement, although normal saline usually is used for rehydration and to raise blood pressure. However, carbohydrate is known to lower blood pressure in autonomic failure [Mathias *et al* 1989], and the potential hypotensive effect of dextrose may have negated the pressor effect of this volume of water intravenously. Finally the time course of water infusion in this study is difficult to interpret, as the time taken to fully absorb ingested water into the systemic circulation in PAF is not known.

The reasons for the differential responses to water ingestion in young and older normal subjects remains unclear. Older subjects are more likely to have a degree of refractoriness of their renal tubules to vasopressin, and thus may be relatively less fluid replete than younger normals. However, other factors, greater vascular responses to pressor stimuli, or less effective baroreceptor reflexes are also possible. This also may account for the pressor response to water (13 to 29 mmHg) observed in four cardiac transplant recipients who lacked cardiac autonomic innervation [Routledge *et al* 2002]. The pressor response to gastric distension with a barostat appears to be greater in older normals despite an attenuation in MSNA increase compared to younger normals. Reduced compliance of central arteries with aging may explain this apparent discrepancy.

The beneficial effects of water ingestion in autonomic failure

The early observations of the pressor effect of water prompted suggestions of potential therapeutic effects on orthostatic and post-prandial and exercise-induced hypotension, in autonomic failure [Mathias 2000]. Subsequent studies have confirmed the therapeutic potential of water ingestion. In autonomic failure, water ingestion raises standing blood pressure (83+6/53+3mm Hg pre water to 114+30/66+18), 35 minutes after water when measurements were made 1 minute after standing [Shannon et al 2002]. Post-prandial hypotension (reduction of 43/20mm Hg after 90 minutes) was reduced when water was ingested immediately before a meal (reduction of 22/12mm Hg after 90 minutes). In a further study comparing PAF and MSA, similar observations have been made in relation to the effectiveness of water in reducing orthostatic hypotension in the different groups; furthermore, in this study there was objective evidence of a reduction in orthostatic symptoms [Young & Mathias 2004]. The effects of repeated water ingestion are not known. Topping up with subsequent smaller drinks of water maintains a large gastric volume thus keeping gastric emptying at a high rate in healthy subjects [Noakes et al 1991]. It is not known whether "topping up" with subsequent small drinks of water can prolong the pressor effect in autonomic failure. However, water ingestion does not appear to raise blood pressure, supine or standing, in Parkinson's disease with autonomic failure [Senard et al 1999] and its effects in the other diseases causing orthostatic hypotension requires further study.

Haemodynamic effects of water ingestion in other autonomic diseases

Water ingestion may benefit disorders such as neurally-mediated syncope and the postural tachycardia syndrome where there is intermittent autonomic dysfunction. Vasovagal syncope is the most common form of neurally-mediated syncope [Mathias *et al* 2000] with cardio-inhibitory, vasodepressor and mixed components. Oral water drinking was suggested as a

treatment in children with vasodepressor syncope [Younoszai *et al* 1998]. In normal subjects water ingestion increases orthostatic tolerance (by up to 5 minutes) when tested with a combination of head-up tilt (to 60^{o}) and lower body negative pressure (at levels of -20, -40 and -60mm Hg for 10 minutes each, or until pre-syncope occurred) [Schroeder *et al* 2000]. Water increased peripheral vascular resistance and reduced the fall in stroke volume. Thus, water may benefit subjects with vasovagal syncope prone to episodes especially whilst standing still or in hot weather.

In the postural tachycardia syndrome (PoTs) there is a substantial rise in heart rate (of over 30 bpm), usually without a fall in blood pressure, on sustaining an upright posture. Following water ingestion standing levels of blood pressure were not affected, but standing heart rate was lowered, from 123$\pm$23 bpm after 3 minutes of standing pre water, to 108$\pm$21 bpm after water [Shannon *et al* 2002]. The effects of water ingestion on symptoms however, were not reported.

Concluding Remarks

Substantial data now clearly indicate that ingestion of 480-500 ml of water result in a pressor response in older normal subjects and an even greater response in autonomic failure due to PAF and MSA whilst not changing blood pressure in young normal subjects. A variable pressor response also occurs in tetraplegics with high spinal cord lesions. The mechanisms of this pressor response to water remain unclear. However, water is effective in autonomic failure in reducing both orthostatic and post-prandial hypotension and also improves symptoms resulting from orthostatic hypotension. Longer-term effects remain to be established, especially in MSA where an expected later diuresis might offset some of these early benefits.

Although much work has been performed over the last few years in both confirming and investigating the aetiology of the pressor effect of water, its mechanisms remain elusive. Further work is needed in this area including in the measurement of vasoactive neurohumeral substances resulting from water ingestion in both normal subjects and those with autonomic failure. Improved understanding of the pressor effect would be of benefit in aiding better understanding of the pathophysiology of autonomic failure. In addition, the pressor effects observed in older subjects prompt the important question of the possible role water ingestion may play in hypertension, an endemic problem in our aging population.

These studies therefore indicate that, although the magnitude of pressor response is similar in PAF and MSA, there are differences in the speed of response. The reasons for this could be multiple, and need to be dissected further. In both PAF and MSA water increased the standing BP as compared to baseline, and reduced symptoms due to OH. In previous studies in autonomic failure orthostatic challenge was compared at 35 minutes only after water ingestion; in our subjects we predicted that the beneficial response on standing might occur earlier, as in previous studies in PAF the seated pressor response was observed within 5 minutes of water ingestion. The haemodynamic analysis indicated that the pressor response was associated with an increase in TPR, again favouring vasoconstrictor mechanisms as being responsible. However, the increase in standing BP appeared to be related to the increase in baseline BP after water rather than to increased sympathetic nerve activation following standing, as the differences in absolute falls of BP following standing at 15 and 35 minutes post-ingestion were similar to those found at baseline. Regardless of the mechanisms, water improved symptoms in all the patients who had been symptomatic pre-water ingestion. Thus water ingestion may be a valuable adjunct in the management of OH, especially in the morning when patients are usually most affected by OH.

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3.5: Cardiovascular Responses on Tilt-Reversal in MSA and PAF

ABSTRACT

Objective: To assess the effects of tilt reversal back to supine after head up tilt (HUT) on blood pressure (BP), heart rate (HR) and plasma Arginine Vasopressin (AVP) in multiple system atrophy (MSA) and in pure autonomic failure (PAF).

Background: In MSA and PAF Head up Tilt (HUT) is associated with significant orthostatic hypotension. On tilt reversal back to supine posture there may be an initial overshoot in the blood pressure in PAF not MSA, resulting in transiently higher supine values than those obtained prior to HUT. AVP is a hormone produced in the hypothalamus and released from the posterior pituitary in response to head-up posture and may be important in helping to maintain BP in the upright position by virtue of its vasopressor actions. As a previous study had suggested that this AVP response may be preserved in PAF but not in MSA (where central lesion sites include the hypothalamus), this study sought to ascertain if there was a difference in the BP overshoot between MSA and PAF, and if so whether this was related to the AVP response. The working hypothesis was that HUT would result in a significant AVP release in PAF but not MSA and that on subsequent return to supine, the residual AVP action would cause an exaggerated pressor response in PAF, in whom there is a known pressor supersensitivity. Thus the expected result would be an initial rise in plasma AVP in PAF but not MSA, and on tilt reversal back to supine a brief BP overshoot in PAF but not MSA.

Methods: An initial pilot study was performed to confirm the presence of BP overshoot in PAF not MSA. A further study was then performed to help ascertain if this BP overshoot appeared to be correlated to plasma AVP levels. In the second study 14 subjects, 7 MSA and 7 PAF were studied. BP was recorded both intermittently and continuously with Dinamap and Portapres II devices respectively. The subject lay supine for 20 minutes then underwent 10 minutes of head up tilt (HUT), immediately following which was a tilt reversal back to supine where the subject remained for the rest of the study. Plasma AVP levels were taken from the

intravenous line at baseline, at the end of 10 minutes HUT, and then 10 and 20 minutes following the return to supine position.

Results: Both studies demonstrated a similar decrease in SBP and DBP in MSA and PAF during HUT, with MSA but not PAF demonstrating a significant increase in HR. In both studies following tilt-reversal there was an increase in supine SBP, compared to pre HUT values, in PAF but not MSA. This increase reached a peak at about 7 minutes post tilt reversal. Following tilt-reversal there was a non-significant increase in DBP and TPR in PAF in both studies and a trend towards HR reduction in the MSA group. Although the mean plasma AVP basal level and increase following HUT was greater in PAF, differences did not reach significance.

Conclusions:

These studies confirm the presence of transient BP overshoot in PAF not MSA following tilt reversal, and have identified that SBP rather than DBP increase appeared to explain the MAP increase in PAF [Chandler and Mathias 2002]. The mechanism, however, is less clear with factors such as TPR and HR likely to contribute to differences in the SBP to tilt-reversal between MSA and PAF. The increased levels of plasma AVP in PAF may be an additional factor. These results have practical implications in the management of BP in PAF.

Introduction

Although much work has been performed on the effects of HUT in MSA and PAF, less attention has been paid to the subsequent tilt reversal to supine. Both MSA and PAF patients have impaired baroreflexes [Omboni *et al* 1996]. Because of this the normal buffering of the tendency to hypertension on change from tilt-up to supine posture would be impaired. Thus an overshoot in BP on resumption of supine position might be expected. In provisional case studies we have shown that, when returning PAF patients back to supine after head-up tilt,

there is an increase in systolic BP compared to their pre-tilt values (see Figs 3.5.1 & 3.5.2). This is greater than in MSA patients who return to supine after tilt. Normal subjects do not usually show either a decrease in BP on HUT, or a BP overshoot after head-up tilt reversal [Wieling et al 1998; Chandler & Mathias 2002; Toska & Walløe 2002], although they may show a spiky increment above pre-tilt baseline levels within the first few seconds after tilt reversal [Wieling et al 1998]. However, the situation appears to be different in PAF where one earlier study had suggested a transient increase in supine BP after tilt-reversal compared to supine values before head-up tilt (HUT) [Chandler & Mathias 2002]. This study by Chandler and Mathias had noted a possible overshoot of MAP in PAF but not MSA subjects during the tilt-reversal following a 10-minute HUT. Although supine hypertension is a well recognized complication in both MSA and PAF, MSA supine BP did not appear to be significantly altered following tilt reversal. The mechanism was postulated to involve TPR, as TPR was increased by about 12% from baseline values in PAF following tilt-reversal but was unchanged in MSA. In view of this, speculation of the involvement of systemic vasoconstricting agents was undertaken; given the extensive sympathetic denervation in PAF this seemed highly unlikely to be related to increased sympathetic activity. Postganglionic sympathetic dysfunction appears to enhance the pressor effect of AVP, as shown in rats pretreated with 6-hydroxydopamine,a drug which destroys peripheral sympathetic postganglionic nerve terminals [Laycock and Lightman 1989]. Additional evidence had seemingly discounted significant adrenomedullary involvement [Polinsky 1999]. Studies in rats have shown an enhanced pressor response to AVP. Whilst other systemic vasoconstrictors such as angiotensin II or endothelin could theoretically be involved, there did not seem to be a clear reason why this should involve PAF and not MSA subjects.

As AVP has a potent pressor effect in AF, it may well be that this hormone explains the discrepancy in overshoot on return to supine [Mohring et al 1980]. The central involvement of MSA, including the hypothalamus, leads to the impaired secretion of substances such as Growth Hormone (GH) and AVP [Kimber et al 1997; Ozawa et al 1999]. AVP secretion in

AF has not been examined as fully as with GH. The pressor actions of AVP seem to be greatly enhanced in autonomic failure [Mohring et al 1980]. AVP secretion is increased on upright posture in healthy subjects, and increases can be detected within 4 minutes of upright tilt in these subjects [Davies et al 1976]. Because of the central involvement in MSA, this rise would be expected to be impaired. Indeed, although the osmotic-induced increase in AVP appears to be preserved in MSA, the ability to increase AVP secretion in response to orthostatic stimulus is impaired [Lightman et al 1992]. Kaufmann et al have shown that upright AVP levels do not increase in MSA, but did so in 4 PAF subjects [Kaufmann et al 1992]. The AVP levels on returning the subject to supine horizontal are not known, and would be important in establishing the pathophysiology of supine BP overshoot in PAF, as this reaches a maximum 5-8 minutes after returning to the supine position in our provisional studies. We would thus expect to confirm increased AVP in PAF but not MSA patients on head-up tilt, and will assess the AVP levels on return to the supine position. Such a finding would not only help in the differential of MSA and PAF as well as having potential important clinical importance; the long term consequences of supine hypertension in autonomic failure is known to be associated with the development of ventricular hypertrophy as seen in essential hypertension whilst theoretical concerns about acute elevation of BP and intracerebral haemorrhage also deserve attention.

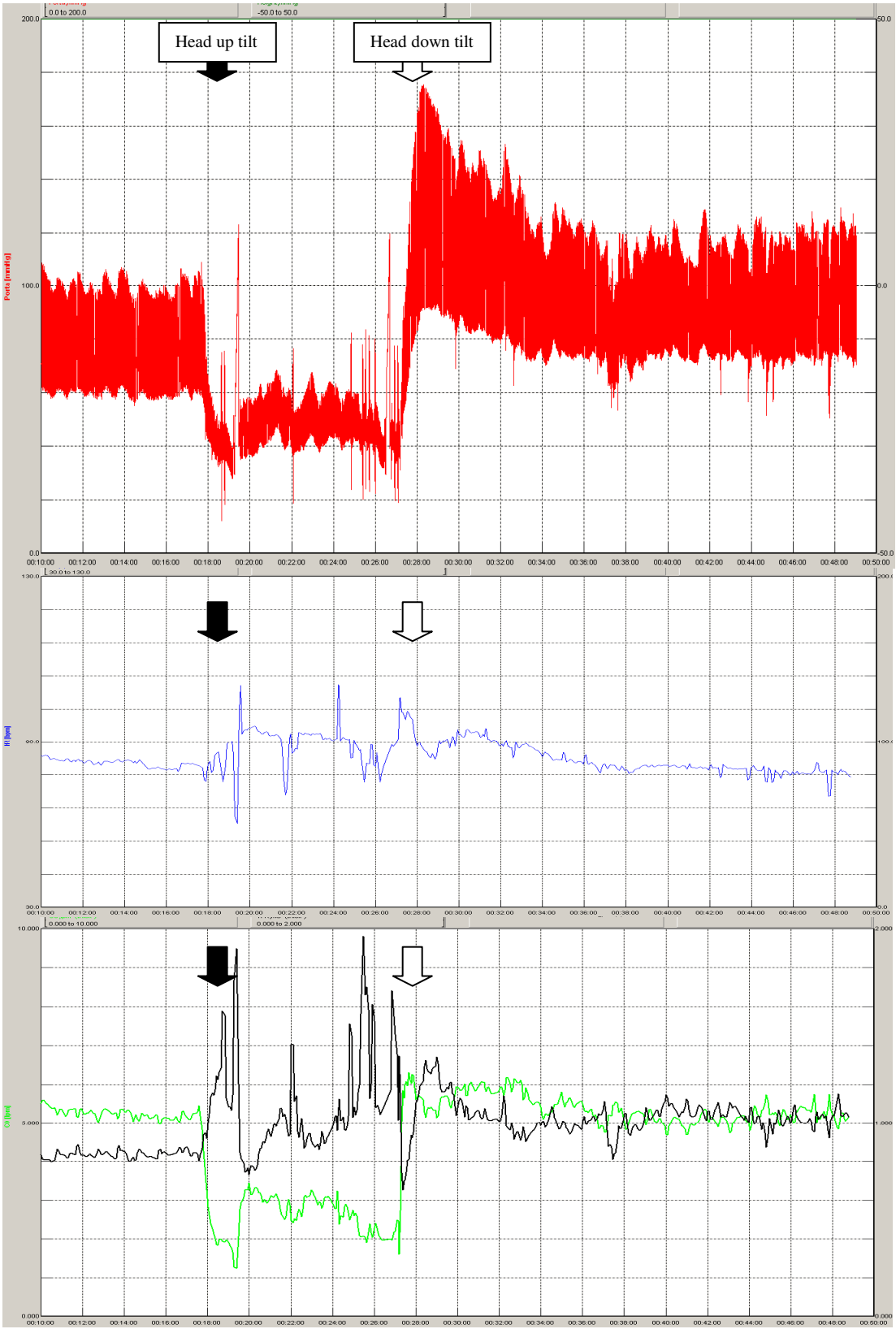

Figure 3.5.1: Continuous Haemodynamics following Head-up Tilt for 10 minutes (black arrow= start) and Tilt Reversal (white arrow= start) in a PAF subject. Upper Trace: BP (red); Middle Trace HR (blue); Lower Trace: Cardiac Output (green) and TPR (black). X-Axis Time in minutes.

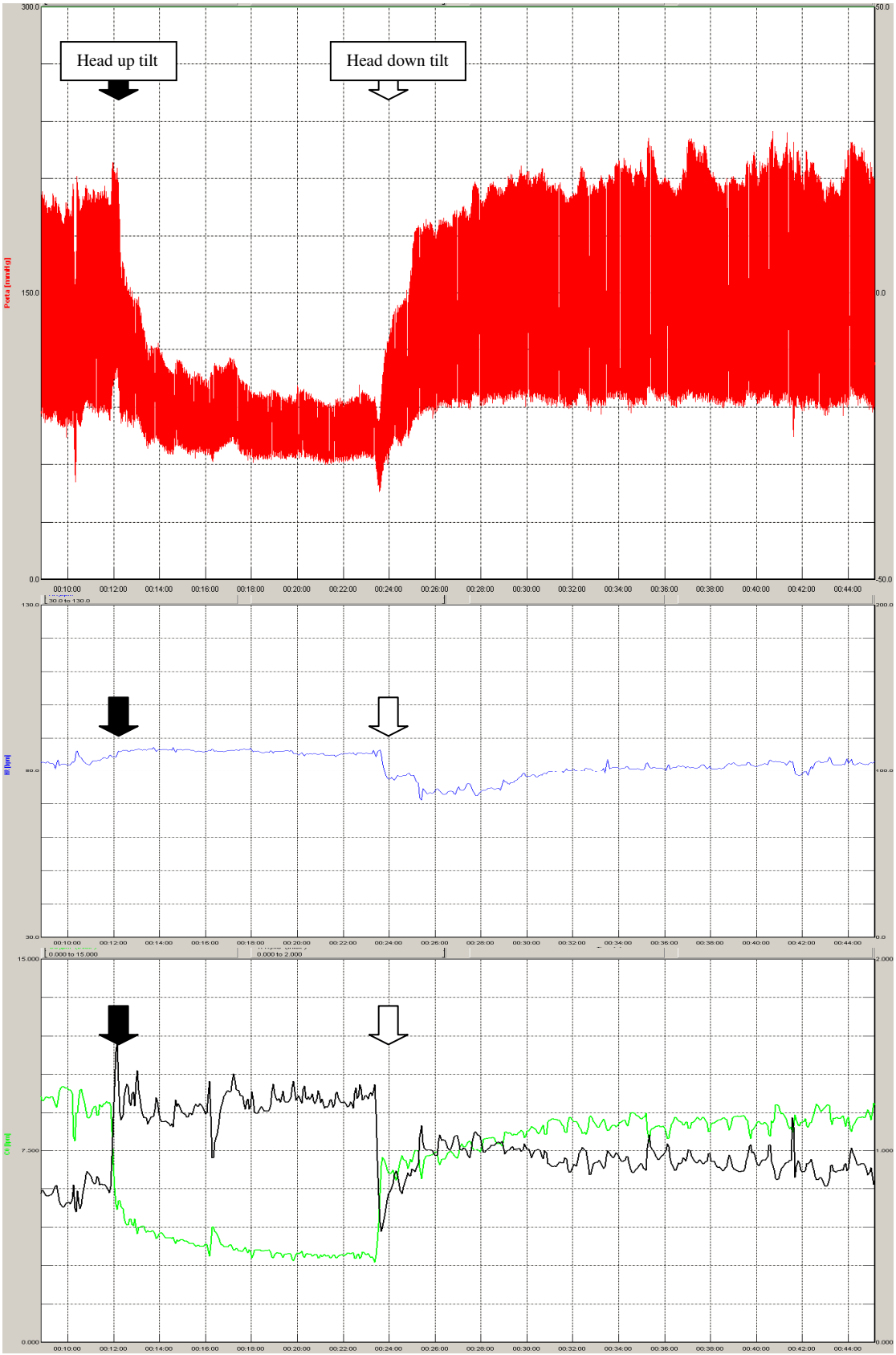

Figure 3.5.2: Continuous Haemodynamics following Head-up Tilt for 10 minutes (black arrow= start) and Tilt Reversal (white arrow= start) in an MSA subject. Upper Trace: BP (red); Middle Trace HR (blue); Lower Trace: Cardiac Output (green) and TPR (black). X-Axis Time in minutes.

Pilot Study

An initial pilot study was performed [Asahina & Young *et al* 2005] primarily using retrospective analysis of cases. Sixteen patients with chronic orthostatic hypotension (fall of BP>20mmHg) were studied, including 8 PAF (6 males and 2 females; mean age 64±13 years) and 8 MSA patients (4 males and 4 females; 66±10 years). Both MSA and PAF patients had characteristic clinical manifestations according to the criteria established by the consensus statement on the diagnosis of MSA and PAF 1998 [Gilman et al 1999]. Table 3.5.1 shows clinical profiles of the PAF and MSA patients. Mean disease duration in PAF (9±5 years) was significantly higher than that in MSA (4±2 years; $p<0.05$). One PAF and one MSA patient was on no drug treatment; 7 PAF and 6 MSA patients were on drug treatment for orthostatic hypotension (fludrocortisone 0.05–0.2 mg/day in 7 PAF and 6 MSA patients; ephedrine 15–90 mg/day in 3 PAF and 3 MSA patients; midodrine 7.5–10 mg/day in one PAF and one MSA patient). Three MSA patients took oxybutynin (5–10 mg/day), with two MSA patients on levodopa with dopadecarboxylase inhibitor (200–400 mg/day). No patients were taking antihypertensive medicine; all MSA patients and 7 out of 8 PAF patients had supine hypertension (systolic BP>140mmHg). Systolic and diastolic BP and heart rate (HR) were measured intermittently, using the automatic sphygmomanometer (Dinamap, Critikon A, UK), on the right arm. In addition, the Portapres model 2 device (TNO-TPD Biomedical Instrumentation, the Netherlands) continuously measured beat-to-beat arterial pressure through a cuff wrapped around the middle phalanx of the left middle finger, and stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) were derived using the Beatscope Software for Windows, ver. 1 Pre-tilt supine values (baseline) for SV, CO and TPR were set at 100%, and changes were expressed in percentages from baseline. As values of SV, CO and TPR, 30-seconds averages were used. Vasoactive medication was omitted the evening before the study. Subjects were studied following an overnight fast. Patients rested supine until the BP and HR stabilised, following which baseline (pre-tilt) HR and BP were

recorded. After 5 minutes of baseline measurements, patients underwent HUT to 60° on an electrical tilt table for 10 minutes, and then returned to the horizontal supine position (tilt reversal). Measurements in the tilt reversal supine position were performed until BP was stabilized. Mann-Whitney U tests were used to compare the PAF and MSA groups. The level of significance was set at p-value<0.05.Data were reported as mean ± SD.

	PAF (n = 8)	MSA (n = 8)	p value*
Age (years)	64±13	66±10	NS
Sex (M: F)	6:2	4:4	NS
Disease duration (years)	9±5	4±2	p < 0.05
Baseline			
SBP (mmHg)	172±17	170±36	NS
DBP (mmHg)	94±10	89±18	NS
HR (beats/min)	68±14	74±11	NS
Change during HUT			
ΔSBP (mmHg)	−90±24	−88±28	NS
ΔDBP (mmHg)	−39±8	−32±16	NS
ΔHR (beats/min)	−1±7	11±9	p < 0.05

* compared between PAF and MSA (Mann-Whitney U Test)
PAF pure autonomic failure; *MSA* multiple system atrophy; *SBP* systolic blood pressure; *DBP* diastolic blood pressure; *HR* heart rate; *HUT* head-up tilt; *NS* not significant

Table 3.5.1: Characteristics of the MSA and PAF subjects studied for pilot study. From: Asahina M, Young TM, Bleasdale-Barr K, Mathias CJ Differences in overshoot of blood pressure after head-up tilt in two groups with chronic autonomic failure: pure autonomic failure and multiple system atrophy *J Neurol* 2005;252:72–77

There were no significant differences in mean baselines of systolic and diastolic BP between the two groups. Baseline HR in MSA was higher than that in PAF, although there was no statistically significant difference. During HUT, both PAF and MSA had similar mean falls of systolic and diastolic BP. The mean HR increase in MSA was significantly greater than PAF. After tilt reversal, 5 out of 8 PAF patients showed a systolic overshoot of 15mmHg between 5 and 11 min after tilt reversal compared with pre-tilt readings, while one of the 8 MSA patients had a BP overshoot. In patients with BP overshoot, BP reached a peak around 5–11 minutes after tilt reversal, followed by a plateau, and gradually returned to baseline readings between

10 minutes and 35 minutes after tilt reversal. In the PAF group, means of systolic and diastolic BP passed over pre-tilt supine readings after tilt reversal, while those in the MSA group returned to baseline readings without overshoot (Fig. 3.5.3). The mean increments in systolic BP at the peak of systolic BP overshoot after tilt reversal in PAF (17±9mmHg) were significantly greater than those in MSA (5±10mmHg) ($p<0.05$), although there was no significant difference in the mean increments in diastolic BP between the PAF (8±7mmHg) and MSA (6±8mmHg) groups. HR during the post-tilt period did not differ from pre-tilt readings between the PAF and MSA groups, and there was no significant difference in a mean change of HR from the baseline level to the post-tilt readings between the PAF (1±3 beats/min) and MSA (–1±6 beats/min) groups. SV and CO decreased during HUT in both PAF and MSA. The fall of SV in MSA was greater than in PAF. However, there was no difference in the fall of CO between the 2 groups, because HR increased in MSA during HUT, unlike PAF. TPR decreased in PAF during HUT, while it did not change in MSA. After tilt-reversal, SV and CO returned to the baseline levels without overshoot in both PAF and MSA. There were no significant differences in changes in SV and CO from the baseline level to the post-tilt supine level between the PAF and MSA groups. Although TPR increase over baseline was greater in PAF (19±16%) than MSA (6±14%), this did not reach significance.

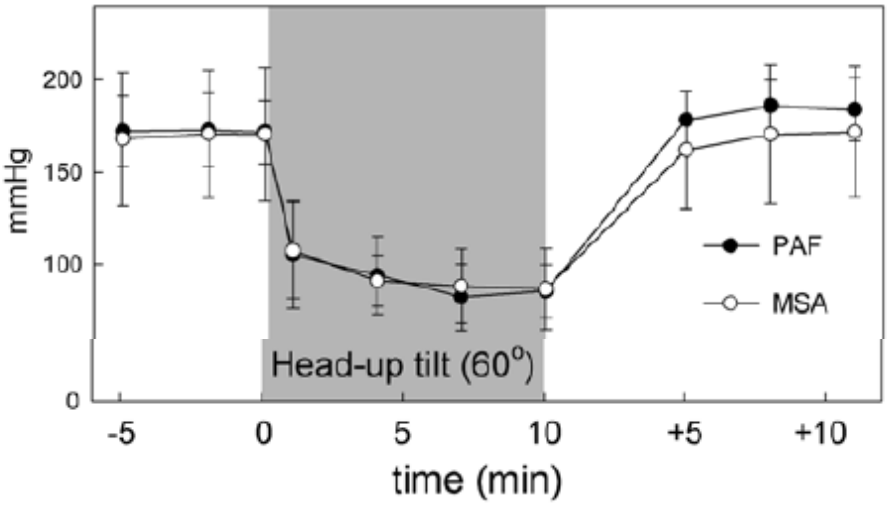

Figure 3.5:3 SBP in 7 MSA and 7 PAF subjects before, during and after HUT. Values = mmHg +/- SD. From Asahina M, Young TM, Bleasdale-Barr K, Mathias CJ Differences in overshoot of blood pressure after head-up tilt in two groups with chronic autonomic failure: pure autonomic failure and multiple system atrophy *J Neurol* 2005;252:72–77 [Ref 12]

Follow Up Study: BP Overshoot and plasma AVP:

Subjects and Methods

Fourteen patients with chronic orthostatic hypotension (fall of BP>20mmHg), including 7 PAF (3 males and 4 females; mean age 67.4±2.5 years) and 7 MSA patients (4 males and 3 females; 54.3 ±3 years) were studied. The PAF patients had no neurological symptoms or signs except for autonomic dysfunction. The MSA patients had characteristic clinical manifestations according to the criteria established by the consensus statement on the diagnosis of MSA in 1999 [Gilman et al 1999]. One PAF and two MSA patients were on no drug treatment; 6 PAF and 5 MSA patients were on drug treatment for orthostatic hypotension (fludrocortisone 0.05–0.2 mg/day in 6 PAF and 5 MSA patients; ephedrine 15–90 mg/day in 3 PAF and 2 MSA patients; midodrine 7.5–10 mg/day in two PAF and one MSA patient). Two MSA patients took oxybutynin (5–10 mg/day), with no MSA patients on anti-parkinsonian medication. No patients were treated with antihypertensive medicine; 6 out of 7 MSA patients

and 6 out of 7 PAF patients had supine hypertension (systolic BP>140mmHg). Systolic and diastolic BP and heart rate (HR) were measured intermittently, using the automatic sphygmomanometer (Dinamap, Critikon, UK), in the right arm. In addition, the Portapres model II device (TNO-TPD Biomedical Instrumentation, the Netherlands) continuously measured beat-to-beat arterial pressure through a cuff wrapped around the middle phalanx of the left middle finger, and stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) were derived using the Beatscope Software for Windows, Version. 1 (TNO-TPD Biomedical Instrumentation). Pre-tilt supine values (baseline) for SV, CO and TPR were set at 100%, and changes were expressed in percentages from baseline. For values of SV, CO and TPR, 60-seconds averages were used. Vasoactive medication was omitted the evening before the study. Subjects were studied after an overnight fast. The studies were performed in dedicated autonomic laboratories between 9:00 and 14:00 hours. Patients rested in the supine position until the BP and HR stabilised, following which baseline (pre-tilt) HR and BP were recorded. After 5 minutes of baseline measurements, patients were tilted head up at a level of 60° on an electrical tilt table for 10 minutes, and then returned to the horizontal supine position (tilt reversal). Measurements in the tilt reversal supine position were performed until BP was stabilized.

A 20G (Venflon) intravenous line was inserted into the subject's right antecubital fossa before baseline and kept patent with 0.9% saline. The subject then lay quietly supine for 20 minutes to obtain a stable baseline. Baseline plasma AVP was taken after 20 minutes supine and the patient then underwent a 60^0 head-up tilt for 10 minutes. AVP levels were taken after 10 minutes of HUT, and the patient underwent tilt reversal back to supine position. Further AVP samples were taken after 10 and 20 minutes after tilt reversal as illustrated below:

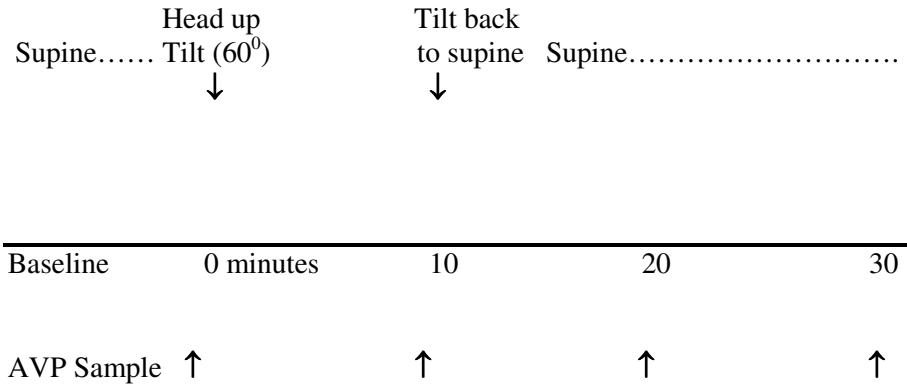

Figure 3.5:4 Graphical layout of study design

Results

Dinamap data:

i) Values for SBP, DBP and HR before and during HUT:

Baseline Supine values for MSA and PAF were similar (Tables 3.5.2-3.5.7). Baseline SBP in MSA was 172.1 +/- 10.4 vs. 167.1 +/- 10.7mmHg in PAF with no significant difference. There was also no significant difference in the DBP between groups, although baseline HR was higher in MSA (78.1 +/- 3.4bpm) vs. than PAF (65.7 +/- 4.2 bpm). After 10 minutes of HUT there were similar drops in SBP (MSA –82.1 +/- 13.7 mmHg vs. PAF -87.0 +/- 10 mmHg) and DBP (MSA –38.7 +/- 7.9 mmHg vs. PAF –35.1 +/- 3.1 mmHg). There was a greater increase in HR in MSA (11.7 +/- 2.1 bpm) compared with PAF (5.6 +/- 3.4 bpm).

	Supine	HUT	HUT	HUT
	SBPBase	SBP3min	SBP5min	SBP10min
MSA 1	145	94	98	91
MSA 2	174	120	121	112
MSA 3	193	113	103	82
MSA 4	212	93	90	76
MSA 5	134	106	107	105
MSA 6	186	126	107	95
MSA 7	161	111	101	67
Mean	**172.1**	**109.0**	**103.9**	**89.7**
Standard Error	**10.4**	**4.7**	**3.6**	**6.0**
Standard Deviation	**27.5**	**12.4**	**9.6**	**15.9**

Table 3.5.2: Dinamap SBP in mmHg before and during HUT in 7 MSA subjects. (SBPBase = supine baseline value pre-tilt; SBP3min = SBP after 3 minutes of HUT; SBP5min = SBP after 5 minutes of HUT; SBP10min = SBP after 10 minutes of HUT)

	Supine	HUT	HUT	HUT
	SBPBase	SBP3min	SBP5min	SBP10min
PAF 1	175	79	65	66
PAF 2	176	122	136	122
PAF 3	205	106	100	84
PAF 4	131	72	79	73
PAF 5	155	72	67	58
PAF 6	134	66	70	65
PAF 7	194	120	111	93
Mean	**167.1**	**91.0**	**89.7**	**80.1**
Standard Error	**10.7**	**9.2**	**10.2**	**8.3**
Standard Deviation	**28.4**	**24.2**	**26.9**	**22.0**

Table 3.5.3: Dinamap SBP in mmHg before and during HUT in 7 PAF subjects. (SBPBase = supine baseline value pre-tilt; SBP3min = SBP after 3 minutes of HUT; SBP5min = SBP after 5 minutes of HUT; SBP10min = SBP after 10 minutes of HUT)

	Supine	HUT	HUT	HUT
	DBPBase	DBP3min	DBP5min	DBP10min
MSA 1	80	64	63	57
MSA 2	105	81	83	77
MSA 3	103	68	60	50
MSA 4	117	52	51	46
MSA 5	88	78	75	79
MSA 6	103	78	74	67
MSA 7	95	66	68	44
Mean	**98.7**	**69.6**	**67.7**	**60.0**
Standard Error	**4.6**	**3.9**	**4.0**	**5.5**
Standard Deviation	**12.2**	**10.2**	**10.7**	**14.5**

Table 3.5.4: Dinamap DBP in mmHg before and during HUT in 7 MSA subjects. (DBPBase = supine baseline value pre-tilt; DBP3min = DBP after 3 minutes of HUT; DBP5min = DBP after 5 minutes of HUT; DBP10min = DBP after 10 minutes of HUT)

	Supine	HUT	HUT	HUT
	DBPBase	DBP3min	DBP5min	DBP10min
PAF 1	92	55	48	44
PAF 2	88	66	72	70
PAF 3	90	63	63	57
PAF 4	77	47	49	44
PAF 5	86	52	48	44
PAF 6	84	43	47	43
PAF 7	104	90	83	73
Mean	**88.7**	**59.4**	**58.6**	**53.6**
Standard Error	**3.1**	**6.0**	**5.4**	**5.0**
Standard Deviation	**8.3**	**15.8**	**14.4**	**13.2**

Table 3.5.5: Dinamap DBP in mmHg before and during HUT in 7 PAF subjects. (DBPBase = supine baseline value pre-tilt; DBP3min = DBP after 3 minutes of HUT; DBP5min = DBP after 5 minutes of HUT; DBP10min = DBP after 10 minutes of HUT)

	Supine	HUT	HUT	HUT
	HRBase	HR3min	HR5min	HR10min
MSA 1	74	77	80	83
MSA 2	76	86	87	85
MSA 3	93	96	98	102
MSA 4	80	86	85	85
MSA 5	68	86	86	90
MSA 6	71	80	88	87
MSA 7	85	94	94	97
Mean	**78.1**	**86.4**	**88.3**	**89.9**
Standard Error	**3.3**	**2.6**	**2.3**	**2.7**
Standard Deviation	**8.6**	**6.8**	**6.0**	**7.1**

Table 3.5.6: Dinamap Heart rate in bpm before and during HUT in 7 MSA subjects. (HRBase = supine baseline value pre-tilt; HR3min = HR after 3 minutes of HUT; HR5min = HR after 5 minutes of HUT; HR10min = HR after 10 minutes of HUT)

	Supine	HUT	HUT	HUT
	HRBase	HR3min	HR5min	HR10min
PAF 1	68	70	70	70
PAF 2	58	61	63	58
PAF 3	86	82	79	82
PAF 4	72	84	81	77
PAF 5	65	79	83	84
PAF 6	53	53	53	53
PAF 7	58	74	76	75
Mean	**65.7**	**71.9**	**72.1**	**71.3**
Standard Error	**4.2**	**4.3**	**4.1**	**4.5**
Standard Deviation	**11.1**	**11.4**	**10.9**	**11.8**

Table 3.5.7: Dinamap Heart rate in bpm before and during HUT in 7 PAF subjects. (HRBase = supine baseline value pre-tilt; HR3min = HR after 3 minutes of HUT; HR5min = HR after 5 minutes of HUT; HR10min = HR after 10 minutes of HUT

Portapres beat-to-beat values for SBP, DBP and HR before and during HUT were very similar to those obtained with the Dinamap as outlined above. Mean Portapres values for the 7 MSA subjects and 7 PAF subjects were as follows:

	SBPBase	SBP10min	DBPBase	DBP10min	HRBase	HR10min
MSA	158.2 +/- 12.7	83.2 +/- 4.5	78.0 +/- 3.5	49.4 +/- 3.6	80.8 +/- 3.9	91.1 +/- 2.3
PAF	156.4 +/- 11.2	81.1 +/- 11.1	81.4 +/- 6.5	48.6 +/- 4.4	67.2 +/- 3.0	71.7 +/- 3.5

Table 3.5.8: Mean Portapres values for SBP, DBP, HR (+/- SE) for pre-HUT supine baseline and at the end of 10 minute HUT

i) Dinamap values for SBP, DBP and HR following tilt-reversal

Following tilt-reversal 1 out of the 7 MSA subjects showed a SBP overshoot of at least 15mmHg in the first 11 minutes compared with baseline versus 6 out of 7 PAF who showed a SBP overshoot of at least this magnitude in this time. Mean values of delta SBP showed a significantly greater increase in the 7 PAF subjects compared to MSA with maximum difference occurring at 7 minutes post tilt-reversal ($p=0.03$ Mann-Whitney U test). Wilcoxan matched pairs (mean for group compared with baseline) confirmed a significant ($p=0.02$) SBP overshoot in PAF with maximum increase again at 7 minutes post-tilt-reversal. By contrast there was no significant SBP overshoot in the MSA group. As with the study by Chandler & Mathias and the pilot study, DBP did not show overshoot in either group [Chandler & Mathias 2002]. Comparison of HR response showed no significant difference between the MSA and PAF, although MSA showed a significant reduction in HR compared with baseline

following tilt reversal-mean –6 +/- 1.1 bpm at 7 minutes post-tilt-reversal (p=0.02), whilst PAF showed little change (-0.6 +/- 1.5 bpm)

SBP BASE	SBP 1min	SBP 2min	SBP 3min	SBP 4min	SBP 5min	SBP 6min	SBP 7min	SBP 8min	SBP 9min	SBP 10min
MSA										
145	105	122	136	123	137	116	128	132	131	108
174	164	168	178	182	188	188	169	178	186	184
193	148	168	182	185	194	192	195	188	193	193
212	142	165	194	209	215	219	219	219	216	217
134	127	144	151	136	146	137	135	139	145	136
186	169	175	180	184	181	178	176	168	171	164
161	77	129	156	152	159	157	159	155	162	162
145	105	122	136	123	137	116	128	132	131	108
MEAN										
172.1	**133.1**	**153.0**	**168.1**	**167.3**	**174.3**	**169.6**	**168.7**	**168.4**	**172.0**	**166.3**
+/-SE	12.5	8.0	7.8	11.7	10.6	13.3	12.1	11.3	11.0	13.7
PAF										
175	136	162	184	185	189	191	188	181	182	181
176	171	191	202	203	198	195	201	201	199	201
205	168	194	216	221	213	221	222	219	217	218
131	145	193	187	174	164	157	148	145	136	142
155	143	163	169	172	163	162	157	153	155	152
134	110	116	128	142	147	149	149	149	146	147
194	186	202	196	195	196	208	203	207	197	192
175	136	162	184	185	189	191	188	181	182	181
MEAN										
167.1	**151.3**	**174.4**	**183.1**	**184.6**	**181.4**	**183.3**	**181.1**	**179.3**	**176.0**	**176.1**
+/- SE	9.7	11.4	10.8	9.6	9.0	10.4	11.2	11.6	11.6	11.2

Table 3.5.9: Dinamap SBP from baseline supine for each minute post-tilt reversal in 7 MSA and 7 PAF. (mmHg +/- SE); N/S difference for MSA compared with baseline; significant increase in SBP vs. baseline in PAF, maximum at 7 minutes post tilt-reversal (p=0.02 Wilcoxan Matched pairs)

SBP BASE	ΔSBP 1min	ΔSBP 2min	ΔSBP 3min	ΔSBP 4min	ΔSBP 5min	ΔSBP 6min	ΔSBP 7min	ΔSBP 8min	ΔSBP 9min	ΔSBP 10min
MSA										
145	-30	-23	-9	-22	-8	-29	-17	-13	-14	-37
174	-10	-6	4	8	14	14	-5	4	14	10
193	-45	-25	-11	-8	1	-1	-2	-5	0	0
212	-70	-47	-18	-3	3	7	7	7	4	5
134	8	10	17	2	12	3	1	5	11	2
186	-17	-11	-6	-2	-5	-8	-10	-18	-15	-18
161	-84	-32	-5	-9	-2	-4	-2	-6	1	1
MEAN										
172.1	**-35.4**	**-19.1**	**-4.0**	**-4.9**	**2.1**	**-2.6**	**-4.0**	**-3.7**	**0.1**	**-5.3**
+/- SE	12.5	7.0	4.3	3.6	3.1	5.2	2.9	3.6	4.2	6.2
PAF										
175	-39	-13	9	10	14	16	13	6	7	6
176	-5	15	26	27	22	19	25	25	23	25
205	-37	-11	11	16	8	16	17	14	12	13
131	14	62	56	43	33	26	17	14	5	11
155	-10	8	14	17	8	7	2	-2	0	-3
134	-24	-18	-6	8	13	17	17	15	12	13
194	-8	8	2	1	2	14	9	13	3	-2
MEAN										
167.1	**-15.6**	**7.3**	**16.0***	**17.4****	**14.3***	**16.4****	**14.3*****	**12.1****	**8.9**	**9.0**
+/- SE	7.2	10.3	7.7	5.3	3.9	2.1	2.7	3.2	2.9	3.7

Table 3.5.10: Change (Δ) in SBP from baseline supine for each minute post-tilt reversal in 7 MSA and 7 PAF. PAF showed significantly greater increase in SBP over this 10 minute time period (Mann-Whitney max at 7 minutes post tilt-reversal p= 0.03) (PAF versus MSA)

DBP BASE	DBP 1min	DBP 2min	DBP 3min	DBP 4min	DBP 5min	DBP 6min	DBP 7min	DBP 8min	DBP 9min	DBP 10min
MSA										
80	71	77	78	74	85	66	79	75	81	68
105	97	102	111	104	107	110	106	112	109	107
103	98	104	112	113	115	115	110	105	109	113
117	87	105	122	121	126	124	124	120	119	119
88	87	91	89	90	89	85	87	89	87	89
103	101	100	101	101	99	103	97	96	94	93
95	48	77	91	86	87	82	81	82	83	89
80	71	77	78	74	85	66	79	75	81	68
MEAN										
98.7	**84.1**	**93.7**	**100.6**	**98.4**	**101.1**	**97.9**	**97.7**	**97.0**	**97.4**	**96.9**
+/- SE	7.1	4.6	5.8	6.1	5.9	7.8	6.3	6.2	5.6	6.6
PAF										
92	90	98	105	102	100	97	97	93	92	93
88	89	85	103	94	89	92	99	90	100	95
90	84	94	100	100	100	102	101	100	99	97
77	88	104	103	99	88	84	80	80	76	79
86	91	94	97	96	92	91	89	87	87	80
84	81	77	84	80	81	85	78	86	83	84
104	101	98	106	105	99	101	99	104	103	104
92	90	98	105	102	100	97	97	93	92	93
MEAN										
88.7	**89.1**	**92.9**	**99.7**	**96.6**	**92.7**	**93.1**	**91.9**	**91.4**	**91.4**	**90.3**
+/- SE	3.1	2.4	3.4	2.9	3.1	2.8	2.7	3.6	3.1	3.8

Table 3.5.11: Dinamap DBP compared with baseline supine for each minute post-tilt reversal in 7 MSA and 7 PAF. (mmHg +/- SE). No significant difference with either MSA or PAF.

DBP BASE	ΔDBP 1min	ΔDBP 2min	ΔDBP 3min	ΔDBP 4min	ΔDBP 5min	ΔDBP 6min	ΔDBP 7min	ΔDBP 8min	ΔDBP 9min	ΔDBP 10min
MSA										
80	-9	-3	-2	-6	5	-14	-1	-5	1	-12
105	-8	-3	6	-1	2	5	1	7	4	2
103	-5	1	9	10	12	12	7	2	6	10
117	-30	-12	5	4	9	7	7	3	2	2
88	-1	3	1	2	1	-3	-1	1	-1	1
103	-2	-3	-2	-2	-4	0	-6	-6	-9	-10
95	-47	-18	-4	-9	-8	-13	-14	-13	-12	-6
MEAN										
98.7	**-14.6**	**-5**	**1.9**	**-0.3**	**2.4**	**-0.9**	**-1**	**-1.6**	**-1.3**	**-1.9**
+/- SE	2.8	1.8	2.4	2.6	3.7	2.8	2.6	2.5	2.9	2.8
PAF										
-2	6	13	10	8	5	5	1	0	1	-2
1	-3	15	6	1	4	11	2	12	7	1
-6	4	10	10	10	12	11	10	9	7	-6
11	27	26	22	11	7	3	3	-1	2	11
5	8	11	10	6	5	3	1	1	-6	5
-3	-7	0	-4	-3	1	-6	2	0	0	-3
-3	-6	2	1	-5	-3	-5	0	0	0	-3
MEAN										
88.7	**0.43**	**4.1**	**11**	**7.9**	**4**	**4.4**	**3.1**	**2.7**	**3**	**1.6**
+/- SE	2.2	4.4	3.3	3.1	2.4	1.8	2.6	1.3	2.0	1.7

Table 3.5.12: Change (Δ) in DBP from baseline supine for each minute post-tilt reversal in 7 MSA and 7 PAF. No significant difference between MSA and PAF

HR BASE	HR 1min	HR 2min	HR 3min	HR 4min	HR 5min	HR 6min	HR 7min	HR 8min	HR 9min	HR 10min
MSA										
74	61	55	58	63	68	71	64	74	74	71
76	71	72	70	68	71	71	74	76	73	74
93	86	82	81	80	81	85	84	86	85	87
80	77	77	72	74	74	76	77	78	78	79
68	70	60	62	61	60	60	63	64	65	65
71	61	62	63	64	68	67	65	68	68	67
85	94	80	70	74	80	77	78	81	85	86
MEAN										
78.1	**74.3**	**69.7**	**68.0**	**69.1**	**71.7**	**72.4**	**72.1**	**75.3**	**75.4**	**75.6**
+/- SE	4.7	4.0	2.9	2.7	2.8	3.0	3.1	2.8	2.9	3.3
PAF										
68	71	70	70	69	69	69	69	69	68	69
58	57	53	56	56	54	57	58	61	61	58
86	85	84	84	82	84	85	85	86	87	86
72	87	75	77	78	82	78	78	74	72	72
65	61	60	64	64	63	61	60	63	64	64
53	53	53	53	53	54	54	54	54	54	54
58	53	51	50	51	47	53	52	52	52	54
68	71	70	70	69	69	69	69	69	68	69
MEAN										
65.7	**66.7**	**63.7**	**64.9**	**64.7**	**64.7**	**65.3**	**65.1**	**65.6**	**65.4**	**65.3**
+/- SE	5.5	4.8	4.8	4.6	5.4	4.7	4.8	4.5	4.5	4.4

Table 3.5.13: HR from baseline supine for each minute post-tilt reversal in 7 MSA and 7 PAF. MSA showed significant increase in HR vs. baseline (Wilcoxan matched pairs p=0.02) but no significant difference from PAF where HR increase was N/S

HR BASE	ΔHR 1min	ΔHR 2min	ΔHR 3min	ΔHR 4min	ΔHR 5min	ΔHR 6min	ΔHR 7min	ΔHR 8min	ΔHR 9min	ΔHR 10min
MSA										
74	-13	-19	-16	-11	-6	-3	-10	0	0	-3
76	-5	-4	-6	-8	-5	-5	-2	0	-3	-2
93	-7	-11	-12	-13	-12	-8	-9	-7	-8	-6
80	-3	-3	-8	-6	-6	-4	-3	-2	-2	-1
68	2	-8	-6	-7	-8	-8	-5	-4	-3	-3
71	-10	-9	-8	-7	-3	-4	-6	-3	-3	-4
85	9	-5	-15	-11	-5	-8	-7	-4	0	1
MEAN										
78.1	**-3.9**	**-8.4**	**-10.1**	**-9**	**-6.4**	**-5.7**	**-6**	**-2.9**	**-2.7**	**-2.6**
+/- SE	2.8	2.1	1.6	1.0	1.1	0.8	1.1	0.9	1.0	0.8
PAF										
68	3	2	2	1	1	1	1	1	0	1
58	-1	-4	-2	-2	-4	-1	0	3	3	0
86	-1	-2	-2	-4	-2	-1	-1	0	1	0
72	15	3	5	6	-10	6	6	2	0	0
65	-4	-5	-1	-1	-2	-4	-5	-2	-1	-1
53	0	0	0	0	1	1	1	1	1	1
58	-5	-7	-8	-7	-11	-5	-6	-6	-6	-4
MEAN										
65.7	**1.0**	**-1.9**	**-0.9**	**-1.0**	**-3.9**	**-0.4**	**-0.6**	**-0.1**	**-0.3**	**-0.4**
+/- SE	2.5	1.4	1.5	1.5	1.8	1.4	1.5	1.1	1.1	0.6

Table 3.5.14: Change (Δ) in HR from baseline supine for each minute post-tilt reversal in 7 MSA and 7 PAF. N/S difference between MSA and PAF

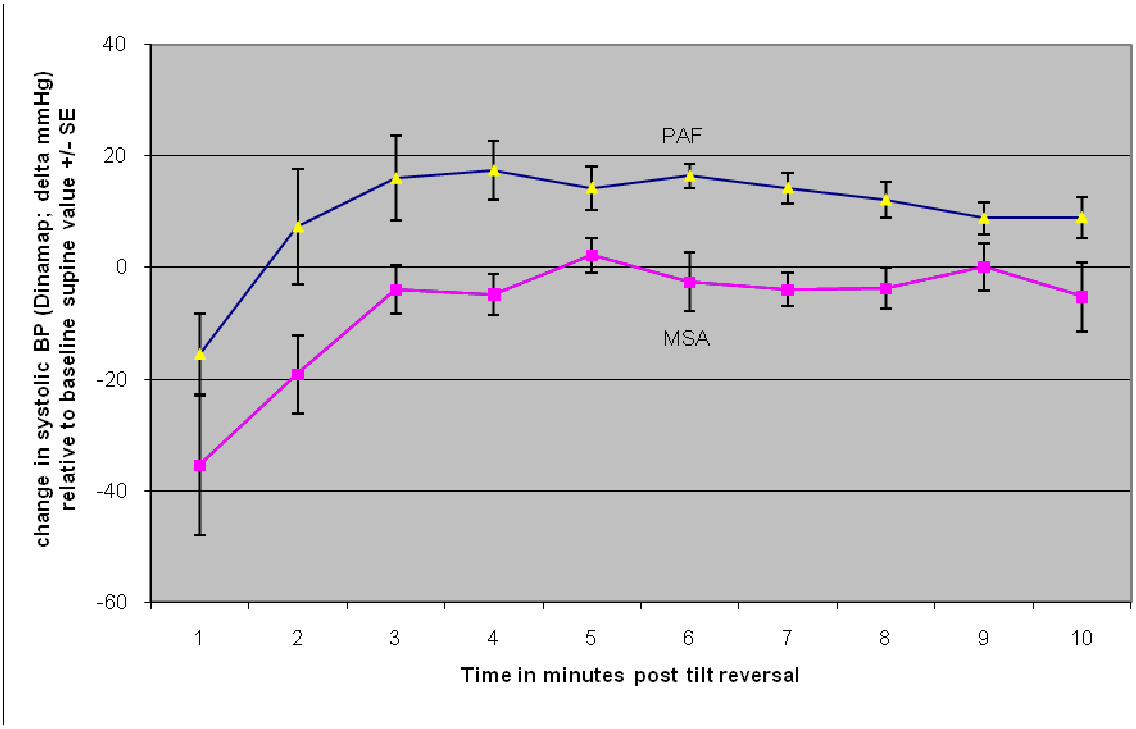

Figure 3.5.5: Change in systolic BP (Dinamap; delta mmHg) relative to baseline supine value +/- SE in 7 PAF and 7 MSA immediately following tilt-reversal from HUT. Significantly greater increase in SBP relative to baseline in PAF vs. MSA-maximal at 7 minutes post tilt-reversal

2) Portapres Beat-to-beat data following tilt-reversal

Portapres data for this study were primarily used to allow calculation of additional haemodynamic variables, including TPR, SV and CO, using the Beatscope software for windows. The Beatscope results also demonstrated a SBP overshoot in PAF but not MSA (Table 3.5.15). Between 7 & 8 minutes corresponding to the greatest BP overshoot in PAF, TPR increase compared with baseline was 7% in MSA and 16% in PAF.

SBP BASE	ΔSBP 1min	ΔSBP 2min	ΔSBP 3min	ΔSBP 4min	ΔSBP 5min	ΔSBP 6min	ΔSBP 7min	ΔSBP 8min	ΔSBP 9min	ΔSBP 10min
MSA										
157.9	**-31.6**	**-9.4**	**-4.9**	**-1.2**	**3.4**	**6**	**6.7**	**5.8**	**5.4**	**6.7**
+/- SE	14.4	9.3	6.9	4.6	3.8	4.5	5	5.7	5.6	3.7
PAF										
156.4	**-35.3**	**8.5**	**17**	**19.2**	**19.5**	**18.6**	**14.7**	**18.2**	**17**	**17.1**
+/- SE	11.6	7.2	6.5	5.5	5.8	5.1	5.1	4.9	6.4	11.6

Table 3.5.15: Mean Portapres ΔSBP mmHg (+/- SE) for 7 MSA and 7 PAF subjects for each minute post-tilt-reversal. Significantly greater increase in SBP vs. baseline in PAF vs. MSA (Mann-Whitney U test p=0.04)

DBP BASE	ΔDBP 1min	ΔDBP 2min	ΔDBP 3min	ΔDBP 4min	ΔDBP 5min	ΔDBP 6min	ΔDBP 7min	ΔDBP 8min	ΔDBP 9min	ΔDBP 10min
MSA										
78.0	**-8.0**	**1.5**	**2.4**	**3.4**	**3.6**	**3.3**	**3.0**	**2.8**	**2.4**	**1.8**
+/- SE	5.0	2.9	2.4	1.3	1.5	1.9	2.2	1.9	2.4	1.9
PAF										
81.5	**-1.6**	**2.4**	**2.2**	**0.7**	**0.1**	**-2.4**	**-0.9**	**-4.0**	**3.7**	**-1.6**
+/- SE	8.2	8.2	8.2	8.3	8.0	7.5	9.1	8.5	2.8	8.2

Table 3.5.16: Mean Portapres ΔDBP mmHg (+/- SE) for 7 MSA and 7 PAF subjects for each minute post-tilt-reversal. N/S difference MSA vs. PAF

TPR BASE	Δ TPR 1min	Δ TPR 2min	Δ TPR 3min	Δ TPR 4min	Δ TPR 5min	Δ TPR 6min	Δ TPR 7min	Δ TPR 8min	Δ TPR 9min	Δ TPR 10min
MSA										
0.9	0.7	0.2	0.2	0.1	0	-0.1	-0.1	-0.1	-0.1	-0.2
0.7	0.1	0.2	0.2	0.3	0.1	0.1	0.1	0.1	0	0
0.9	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0	0	0
0.8	0	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1
0.8	0	0.1	0	0	0	0	0	0	0	0
0.7	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1
1.0	-0.2	-0.1	-0.1	0.2	0	0	0.1	0.1	0	0.1
MEAN										
0.8	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.0
+/- SE	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
PAF										
1.8	-0.4	0.2	0.5	0.6	0.4	0.4	0.3	0.3	0.2	0.2
4.3	-3.4	-3.3	-3.3	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4
1.6	-0.4	0.1	0.4	0.5	0.4	0.6	0.3	1.5	0.2	0.1
0.8	0.1	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2
0.8	-0.1	0.1	0.3	0.2	0.2	0.1	0.1			
1.8	-0.2	0	0.1	0.1	0.1	0	-0.1	-0.1	-0.2	-0.2
1.6	-0.2	0.5	0.4	0.3	0.2	0.1	0.1	0.1	0	0
MEAN										
1.8	-0.7	-0.3	-0.2	-0.2	-0.3	-0.3	-0.4	-0.2	-0.5	-0.5
+/- SE	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.7	0.6	0.6

Table 3.5.17: Change (Δ) in TPR from baseline supine for each minute post-tilt reversal in 7 MSA and 7 PAF. There was no significant overall difference, although at 7-8 minutes post tilt-reversal (time of maximal SBP overshoot) PAF demonstrated a 16% increase over baseline vs. 7 % for MSA

Plasma AVP levels were variable with the greatest increase in both MSA and PAF. Overall levels did not show a clear trend of increase in either group (Friedman ANOVA showing $p = 0.28$ in MSA and $p = 0.31$ for PAF. However, the greatest increase in AVP occurred at the end of 10 minutes HUT as would be expected by the underlying mechanism of AVP release in response to postural challenge. A more important statistic therefore appears to be the comparison of AVP levels at the end of the 10-minute HUT with baseline in both groups.

This showed a non-significant increase in AVP levels in both groups with Wilcoxan match pairs (AVP level end of HUT vs. baseline) producing a p = 0.13 for MSA and 0.07 PAF.

Patient number	**AVP 1 (baseline)**	**AVP 2 (after 10min of HUT)**	**AVP 3 (10 minutes after tilt-reversal**	**AVP 4 (20 minutes after tilt-reversal)**
MSA 1	1.03	5.42	1.00	1.91
MSA 2	5.43	5.16	4.73	5.98
MSA 3	6.60	12.42	4.28	2.28
MSA 4	3.00	1.15	5.63	2.06
MSA 5	2.04	2.67	2.28	1.88
MSA 6	6.33	12.38	8.83	5.08
MSA 7	3.03	4.79	3.25	3.21
Mean MSA +/- SE	**3.9 +/- 0.8**	**6.3 +/- 1.7**	**4.3 +/-1.0**	**3.2 +/- 0.6**
PAF 1	GROSSLY HAEMOLYSED	GROSSLY HAEMOLYSED	GROSSLY HAEMOLYSED	GROSSLY HAEMOLYSED
PAF 2	7.63	5.17	3.75	8.75
PAF 3	6.21	8.69	5.88	5.46
PAF 4	3.95	20.53	23.98	12.60
PAF 5	3.77	34.32	7.21	6.19
PAF 6	2.44	11.57	5.05	15.50
PAF 7	4.83	6.20	4.17	2.25
Mean PAF	**4.8 +/- 0.8**	**14.4 +/- 4.6**	**8.3 +/- 3.2**	**8.5 +/- 2.0**

Table 3.5.18: Plasma AVP (pg/ml) in 7 MSA and 7 PAF subjects before HUT (AVP 1), at the end of 10 minutes HUT (AVP 2) and at 10 and 20 minutes post-tilt-reversal (AVP 3 & 4 respectively). Wilcoxan matched pair AVP after 10 minute HUT vs. Base line: p= 0.13 MSA and p= 0.07 (PAF)

	Baseline	10 min head up tilt	10min tilt-reversal	20 min tilt-reversal
MSA				
1	100.00	526.20	99.90	185.40
2	100.00	95.00	87.10	110.10
3	100.00	188.20	64.80	34.50
4	100.00	38.30	187.70	68.70
5	100.00	130.90	111.80	92.30
6	100.00	195.60	139.40	80.30
7	100.00	158.10	107.30	105.90
Mean +/- SE	**100.00**	**190.3 +/ 59.7**	**114.0 +/- 15.0**	**96.7 +/- 17.6**
PAF				
1	GROSSLY HAEMOLYSED	GROSSLY HAEMOLYSED	GROSSLY HAEMOLYSED	GROSSLY HAEMOLYSED
2	100.00	67.80	49.10	114.70
3	100.00	139.90	94.70	87.90
4	100.00	519.70	607.10	319.00
5	100.00	910.30	191.20	164.20
6	100.00	474.20	207.00	635.20
7	100.00	128.40	86.30	46.60
Mean +/- SE	**100.00**	**328.2 +/- 120.9**	**183.2 +/- 74.7**	**198.7 +/- 81.6**

Table 3.5.19: Plasma AVP levels expressed as percentage of baseline in 7 MSA and 7 PAF at baseline, after 10 minutes head up tilt and then supine 10 and 20 minutes after tilt-reversal

Wilcoxan comparing AVP 1 and AVP 2 showed a non-significant increase (p=0.13) for MSA and PAF (p=0.07). This is likely to reflect the haemolysed PAF sample from PAF patient 1, thus reducing the n number to 6 for the study. Spearman correlation between the AVP values following 10 minutes of HUT and peak increase in SBP in PAF failed to show correlation (p=0.7). Thus this provisional assessment of AVP and BP overshoot could not demonstrate a clear relationship between AVP and SBP overshoot despite a trend towards correlation.

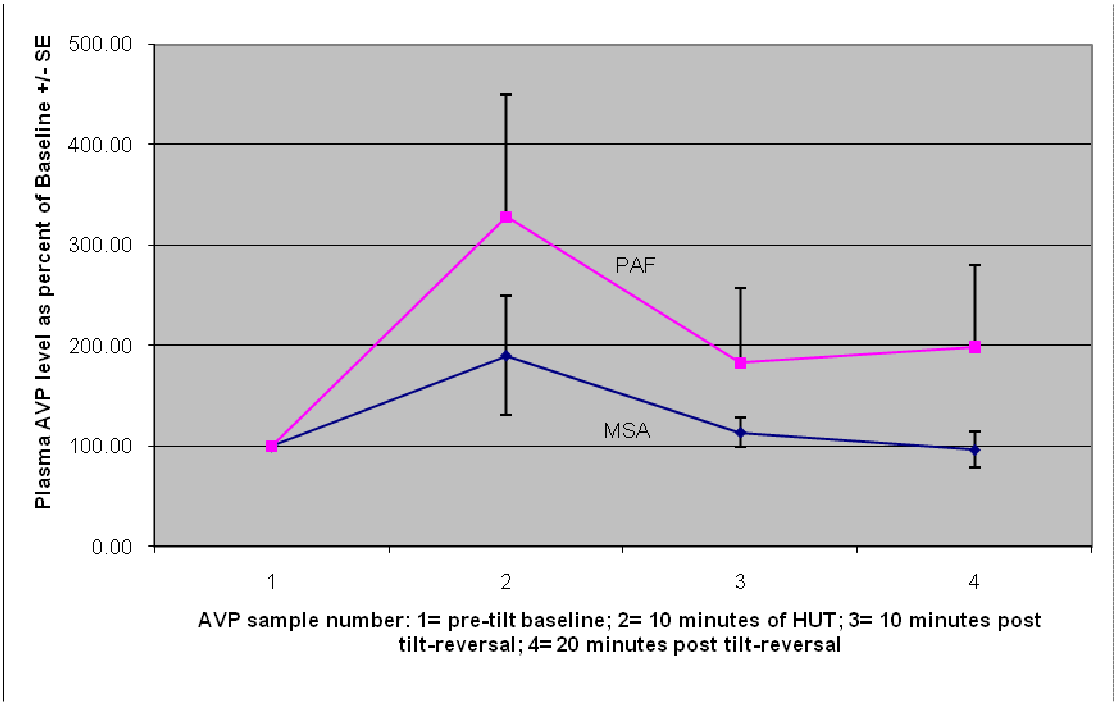

Figure 3.5.6: Plasma AVP as a percentage of baseline (Value 1) at end of 10 minute HUT (Value 2), after 10 minutes of tilt-reversal (Value 3) and after 20 minutes of tilt-reversal (Value 4).

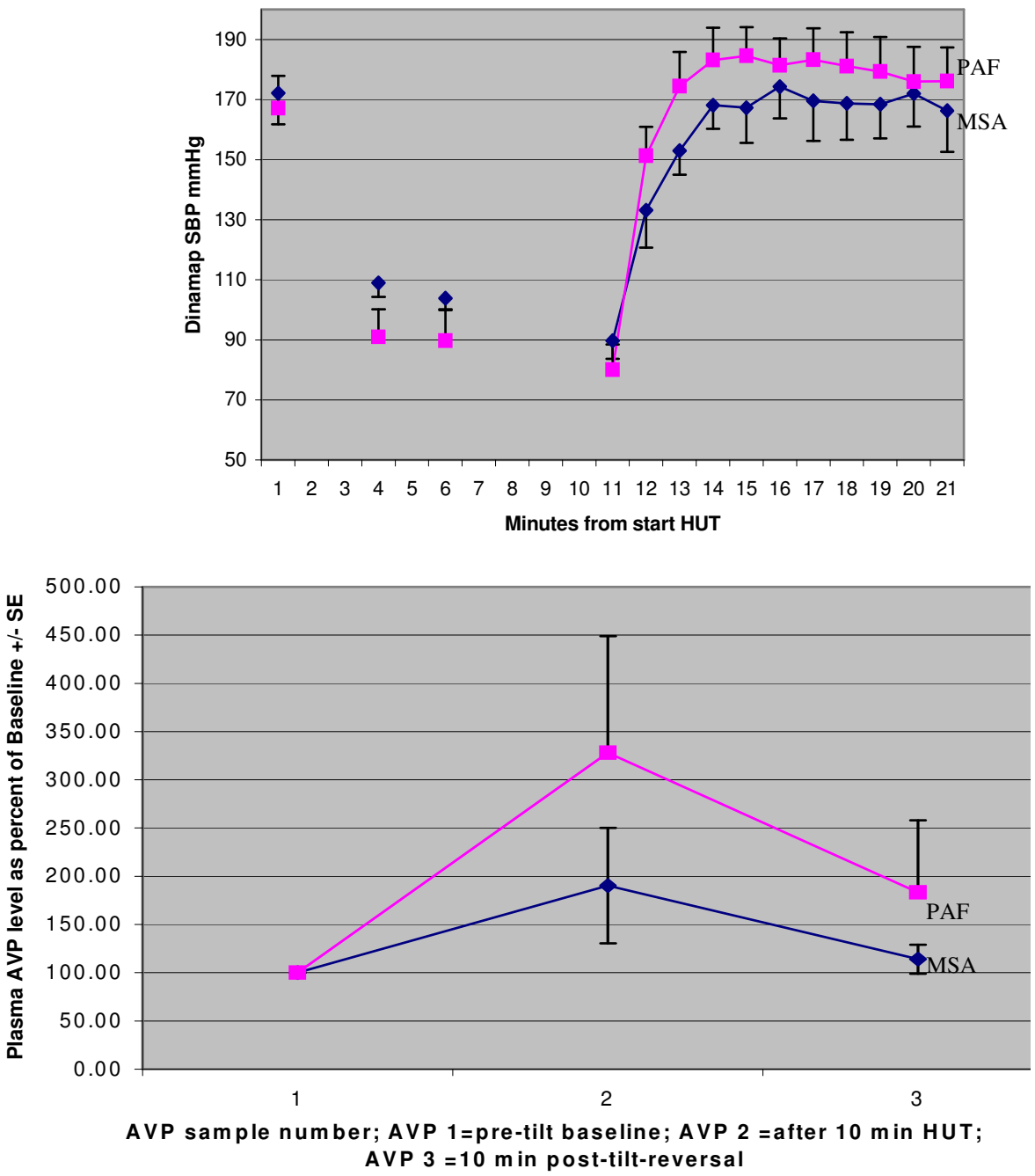

Figure 3.5.7: Upper Graph: Systolic BP (Dinamap; mmHg) +/- SE in 7 PAF and 7 MSA during the 10 minutes of HUT, and subsequent 10 minutes supine following tilt reversal; Lower Graph: Plasma AVP values for MSA and PAF at corresponding time points. Spearman's correlation was non-significant between SBP overshoot and increase in ADH in MSA and PAF.

Discussion

Both the pilot and subsequent AVP study primarily focussed on tilt-reversal. The baseline values and drop in BP on HUT were consistent with expected results. There was not a significant difference between the BP values in MSA and PAF either before or during HUT. Baseline HR was higher in MSA (78.1 +/- 3.4bpm) vs. than PAF (65.7 +/- 4.2 bpm) consistent with the findings described elsewhere in this thesis (chapter 3.2: Validating Standardised Pressor Responses in MSA and PAF). Similarly the HR variability was greater in MSA than in PAF (shown as greater increase of HR on HUT in MSA not PAF, in keeping with results elsewhere in this thesis.)

Following tilt-reversal, in the provisional pilot study, the PAF group showed a significant increase in SBP compared to pre HUT baseline unlike MSA. Within the group 5 PAF patients (62.5 %) had a BP overshoot (increase in supine SBP by at least 15mmHg 5–11 minutes after tilt reversal, with only one MSA patient (12.5 %) demonstrated BP overshoot. There were similar findings in the follow-up AVP study with only 1/7 MSA but 6/7 PAF showing at least 15mmHg SBP overshoot in the first 11 minutes following tilt-reversal. Both studies showed a significantly greater increase in SBP in the first 11 minutes post tilt-reversal in PAF compared to MSA. Neither study showed a significant change in DBP however. There were no prior reports specifically about BP overshoot after tilt reversal, except for incidental observations by Chandler and Mathias , who noted that mean arterial pressure in the post-tilt period was higher than in the pre-tilt period in PAF patients in a study primarily concerned with the cardiovascular changes during the HUT period rather than the subsequent post-tilt supine period. Furthermore, the differences in degree of BP overshoot between patients with PAF and MSA were not analysed. It appears likely that this original observation of MAP overshoot

in PAF reflected the SBP only overshoot shown in both the pilot study and subsequent AVP study described in this thesis.

In all three studies on overshoot to date (Chandler & Mathias and the two studies forming this chapter), there appears to be a consistent, small increase in TPR of about 12-19% from baseline. SV and CO in the post-tilt supine period did not differ from those in the pre-tilt supine period in either PAF or MSA. This leads to speculation regarding the possible existence of systemic vasopressor factors released during HUT in PAF but not in MSA. Increased sympathetic nerve activity seems unlikely to be important as PAF subjects have post-ganglionic lesions, low noradrenaline levels, and do not respond to pressor stimuli that work by increasing sympathetic nerve activity. Humoral factors may have a role in the observed overshoot, with AVP the candidate investigated in this chapter. It is known that upright posture and hypotension elicit AVP release in normal subjects, and that it produces potent pressor effect in autonomic failure [Mohring et al 1980; Williams et al 1986]. Furthermore, whilst this hypotension-induced release of AVP appears to be preserved in PAF, it is blunted in MSA [Kaufmann et al 1992; Kimber et al 1999] where the lesions are central. In addition, adrenoreceptor and pressor supersensitivity in PAF is often greater than in MSA [Polinsky et al 1999], reflecting the postganglionic lesions. Even though basal plasma noradrenaline levels are characteristically very low in PAF, and do not increase on standing, noradrenaline clearance is reduced in patients with PAF [Mathias et al 1990; Gilman et al 1999; Mathias & Bannister 2002; Chandler & Mathias 2002],. A combination of adrenoreceptor supersensitivity and delayed noradrenaline clearance may contribute to the prolonged pressor effect and BP overshoot after tilt reversal in PAF. However, nearly all subjects in both the pilot and subsequent AVP study had been taking fludrocortisone medication for postural hypotension previously. This tends to suppress the renin-angiotensin-aldosterone system, making it even less likely to play an important role in generating the BP overshoot on tilt-reversal. Theoretically a reduction of existing vasodilators might explain a BP overshoot effect, but the rapid time course of the overshoot makes this also seem

implausible. Finally, the possible role of other systemic vasoconstrictors such as endothelin must remain speculative whilst we do not yet know whether levels and activity are comparable to those seen in normal subjects.

AVP levels were expected to be greater in PAF than MSA based on previous results. Given that upright posture is the mechanism resulting in AVP release, maximal levels at the end of HUT would have been predicted as indeed was found (see Figure 5). The increase in AVP relative to baseline was greater in PAF than MSA but just missed significance, possibly related to one set of PAF values being insubmissible because of excessive haemolysis of the blood sample. Overall however the relatively small subject numbers employed in all three BP overshoot studies to date have been sufficient to consistently show a significant SBP overshoot in PAF whilst TPR increase from baseline was not as clear-cut. Given additional factors such as the increase in HR in MSA during HUT but reduction in HR on tilt-reversal, the possible contribution of more severely affected baroreceptors needs consideration. It may be that the preservation of AVP response to HUT exaggerates the tendency towards BP overshoot caused by impairment of the baroreflexes. In MSA however AVP levels appear to be lower than MSA following upright posture, and there appears to be at least some residual buffering capacity provided by reduced heart rate to further lessen any BP overshoot.

Regarding the limitations of the studies outlined above, the use of medication by subjects needs consideration. Because of ethical considerations it was not possible to withhold medication for longer than in the protocol. However, MSA and PAF subjects were fairly well matched by medication use, and the consistent findings of BP overshoot across three consecutive studies make it seem unlikely that this resulted in significant bias. It is unclear if disease duration, which in PAF was longer than in MSA, is a factor. As elsewhere in this thesis a major difficulty inherent in eliminating this problem is the relatively long period of time before PAF subjects are diagnosed after the onset of their symptoms. As the mean life expectancy in MSA is under 10 years, it is therefore almost inevitable that a random

PAF sample will have had longer disease duration where patients can live for decades post-diagnosis.

Whether BP overshoot reflects differences in sites of lesions, partial lesions in MSA, degree of pressor supersensitivity, changes in associated vascular hormones or other factors is unclear. It is possible that MSA subjects may partially compensate for a relative lack of AVP by enhanced sensitivity to the pressor actions of NA and Angiotensin II as seen in Diabetes Insipidus subjects on upright tilt [Williams et al 1988]. What does seem certain however is that these findings have potential clinical relevance. Increased hypertensive load, as experienced during BP overshoot, is likely to have deleterious effects. As already mentioned PAF subjects may survive for decades and so the accumulative effect of BP overshoot (presumably happening at least several times a day with normal positional change) is of concern. It is known that MSA or PAF patients who have supine hypertension, like patients with essential hypertension, have been reported to develop ventricular hypertrophy [Vagaonescu et al 2000]. Long term damage in different vascular territories (such as renal and retinal) should be considered. More acute deleterious effects of sudden SBP increase are known from general medicine such as intracerebral haemorrhage. These studies reinforce the need for head-up tilt while resting and sleeping, for long-term preventative as well as short-term therapeutic reasons. It has been customary to advise patients with PAF (as with MSA) to assume the upright posture only gradually to reduce the risks of symptomatic hypotension. The results of the studies outlined in this study suggest that similar care should be exercised in returning to the supine position, at least in PAF. In conclusion, we report BP overshoot after tilt in PAF but not MSA. The underlying mechanisms need to be evaluated further and their clinical relevance considered when treating orthostatic hypotension.

With gratefully acknowledgment to the Sarah Matheson Trust for their support with this study.

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3.6: Vasomotor Responses to Sympatho-excitatory stimuli in MSA and PAF

Abstract

Background & Aim: A variety of stimuli such as deep inspiration, isometric exercise and mental arithmetic, result in a transient vasoconstriction, mediated by sympathetic efferent nerves, in the skin of the fingers and toes of healthy controls [Skin Vasomotor Reflex: SkVR]. Multiple system atrophy (MSA) and pure autonomic failure (PAF) provide contrasting models of autonomic failure. In MSA the lesion is central and preganglionic, whilst in PAF the lesion site is peripheral and postganglionic. We evaluated the SkVR in response to various stimuli in MSA and PAF, to determine differences in skin vasomotor involvement between these two patient groups.

Methods: A total of 25 subjects (10 MSA, 7 PAF, 8 healthy controls) were studied. Baseline recordings of skin blood flow were obtained with a laser Doppler probe on the left index finger pulp and forearm. The subject then underwent a variety of stimuli with rest periods in between to re-establish baseline skin blood flow (SkBF). These stimuli were: single deep inspiration (inspiratory gasp); mental arithmetic; bilateral leg elevation and cutaneous cold.

Results: Healthy control subjects demonstrated marked SkVRs on the finger pulp to each of the stimuli of a magnitude similar to those seen in previous studies, but no SkVRs on the forearm. In MSA SkVRs to inspiratory gasp on the finger pulp were reduced relative to controls. In PAF SkVRs were reduced relative to controls or MSA. The magnitude of SkVR response to gasp and cutaneous cold in PAF was significantly less than in healthy controls. In addition, the magnitude of the response in PAF was significantly less than MSA for inspiratory gasp.

Conclusions: PAF showed a decreased SkVR response to all 4 stimuli, the response being significantly less than controls (for inspiratory gasp and cutaneous cold) or MSA (inspiratory

gasp). The decreased responses in PAF may reflect the extensive postganglionic sympathetic denervation seen in this group.

Introduction

In healthy control subjects, a variety of stimuli elicit a rapid and transient increase in the cutaneous vasoconstriction of the finger tip pulps [Bolton *et al* 1936; Low et al 1983, Saad et al 2001, Asahina et al 2003]. These skin vasomotor reflex (SkVR) responses are mediated via vasoconstrictor sympathetic efferent fibres, which richly innervate the small blood vessels of the finger pulp with [Johnson et al 1995]. This reduction in skin blood flow (SkBF) can be measured non-invasively by use of Laser Doppler flowmetry [Low et al 1983; Asahina et al 2003].

Multiple system atrophy (MSA) and pure autonomic failure (PAF) provide contrasting models of chronic autonomic failure. In MSA the lesion site is central and preganglionic, whilst in PAF it is peripheral and postganglionic [Daniel 2002; Matthews 2002]. It recently has been reported that SkVR responses to a number of stimuli are relatively preserved in MSA [Asahina et al 2003]. There is extensive postganglionic denervation in PAF; however the SkVR response has not previously been studied in this group. We hypothesised that the SkVR response would be impaired in PAF, but partially preserved in MSA. We therefore set out to assess the SkVR response in MSA and PAF subjects and to determine if there was a difference in the SkVR response between these two disorders.

Subjects

We studied a total of 17 subjects with chronic autonomic failure: 10 MSA (6 females and 4 males, mean age 58 ± 7 years) and 7 PAF (4 females and 3 males, 69 ± 6 years) patients and 8

healthy control subjects (3 female and 5 males, 54 ± 11years) (Table 3.6.1). MSA and PAF patients had the diagnosis made using existing criteria [Gilman et al 1999, Mathias 2004]. All MSA and PAF patients had documented sympathetic and parasympathetic dysfunction with severe orthostatic hypotension. The mean disease duration was 5.5 ± 3 years in the MSA patients and 14.4 ± 6.5 years in the PAF patients. Supine BP was higher in PAF (175 ± 30 / 92 ± 9) than MSA (152 ± 25 / 87 ± 15), but this difference did not reach statistical significance (Table 3.6.1). No subjects were on anti-parkinsonian medication. Vasoactive medication was withdrawn from the night prior to the study. All patients gave informed consent to participate in the study, which had ethical approval from the National Hospital for Neurology & Neurosurgery and St. Mary's Hospital London. Studies were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Methods

All studies were performed in a dedicated, temperature controlled (24+/-1^{0}C), autonomic laboratory. Skin blood flow was measured by laser Doppler flowmetry [Periflux 5000/5010, Perimed, UK]. This method utilises fibreoptics to shine a laser light (780 nm wavelength) directly at the skin surface. The light is reflected back to the recording element of the probe from red blood cells in the skin capillaries. The wavelength of the reflected light is altered by the Doppler shift effect caused by the movement of the red blood cells relative to the probe. This altered wavelength is then used to calculate an arbitrary perfusion rate ("Perfusion units"; PU), rather than absolute flow, which is then visualised as a real-time trace. Cutaneous vasoconstriction will thus be shown as a reduction in perfusion units relative to baseline perfusion.

With the subject supine, a small straight Laser Doppler probe was attached to the left index finger tip (pulp) and to the ventral forearm using double-sided adhesive tape, ensuring that the

probe was not placed under pressure. The forearm probe was used as a control to exclude movement or temperature artefact as forearm skin blood flow is under thermoregulatory rather than the "emotional" sympathetic regulation present in the finger tip skin [Johnson et al 1995; Saad et al 2001]. The resulting SkBF signal data were stored for later analysis using PeriSoft for Windows software [Perimed, UK]. The subject remained supine throughout the study. Ambient noise was kept to a minimum for the duration of testing. After a 20-minute period to allow for a stable basal recording of SkBF the application of sympathetic activating procedures commenced:

1) Inspiratory gasp (IG): The subject was asked to take the deepest breath they could, hold it in inspiration and then breathe out.
2) Mental arithmetic (MA): The subject was required to give verbal answers to mathematical sums (Continually subtracting 7 from 100) for 15 seconds.
3) Bilateral leg elevation (BLE): The subject raised both legs 20^0 off the couch for 10 seconds.
4) Cutaneous cold (CC): An ice pack (-4^0C) was applied to the dorsum of the right hand for 90 seconds.

After each stimulus, SkBF was allowed to return to stable baseline levels before commencing the next stimulus. Each study was performed twice, allowing calculation of a mean SkVR reduction rate for each stimulus. The SkVR reduction rate was calculated as the reduction of SkBF seen after each stimulus as a percent of the baseline value. Comparison between groups was performed with ANOVA. Significances of individual differences were evaluated by the Scheffé test if ANOVA was significant.

Results

Means of basal SkBF in the finger tip were 160 ± 115 PU in the MSA patients, 176±93 PU in the PAF patients, and 188±97 PU in the controls (Table 3.6.1), and there were no significant differences in three groups. Each procedure elicited distinct SkVRs on the finger pulp in every control subjects, as shown with the example of inspiratory gasp stimulus shown in Figure 3.6.1. Figure 3.6.2 shows results of SkVR reduction rates. The SkVR reduction rate for IG was significantly less in either MSA (51 ± 16 %, $p<0.05$) or PAF (30 ± 7 %, $p<0.0001$) than the controls (69 ± 15 %), and significantly less in the PAF patients than in the MSA patients ($p<0.05$). The SkVR reduction rate for CC was significantly less in the PAF patients (28 ± 27 %) than the controls (63 ± 24 %) ($p<0.05$). Although the SkVR reduction rate for CC was less in the MSA patients compared with the controls, there was no significant difference. The SkVR reduction rate for MA or BLE was less in the PAF patients (27 ± 10 % and 37 ± 20 %) than the controls (45 ± 30 % and 62 ± 31 %) or MSA patients (39 ± 12 % and 57 ± 14 %), but this difference did not reach statistical significance. There were no significant differences in the SkVR reduction rate for MA or BLE between the MSA patients and controls. The SkVR reduction rate for IG was almost uniform in the controls (small SD). However, the SkVR reduction rates for other procedures were somewhat varied in the controls (large SD).

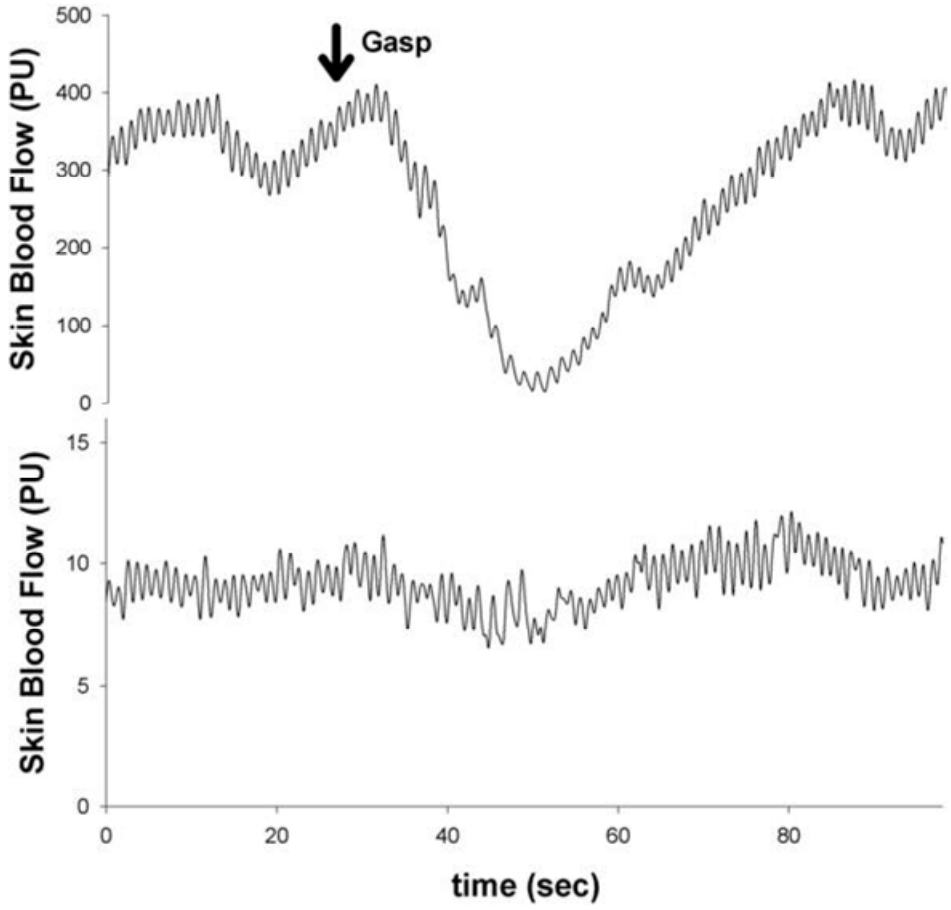

Figure 3.6.1: Skin Blood Flow in the finger tip (upper panel) and ventral forearm (lower panel) following inspiratory gasp (arrowed) in a healthy control subject.

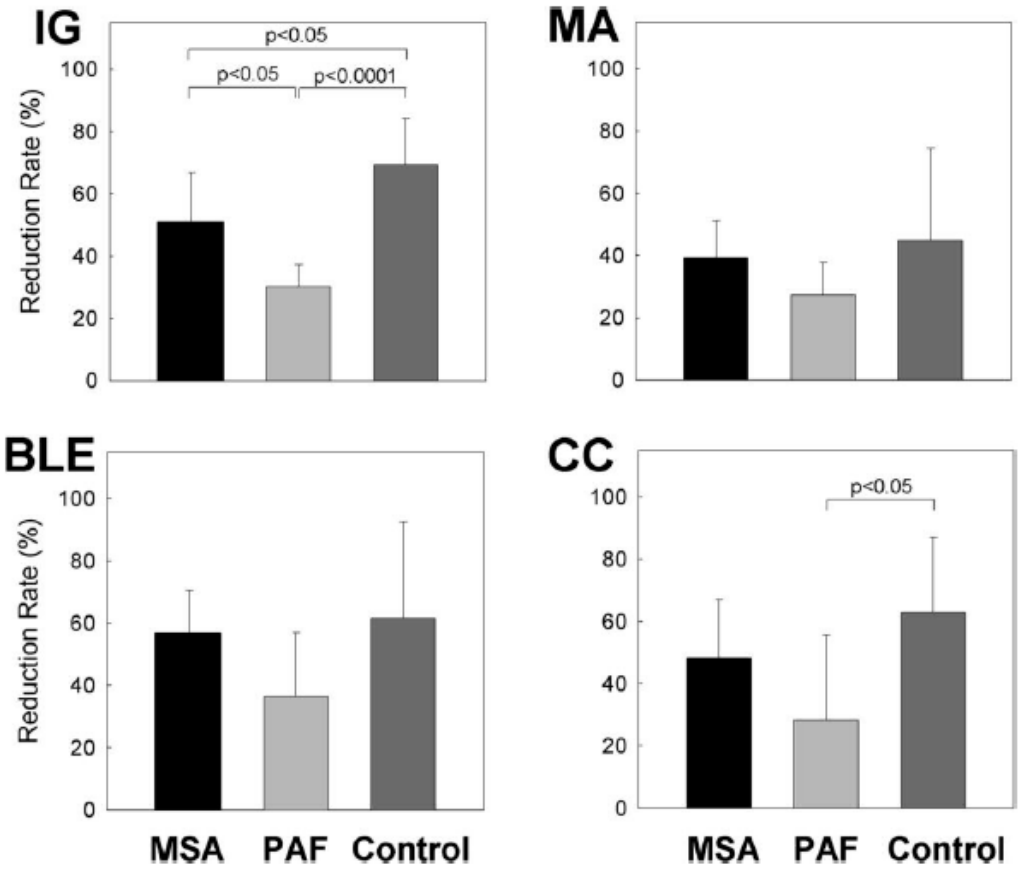

Figure 3.6.2: Mean reduction rates (+ SD) in Skin Blood Flow (SKBF) in the finger tip after inspiratory gasp (IG), Mental Arithmetic (MA), bilateral leg elevation (BLE), and Cutaneous Cold (CC) in 10 MSA patients and 7 PAF patients and 8 healthy controls.

Means of basal SkBF as recorded from the forearm probe were 19±25 PU in MSA, 22±14 PU in PAF, and 16±5 PU in controls (Table 3.6.1), and there were no significant differences in three groups. SkBF in the forearm, unlike the finger tip, did not show any significant changes during sympathetic activation procedures in any group (Figure 3.6.1).

Discussion

In control subjects, the reduction in SkBF on the finger pulp following various vasomotor stimuli was similar in magnitude to previous reports [Low et al 1983, Khan et al 1991, Asahina et al 2003]. The lack of SkVR response in any group from the skin of the forearm is consistent with previous studies [Saad *et al* 2001]; unlike the finger pulp skin, the forearm vasomotor function is more strongly influenced by thermoregulatory factors than by stimuli such as IG and MA.

The SkVRs to all procedures were severely attenuated in PAF, and to a lesser extent in MSA. However, the most significant differential between groups occurred with the SkVR to IG. In the controls, SkVR showed the greatest response to IG (Mean reduction rate of 69%). The response of controls to MA was the least marked (45%). IG may therefore be the best procedure to evoke SkVR.

Although BLE has previously been demonstrated as a means to elicit SkVR in MSA, mild motor impairment in our MSA subjects (although none required anti-parkinsonian treatment), may have contributed to the greater variability of response seen with this test [Asahina et al 2003]. A relationship between laser Doppler recordings of SkVR responses and direct intraneural recordings of skin sympathetic activity has been reported [Lundin et al 1990]. SkVR responses require intact peripheral sympathetic nerves as shown by studies in surgically sympathectomised subjects and temporary nerve blocks [Bolton et al 1936; Netten et al 1995; Rex et al 1998 Lehtipalo et al 2000]. In PAF, the lesion site affects the peripheral, postganglionic autonomic nerves, which is the final common pathway involved in all SkVRs [Matthews 2002]. Attenuated SkVR in patients with amyloidosis and peripheral autonomic neuropathy has been reported, but SkVRs in PAF had not previously been studied [Ando et al

1992]. Our findings of severely diminished SkVR in PAF may reflect involvement of postganglionic sympathetic neurons in this group.

In MSA the postganglionic nerves appear to be spared both morphologically and functionally, while the central nervous system is involved [Matthews 2002, Dotson et al 1990]. Depletion of sympathetic pre-ganglionic neuron in the intermediolateral cell column (IML) is thought to be a major factor involved in sympathetic failure in MSA [Bannister & Oppenheimer 1972]. However, IML involvement does not correlate well with the degree of autonomic failure seen in MSA [Gray *et al* 1988]. This suggests that other lesion sites, besides the IML, contribute to autonomic failure in MSA. Particularly, depletion of catecholaminergic neurons in the rostral ventrolateral medulla (rVLM) may contribute to sympathetic vasomotor failure, such as orthostatic hypotension, in MSA [Benarroch *et al.*1998]. The rVLM, which in turn projects to the intermediolateral cell columns, is thought to be involved in control of sympathetic cardiovascular outflow [Guyenet 1989; Saper 1998]. However, rVLM may not participate in control of skin blood flow [Blessing *et al.*1999]. Although the central pathway of SkVR generation is still unclear, our findings suggest that the reflex arc of SkVR is comparatively preserved in MSA.

SkVRs are highly localised to the palm or sole areas, and we recorded no SkVR from the forearm laser Doppler probe in any subject [Saad *et al* 2001]. Furthermore, skin vasomotor function on the palm or sole is independent of systemic blood pressure, [Lehtipalo et *al* 2000]. Therefore, systemic blood pressure was not measured in this study, or in several previous studies, although procedures, such as mental stress (MA), isometric exercise (BLE) and cold stimuli (CC), affect systemic blood pressure [Ando et al 1992, Asahina et al 2003].

Mean age in PAF (69 ± 6 years) was significantly greater than in controls (54 +/- 11 years: p= 0.007) or MSA (58 ± 7 years: p= 0.004). Whilst a previous study has suggested that SkVR to gasp and cutaneous cold is impaired in elderly compared to young healthy control subjects,

the age difference was far greater than in our study (young healthy control age 26 ± 5 years; older healthy controls 68 ± 4 years) [Khan *et al* 1992]. In addition, the mean SkVR in the older healthy controls to gasp (51%) and cutaneous cold (58%) was greater than we found in the PAF patients of a similar age (30% and 28% respectively). Difference in age therefore does not seem sufficient to explain the reduced SkVRs found in our study.

Disease duration differed between MSA (mean duration 5.5 +/- 3 years) and PAF (14.4 +/- 6.5 years). This difference may have affected our results as greater disease duration might be expected to result in a greater reduction in SkVR. However, several important points need to be made in this regard. Firstly the natural history of disease progression is different in MSA and PAF. In MSA mean survival time after diagnosis is 9 years and as such our subjects had relatively advanced disease state with severe autonomic dysfunction recorded on standard autonomic function tests. By contrast in PAF autonomic dysfunction is severe at initial diagnosis with little, if any progression from that time on. A major advantage of studying PAF subjects with long disease duration is that the diagnosis may then be considered secure. It is this point that also explains the need for improved differential testing of these conditions. As with existing tests used to define autonomic dysfunction, we did find variability of response of SkVR between subjects within each individual group. However, variability was not marked in respect to the SkVR to inspiratory gasp, resulting in significant differences between the groups. We have thus been able to demonstrate proof of concept in this study. Prospective studies of SkVR in chronic autonomic failure patients from the time of diagnosis would be required to explore possible clinical value in the differential diagnosis of MSA and PAF.

In conclusion, the effects of vasomotor stimulation on skin blood flow in PAF have not previously been reported. We have found that SkVRs are impaired to a greater extent in PAF than in MSA. This may reflect the underlying pre- and post-ganglionic lesions in these

conditions. The measurement of changes in relative blood flow provides a non-invasive method which may help to distinguish MSA and PAF.

We gratefully acknowledge the support of the Sarah Matheson Trust for this study

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3.7: Acute Cardiovascular effects of CO_2 Inhalation in MSA and PAF

a) Effects on Systemic Haemodynamics

Abstract

We recorded the haemodynamic effects of inhaled CO_2 in multiple system atrophy (MSA; where the lesion site is preganglionic, n=9), in pure autonomic failure (PAF; where it is postganglionic, n=9), and in normal subjects (n=5). Our hypothesis was that the pressor effects of inhaled CO_2 seen in normal subjects are mediated via sympathetic activation, and as such the pressor effect would be impaired in chronic autonomic failure, as with MSA and PAF. As MSA and PAF provide naturally occurring models of pre-ganglionic and post-ganglionic autonomic failure respectively, we set out to determine if additional differentiation in the degree of pressor response to inhaled CO_2 could be made between these two contrasting types of autonomic failure.

To determine the mechanisms involved, blood Pressure (BP) and heart rate (HR) were measured continuously with a Portapres II Device. A single, maximal, breath of room air was taken by each subject via a mouthpiece. This was compared with response to a single maximal breath of 35% CO_2 in 65% oxygen. After air inhalation there was no significant BP change in any group. After CO_2, BP increased in each group, with the peak value occurring after 32 seconds in controls.

There were no significant haemodynamic effects of air inhalation in any group. With CO_2 inhalation normal subjects demonstrated an initial bradycardia 20 seconds post-inhalation, and then marked pressor response with tachycardia in keeping with previous studies. Neither MSA nor PAF subjects demonstrated significant changes in HR; however, in both MSA and PAF there was a significant pressor response. Compared with normal subjects, the pressor

effect was attenuated and occurred later than in controls. However, there was no significant difference either in magnitude or latency of the pressor response to inhaled CO_2 between MSA and PAF.

Thus, despite the absence of HR changes, a pressor response to CO_2 inhalation was still seen in both MSA and PAF, albeit significantly attenuated and delayed. This may reflect either incompleteness of sympathetic denervation in these conditions, or alternative effector mechanisms for the CO_2 pressor response. There were no significant differences between the BP changes seen in MSA and PAF, suggesting that both pre-and post-ganglionic autonomic mechanisms are important in generating the CO_2 pressor response.

Introduction

In normal subjects maximal inhalation of a single breath of 35% CO_2 in oxygen evokes a rapid pressor response, which is thought to result from stimulation of sympathetic outflow centrally [Kaye *at al* 2004]. The initial response is a transient bradycardia (thought to reflect direct vagal action of CO_2), followed by tachycardia. The acute pressor response that follows is centrally mediated and associated with a significant increase in plasma noradrenaline. We studied two physiological lesion models of chronic autonomic failure, multiple system atrophy (MSA) where the lesion is predominantly central and preganglionic, and pure autonomic failure (PAF) where it is postganglionic [Polinsky 2002]. The effects of air or CO_2 inhalation on systemic haemodynamics have not been determined in these two differing physiological models. We measured continuously the systemic haemodynamic responses before and after a single breath of CO_2. Comparisons were made with inhalation of air.

Subjects

A total of 23 subjects were studied (5 healthy controls, mean age 47+/- 6.8; 9 MSA, mean age 57.1+/- 3.1; 9 PAF, mean age 66.4). The diagnosis of MSA and PAF was made using established criteria [Gilman et al 1998, Mathias 2004]. All had documented sympathetic and parasympathetic cardiovascular autonomic dysfunction (Table 3.7.1). Sequential subjects attending outpatients as well as inpatients were approached to ask if they would be happy to participate. Normal healthy subjects consisted of Research staff. Written, informed consent was obtained from all subjects, the studies conformed to the standards set by the Declaration of Helsinki, and the procedures had been approved by the local ethics committees (The National Hospital for Neurology and Neurosurgery, London and St Mary's Hospital London). No patient was taking anti-parkinsonian medication and all medications were withdrawn from the night before the study. On the morning of the study subjects were fasted.

		MSA	PAF	Normal range
Age		57.1 (3.1)	66.4 (2.1)	
Supine SBP (mmHg)		162.3 (8.6)	170.6 (9.9)	
Standing SBP (mmHg)		79.9 (5.8)	83.3 (5.3)	
Change in SBP	Standing	-82.4 (9.3)	-65.0 (21.8)	< -20
	Hand grip	2.3 (2.6)	2.9 (3.6)	≥17
	Cold Pressor	-3.9 (4.7)	2.6 (2.1)	≥15
Supine DBP (mmHg)		93.3 (4.8)	94.7 (4.4)	
Standing DBP (mmHg)		54.1 (3.5)	53.3 (3.9)	
Change in DBP	Standing	-39.4 (6.3)	-30.9 (8.8)	<-10
	Hand grip	-0.3 (2.1)	1.7 (2.2)	≥11
	Cold Pressor	-3.7 (4.3)	-0.2 (2.1)	≥10
DHR on Deep Breathing (beats per min)		6.3 (1.1)	3.2 (1.3)	>9

Table 3.7.1: Autonomic screening tests in 9 MSA and 9 PAF subjects: mean results shown +/- (SE)

Methods

All tests were conducted between 1000 and 1500 hours in a temperature (24 +/- 1^0C) controlled laboratory. Upon arrival at the testing unit, a 20G (Venflon) intravenous line was

inserted into a subject's right antecubital fossa and kept patent with 0.9% saline. A Portapres II device was applied to the middle finger of the right hand to provide continuous systolic and diastolic blood pressure (SBP and DBP) and heart rate (HR) readings. Subsequent calculation of cardiac output (CO), total peripheral resistance (TPR) and stroke volume (SV) was performed using Modelflow analysis [Wesseling *et al* 1995; Langewouters *et al* 1998]. These haemodynamic indices were calculated at 4 time points:

i) Baseline

ii) At time of peak BP change after air inhalation

iii) Baseline

iv) At time of peak BP change after CO_2 inhalation

The subjects remained seated throughout the study. After 30 minutes of quiet rest physiological monitoring and blood sampling commenced. A 10L silicone 'Douglas' bag (Hans-Rudolph, Kansas City, Missouri, USA) was filled with the gas mixture (35% CO_2 and 65% O2; BOC Gases, Guildford, Surrey, UK). Inspired vital capacity (VC) was determined using an analogue flow meter (Ohmeda, USA) that was attached via a 3-way valve to a 3cm diameter silicone mouth-piece (Hans-Rudolf, Germany) at one end and the 10L silicone bag containing the CO_2 at the other end. The subject took a single VC breath of room air through the mouthpiece whilst occluding their nose with a nose-clip. Baseline inspired VC with a single breath of air was measured and a test breath of room air was considered adequate if it was more than 80% of this baseline breath [Kaye *et al* 2004]. Once the cardiovascular indices had returned to baseline, a single VC breath of CO2 was taken in an identical manner to that for room air. Venous blood sampling for catecholamines was performed at baseline and at 2, 10 and 20 minutes after CO_2 inhalation. Plasma levels of Noradrenaline (NA), Adrenaline (Ad) and Dopamine (DA) were measured at baseline and at 2, 10 and 20 minutes post- CO_2. Electrochemical detection for NA, Ad and DA concentration was performed using high-performance liquid chromatography [Smedes *et al* 1982; May *et al* 1988; Mathias *et al* 1990].

The ethics committees of the National Hospital for Neurology and Neurosurgery, and St Mary's Hospital approved the study and each subject provided written informed consent prior to participation.

Statistics: Data are presented as mean +/- SEM. Within-group differences were compared with paired two-tailed t-tests. One-way analysis of variance was used to determine between-group differences with two-tailed t-tests used to compare single time-point data including baseline differences. Cardiovascular responses for a particular time point were calculated as the mean of +/-3 beats from that point. A p value of <0.05 was considered significant.

Results

Systemic haemodynamics and cardiac function

Blood pressure, heart rate, stroke volume, cardiac output, and total peripheral resistance are described at baseline and following inhalation of either air or CO_2.

Baseline:

There were no significant differences in baseline SBP (122.5 +/- 12.3 mmHg in controls; 132.7 +/- 8.7 in MSA; 128.8 +/- 13.6 in PAF) or DBP (66.9 +/- 3.8 mmHg in controls; 76.1 +/- 7.2 in MSA; 75.7 +/- 4.3 in PAF). Baseline HR was higher in MSA than in controls (77+/-4.1 bpm and 60.9+/-3.9 bpm respectively, p=0.02); baseline HR in PAF was 66.8 +/- 2.6 bpm. In controls SV (82.6 +/- 6.1 ml), was not significantly different from MSA (65.9 +/- 0.9 ml) but was significantly higher than in PAF (43.5 +/- 7.6 ml). CO was similar in controls (4.9 +/-0.9 l/min) and MSA (4.7 +/- 0.6 l/min), but was significantly lower in PAF (2.8 +/- 0.4 l/min).

TPR was similar in controls (1.3 +/- 0.2 MU) and MSA (1.5 +/- 0.3 MU), but was significantly higher in PAF (2.2 +/- 0.2 MU; $p<0.05$).

Post-air inhalation:

There were no significant changes in SBP, DBP, HR, TPR or CO in any group.

Post- CO_2 inhalation:

In controls there was an increase in SBP (+60.2+/-13.9mmHg; +50.3+/-11.8% of baseline) and DBP (21.8+/-5.0mmHg; 32.3+/-7.4% of baseline), p=0.04, 0.04 respectively, with the peak increase at 32.4+/-2.1 seconds post inhalation. Despite a transient bradycardia within 20 seconds of CO_2, HR, CO and SV were not significantly altered at the time of the peak pressor effect. TPR was increased at the time of peak pressor effect although this just failed to reach significance (p=0.08).

In MSA there were increases in SBP (18.3+/-2.7 mmHg; 15.1+/-2.5% of baseline) and DBP (10.5+/-2.7 mmHg; 18.7+/-2.9%) p=0.008 and 0.0015 respectively. However, pressor response peaked later (at 140.2 +/-35.5 seconds post-inhalation) and to a smaller extent than in controls ($p<0.01$). There was no change in HR, CO or SV. TPR increased at the time of peak pressor response, but did not reach significance (p=0.066).

In PAF there was an increase in SBP (26.8+/-3.2mmHg; 23.2+/-3.9%) and DBP (13.7 +/-2.0 mmHg; 14.7+/-3.1%), p=0.008. This pressor effect also occurred later (152.4+/-23.9 seconds) and to a lesser extent than in controls ($p<0.01$). In PAF, TPR was increased relative to baseline at the time of peak pressor response (p=0.008) but there was no significant change in CO, HR or SV.

Plasma Noradrenaline:

Baseline:

Baseline plasma NA levels were significantly lower in PAF (126.8+/-19.8pg/ml) than in controls (288.4+/-31.3pg/ml) or MSA (246.9+/-13.1pg/ml) ($p<0.001$)

Post- CO_2 inhalation:

In controls there was a significant increase in NA levels ($p<0.05$), first reaching significance 2 minutes post-inhalation (Figure 3.7.1). NA levels following CO_2 inhalation did not increase in either MSA or PAF.

Plasma Adrenaline:

Baseline:

Baseline Ad levels were significantly higher in controls (44.2 +/- SE 5.9 pg/ml) than in MSA (20.8 +/-SE 3.6 pg/ml): $p<0.05$. In PAF, Ad levels were undetectable at baseline.

Post- CO_2 inhalation:

In controls there was a significant increase in Ad levels ($p<0.05$), peaking at 90.4 +/- SE 14.0 at 10 minutes post-inhalation. Plasma levels of Ad following CO_2 inhalation did not increase in either MSA or PAF.

Plasma Dopamine:

In 2/5 controls, 5/9 MSA and 9/9 PAF, DA levels were undetectable at baseline. There was no significant change in any group Post- CO_2 inhalation.

	Normal Subject	PAF	MSA	P value compared with Normal (N)
Noradrenaline (pg/ml)				
Δmax	124.2+/-19.5	5.2+/-2.8	20.0+/-6.4	<0.001 (NvPAF)
%Δmax	41.7+/-7.1	4.2+/-2.2	8.0+/-2.4	<0.001 (NvMSA)
SBP (mmHg)				
Δmax	60.2+/-13.9	26.8+/-3.2	18.3+/-2.7	<0.01 (NvPAF)
%Δmax	50.3+/-11.8	23.2+/-3.9	15.1+/-2.5	<0.001 (NvMSA)
DBP (mmHg)				
Δmax	21.8+/-5.0	13.7+/-2.0	10.5+/-2.7	-
%Δmax	32.3+/-7.4	14.7+/-3.1	18.7+/-2.9	
HR (b/min)				
Δmax	-9.1+/-3.5	-4.3+/-2.3	-4.1+/-2.1	-
%Δmax	-16.1+/-6.9	-7.1+/-3.7	-6.4+/-3.2	
CO (L/min)				
Δmax	-0.6+/-0.5	-0.1+/-0.1	-0.1+/-0.1	-
%Δmax	-6.2+/-9.5	-4.5+/-3.7	-3.3+/-4.0	
SV (ml)				
Δmax	19.5+/-34.3	-1.7+/-1.4	0.6+/-2.4	-
%Δmax	18.4+/-14.5	-0.5+/-5.7	2.0+/-4.8	
TPR (PRU)				
Δmax	1.7+/-0.2	2.8+/-0.3	1.8+/-0.4	0.03 (NvPAF)
%Δmax	14.3+/-9.0	24.6+/-5.5	17.8+/-7.5	

Table 3.7.2: Comparison of Seated Noradrenaline and Potapres Cardiovascular indices between groups (only significant values displayed)

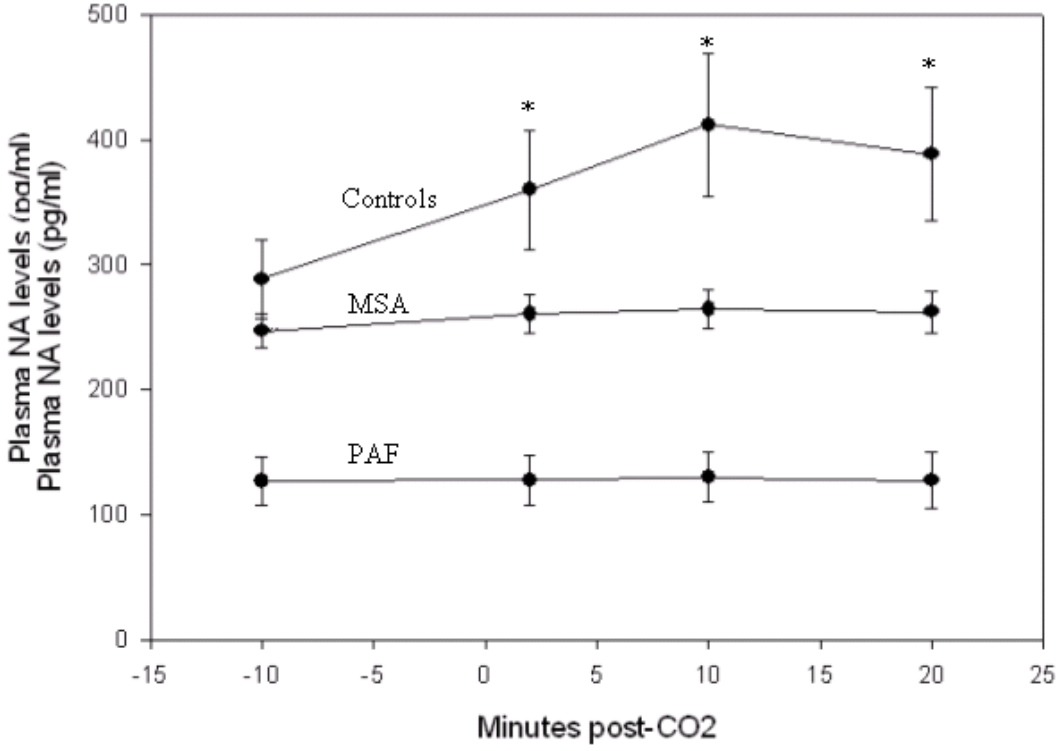

Figure 3.7:1 Changes in NA levels following inhalation of 35% CO_2 in Control Subjects, MSA and PAF. *=p<0.05 relative to baseline levels

Discussion

We observed that the acute inhalation of air in controls, MSA and PAF, resulted in no significant changes in BP. However, following inhalation of 35% CO_2, there was a pressor response in all groups, with the magnitude and speed of onset being greater in controls than in PAF and MSA. The inhalation of air did not result in significant changes in systemic haemodynamics in any of the groups. This was different from the effects observed following CO_2 inhalation. In controls there was a pressor response, peaking at 32.4 seconds post-inhalation. Although there was also a pressor response following CO_2 in MSA and PAF, this was significantly smaller in magnitude and occurred later than in controls. There was no difference between these responses in MSA and PAF. In all 3 groups there was an increase in TPR at the same time as the pressor effect. The reduced pressor response in both MSA and PAF is likely to reflect underlying sympathetic denervation and impaired vasoconstriction. However, neither the magnitude nor latency of response in MSA and PAF differentiated between these pre- and postganglionic models of chronic autonomic failure.

In controls, plasma NA levels were within the normal range and significantly increased 2-minutes post-CO_2 inhalation. This is likely to reflect an increase in sympathetic nerve activity following CO_2. In MSA, baseline plasma NA levels were not significantly different to controls, in keeping with previous observations [Mathias et al 1990] and did not increase following CO_2. In PAF, baseline plasma NA levels were lower than in controls and MSA, again consistent with the known postganglionic lesion site in PAF [Polinsky 2002], and did not change following CO_2. These results provide additional evidence for the inability of CO_2 to increase sympathetic activity in MSA and PAF. The plasma NA response to CO_2 did not differentiate MSA and PAF, suggesting that both pre- and postganglionic pathways are required to elicit an increase in NA following CO_2 inhalation.

Limitations

The mean ages of our subjects differed, being 47 +/- 6.8 years in controls, 57.1 +/- 3.1 years in MSA, and 66.4 +/- 2.1 years in PAF. This reflects the age of onset of disease in these disorders, and the shortened life expectancy in MSA. However, there was no significant difference between the responses seen in MSA or PAF, and thus if one speculated that age was important in the pressor response to CO2 inhalation, one would have to argue that a significant decrease was seen between a mean age of 47 and 57.1, yet no difference in response between 57.1 and 66.4 (with a non-significant increase in pressor response between these 2 age groups). This seems biologically implausible.

Although the Modelflow provides a non-invasive method of obtaining CO estimation, aspects of its precision have been previously investigated. The principle of haemodynamic measurement with the Portapres 2 is based on the volume-clamp method, first described by Jan Penaz in 1973 and the Physiocal system of [Wesseling 1995]. The volume clamp method involves maintaining (clamping) the finger artery diameter, despite changing arterial BP, by means of a finger cuff with inflatable air bladder. Because the pulse contour depends mainly on the stroke volume (SV) of the left ventricle, SV estimates may be made via a mathematical model that makes assumptions of arterial distensibility. This mathematical model is the basis of Modelflow analysis, incorporated in the Portapres software, which also requires details of the subject's age, sex, height and weight as these can influence arterial distensibility [Kelly *et al* 1989]. Subsequent calculation CO is then performed as part of the Modelflow software.

Earlier validation studies have suggested that Modelflow does provide reliable values for cardiac indices such as SV, which have been validated in a variety of settings [Harms et al 1999, Jellema, Imholz et al.1999 Langewouters et al.1998]. However, the reliability of the absolute values of CO have been questioned [Jellema et al 1999, Pitt *et al* 2004], with a view

that relative change of CO values in groups rather than individuals could still provide reliable data. In our study we found possible evidence of the limitation of CO reading by Modelflow in our normal controls in whom CO2 inhalation significantly raised BP but TPR was increased with a p value of 0.08. As there was a slight reduction in CO as estimated by Modelflow at the time of blood pressure peak, it could be suggested that the values of CO were inaccurate. However, in relatively small groups such as studied here, a more likely explanation is that the large variability in TPR response was more likely to have resulted in a less impressive p value than that seen for BP increase. An increase in TPR alone, with CO remaining constant or even falling slightly would still of course be entirely compatible with an increase in BP. It should be remembered, as pointed out by Pitt *et al.* [Pitt *et al* 2004], that even the supposed gold standard of CO measurement, namely thermodilution, has inherent errors of up to +/- 15% [Guyton *et al* 1973]. We therefore feel justified in using the considerably less invasive Portapres/Modelflow methods in investigating the changes of cardiac indices in this study whilst acknowledging the potentially reduced accuracy of CO values thus obtained.

Conclusion

Our results indicate that there was a pressor response following CO_2 in MSA and PAF; however this was significantly smaller in magnitude and occurred later than in controls. There was no difference in the pressor responses seen in MSA or PAF. NA levels at baseline were similar to those previously reported in healthy subjects, MSA and PAF. Following CO_2 NA rose within 2 minutes in controls but showed no change in either MSA or PAF. The lack of HR or NA change following CO_2 is consistent with the suggested importance of autonomic nervous system activation in the haemodynamic response to CO_2, and the known lesions in these two conditions. However, a pressor response still occurred, albeit of diminished magnitude and of greater latency to onset than that seen in normal subjects. These may reflect incomplete denervation in MSA and PAF, or alternative mechanisms by which CO_2 might

result in an increase in BP. There was no significant difference between MSA and PAF in terms of the pressor response. This may well reflect the importance of both pre-and post-ganglionic components of the autonomic nervous system in mounting the pressor response as seen in normal subjects.

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3.8: Acute Cardiovascular effects of CO_2 Inhalation in MSA and PAF

b) Effects on Regional Skin Blood Flow

Abstract

We recorded the haemodynamic effects of inhaled CO_2 on the cutaneous vascular bed in multiple system atrophy (MSA; where the lesion site is preganglionic, n=9), in pure autonomic failure (PAF; where it is postganglionic, n=9), and in normal subjects (n=5). Inhalation of a single breath of CO_2 (35% CO_2 in 65% oxygen) evokes a rapid, transient increase in blood pressure (BP). In addition, it is known that the action of a deep voluntary gasp evokes a reduction in skin blood flow (SkBF) of the finger pulps in healthy subjects, an effect possibly related to activation of a reflex sympathetic vasoconstrictor pathway. To determine the mechanisms involved, acute inhalation of air or CO_2 was compared between normal subjects and two naturally occurring models of chronic autonomic failure, MSA and PAF. Blood Pressure (BP) and heart rate were measured continuously with a Portapres II Device, and SkBF measured with a Laser Doppler probe.

We hypothesised that acute air inhalation would produce less cutaneous vasoconstriction in chronic autonomic failure because of dysfunction of the sympathetic nervous system in these conditions. We furthermore felt it likely that acute inhalation of CO_2 would effectively also act as a gasp stimulus with resultant cutaneous vasoconstriction. As PAF subjects have sympathetic postganglionic denervation with resultant supersensitivity to vasoactive compounds, we further hypothesised that inhalation of CO_2 would in contrast lead to cutaneous vasodilatation as the enhanced direct vasodilatory actions of CO_2 would be relatively unopposed by vasoconstriction from the gasp response.

In controls, after either air or CO_2, there was a rapid reduction in SkBF in keeping with the previously described gasp response. In MSA there was a diminished reduction in SkBF after

air or CO_2. In PAF, after air there was a smaller reduction of SkBF than in either controls or MSA. After CO_2, in PAF a transient *increase* in SkBF was observed seconds after inhalation. The increase in SkBF in PAF after CO_2 was greater than in MSA.

We conclude that these studies suggest that CO_2 has both neural and non-neural cardiovascular autonomic actions. The former effects are diminished in MSA and PAF; the latter probably result in the increased SkBF in PAF. SkBF changes in response to CO_2 may provide a novel means of differentiating pre- from postganglionic sympathetic denervation.

Introduction

In normal subjects maximal inhalation of a single breath of air results in a transient, marked, vasoconstriction in the finger pulp for up to 2 minutes, termed a gasp response [Bolton et al 1936; Asahina *et* al 2004]. This is felt likely to reflect activation of sympathetic vasoconstrictor nerves in a reflex manner. The finger pulp is an area densely innervated by sympathetic vasoconstrictor nerves [Johnson *et al* 1995]. Previous studies have suggested that in the cutaneous vascular bed CO_2 tends to cause vasodilatation [Nishimura *et al* 2002; Schnizer *et al* 1985; Ito *et al* 1989], probably by direct mechanism [Bullard 1964; Ito *et al* 1989]. We anticipated that the inhalation of either air or CO_2 would result in a similar gasp response in normal subjects. In addition we studied two physiological lesion models of chronic autonomic failure, multiple system atrophy (MSA) where the lesion is predominantly central and preganglionic, and pure autonomic failure (PAF) where it is postganglionic [Polinsky 2002]. Our hypothesis was that, in normal subjects, CO_2 inhalation would act as a gasp stimulus as with air inhalation, also resulting in cutaneous vasoconstriction. In MSA and PAF where severe sympathetic dysfunction is present, we anticipated attenuation of the gasp response to either stimulus. Finally, in PAF where the postganglionic lesion site results in

supersensitivity to vasoactive substances, the direct vasodilatory effects of CO_2 would be unmasked resulting in cutaneous vasodilatation with inhalation of CO_2 as oppose to vasoconstriction expected in normal subjects. We measured continuously the systemic haemodynamic and regional skin blood flow responses before and after a single breath of CO_2. Comparisons were made with inhalation of air.

Subjects

A total of 23 subjects were studied (5 healthy controls, mean age 47+/- 6.8; 9 MSA, mean age 57.1+/- 3.1; 9 PAF, mean age 66.4). The diagnosis of MSA and PAF was made using established criteria [Gilman et al 1999, Mathias 2004]. All had documented sympathetic and parasympathetic cardiovascular autonomic dysfunction (Table 3.7.3)

Methods

All tests were conducted between 1000 and 1500 hours in a temperature (24 +/- 1^0C) controlled laboratory. Upon arrival at the testing unit, a 20G (Venflon) intravenous line was inserted into a subject's right antecubital fossa and kept patent with 0.9% saline. A Portapres II device was applied to the middle finger of the right hand to provide continuous systolic and diastolic blood pressure (SBP and DBP) and heart rate (HR) readings. Subsequent calculation of cardiac output (CO), total peripheral resistance (TPR) and stroke volume (SV) was performed using Modelflow analysis [Wesseling *et al* 1995; Langewouters *et al* 1998]. These haemodynamic indices were calculated at 5 time points:

v) Baseline

vi) At time of peak SkBF change after air inhalation

vii) Baseline

viii) At time of peak SkBF change after CO_2 inhalation

A Laser Doppler (Perimed) [Khan *et al* 1991; Kooner *et al* 1991] probe was taped over the pulp of the left index finger with one additional probe over the left anterior forearm.

Skin blood flow (SkBF) was measured by laser Doppler flowmetry [Perimed Periflux 5000/5010 solid state diode laser set at 780nm with a 1mW maximal power output at the probe tip]. Fibre optics permit the Laser Doppler probe to shine a laser light directly at the skin surface. Light is reflected back to the recording element of the probe from red blood cells in the skin capillaries. Light reflected back to the probe has its wavelength altered by the Doppler shift caused by the movement of the red blood cells relative to the probe. This altered wavelength is then used to calculate an arbitrary perfusion rate ("Perfusion units"; PU), rather than absolute flow, which is then visualised as a real-time trace. Cutaneous vasoconstriction

will therefore be shown as a reduction in perfusion units relative to baseline perfusion. The forearm probe was used as a control to exclude movement or temperature artefact, as forearm skin blood flow is under thermoregulatory rather than "emotional" sympathetic regulation, as present in the fingertip skin [Johnson et al 1995; Saad et al 2001].

The subjects remained seated throughout the study. After 30 minutes of quiet rest physiological monitoring and blood sampling commenced. A 10L silicone 'Douglas' bag (Hans-Rudolph, Kansas City, Missouri, USA) was filled with the gas mixture (35% CO_2 and 65% O2; BOC Gases, Guildford, Surrey, UK). Inspired vital capacity (VC) was determined using an analogue flow meter (Ohmeda, USA) that was attached via a 3-way valve to a 3cm diameter silicone mouth-piece (Hans-Rudolf, Germany) at one end and the 10L silicone bag containing the CO_2 at the other end. The subject took a single VC breath of room air through the mouthpiece whilst occluding their nose with a nose-clip. Baseline inspired VC with a single breath of air was measured and a test breath of room air was considered adequate if it was more than 80% of this baseline breath [Kaye *et al* 2004]. Once the SkBF had returned to baseline a single VC breath of the CO_2 was taken in an identical manner to that for room air. Venous blood sampling for catecholamines was performed at baseline and at 2, 10 and 20 minutes after CO_2 inhalation. Plasma levels of Noradrenaline (NA), Adrenaline (Ad) and Dopamine (DA) were measured at baseline and at 2, 10 and 20 minutes post- CO_2. Electrochemical detection for NA, Ad and DA concentration was performed using high-performance liquid chromatography [Smedes *et al* 1982; May *et al* 1988; Mathias *et al* 1990]. The ethics committees of the National Hospital for Neurology and Neurosurgery, and St Mary's Hospital approved the study and each subject provided written informed consent prior to participation.

Statistics: From the individual perfusion unit data, the calculation of percentage reduction in SkBF following inhalation of air or CO_2 was calculated as shown in Figure 3.7.5. This

allowed intra- and inter-groups comparisons. Skin Regional Vascular Resistance (SkRVR) was calculated from the equation: SkRVR = Mean Arterial BP/ SkBF [Lehtipalo *et al* 2000]. The percentage change in SkRVR before and after inhalation of air or CO_2 was then compared between groups. Results (both for SkBF baseline and reduction ratios after gasp of air or CO_2) were compared by ANOVA with Newman-Keuls post-hoc testing. Statistical significance taken as $p<0.05$. All mean data values given +/- SE.

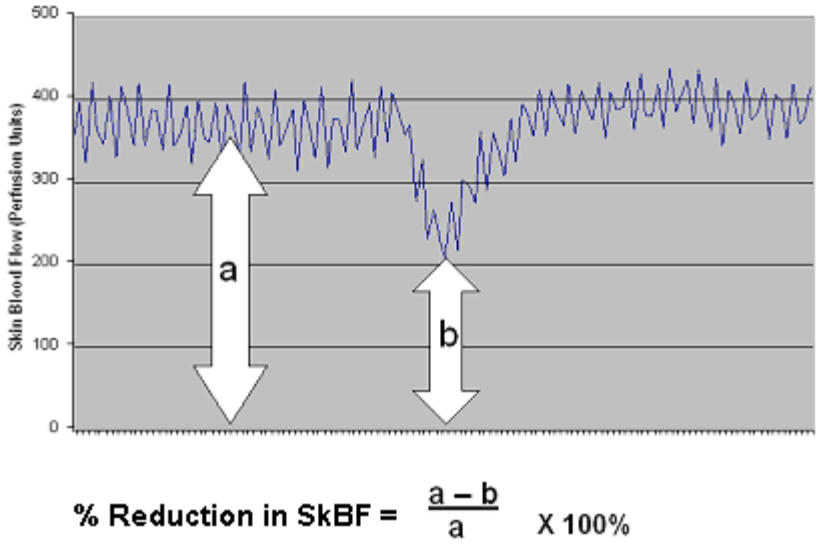

Figure 3.7:2 Calculation of % reduction in Skin Blood Flow (SkBF) following inhalation of air or CO_2

Results

Skin blood flow and vascular resistance:

Baseline:

Baseline SkBF in the forearm was similar in all 3 groups, and did not change significantly in any group following inhalation of either air or CO_2. Baseline finger SkBF was significantly

lower in PAF compared to controls (p=0.000006) and MSA (p=0.026) (Table 3.7.3). MSA baseline SkBF in the finger was lower than in controls (p=0.002).

Post-air inhalation:

In controls there was a reduction in finger SkBF beginning within 3.1+/-0.3 seconds of inhalation and lasting for 87.6 +/-28.9 seconds. In MSA the reduction in SkBF was less than controls ($p<0.012$). In PAF the reduction in SkBF was smaller than in either controls ($p<0.0006$) or MSA ($p<0.04$).

Post- CO_2 inhalation:

In controls there was a reduction in finger SkBF (onset: 2.9 +/- 0.4 seconds after CO_2; duration: 91 seconds +/- 41.3 seconds). (Figures 3.7.6 and 3.7.7). MSA showed a non-significant reduction in finger SkBF after CO_2 (Figure 3.7.7). In MSA the reduction in SkBF to either air or CO_2 occurred over a similar time course to that seen in controls (for air: onset after 2.9 +/-0.3 seconds and duration of 87.6 +/-28.9 seconds). PAF showed no decrease in finger SkBF following CO_2, but instead exhibited a transient *increase* in SkBF in a well defined peak at 45 seconds (+/- 1.1 seconds) post inhalation (Figures 3.7.6 and 3.7.7). Although no increase in SkBF had been noted in controls following CO_2, in MSA there was a variable response with a small increase in SkBF in 5/9 MSA subjects (+28.5% +/- 12.4), which was less than that seen in PAF ($p<0.06$).

Blood pressure, heart rate, cardiac output, stroke volume and total peripheral resistance 43-47 seconds post-inhalation of air or CO_2:

The time point 43-47 seconds post-inhalation corresponded with the approximate mid-SVR response in all 3 groups, as well as encompassing the time period (45 +/- 1.1 seconds) at which the peak of fingertip vasodilatation was seen in PAF following CO_2. The haemodynamics during this time period therefore was assessed in detail both after air and CO_2

inhalation. Following air inhalation during this period there were no significant changes in SBP, DBP, HR, TPR or CO in any group.

In controls following CO_2 inhalation, there was a significant increase in SBP, DBP and HR at 43-47 seconds post-inhalation compared to baseline (ΔSBP: 43.7(+/-30.4mmHg); ΔDBP: 11.3(+/-11.6mmHg); ΔHR: -12.5(+/-18.2bpm)). This was associated with a small increase in TPR (1.3 (+/- 0.6) to 1.6 (+/-0.7MU)). CO was reduced, although this did not reach significance (4.9 (+/-2.1) to 4.3 (+/-1.4).

In MSA, 43-47 seconds post-inhalation following CO_2 inhalation, there was no significant increase in SBP (ΔSBP: 4.5(+/-25mmHg), DBP (ΔDBP: 3.3(+/-9.2mmHg), or HR (ΔHR: 3.2(+/-11.5bpm) (Table 3.7.5). There was no change in TPR or CO.

In PAF, 43-47 seconds post-CO_2, there was no change in SBP (ΔSBP: -3.3 (+/-11.3mmHg), DBP (ΔDBP: -2.9(+/-5.7mmHg), or HR (ΔHR: 1.7(+/-4.5bpm) (Table 3.7.5). TPR was reduced after CO_2 (2.3 (+/-0.8) to 1.8 +/-0.5MU) and CO increase did not reach significance.

The change in SkRVR from baseline in controls before and after inhalation of air or CO_2 was similar, being 661% and 594% after air and CO_2 respectively; for MSA these respective values were: 240% and 107%; and for PAF: 155% and -51%.

Plasma Noradrenaline:

Baseline:

Baseline plasma NA levels were significantly lower in PAF (126.8+/-19.8pg/ml) than in controls (288.4+/-31.3pg/ml) or MSA (246.9+/-13.1pg/ml) ($p<0.001$).

Post- CO_2 inhalation:

In controls there was a significant increase in NA levels ($p<0.05$), first reaching significance 2 minutes post-inhalation (Figure 3.7.8). NA levels following CO_2 inhalation did not increase in either MSA or PAF.

Plasma Adrenaline:

Baseline:

Baseline Ad levels were significantly higher in controls (44.2 +/- SE 5.9 pg/ml) than in MSA (20.8 +/-SE 3.6 pg/ml): $p<0.05$. In PAF, Ad levels were undetectable at baseline.

Post- CO_2 inhalation:

In controls there was a significant increase in Ad levels ($p<0.05$), peaking at 90.4 +/- SE 14.0 at 10 minutes post-inhalation. Plasma levels of Ad following CO_2 inhalation did not increase in either MSA or PAF.

Plasma Dopamine:

In 2/5 controls, 5/9 MSA and 9/9 PAF, DA levels were undetectable at baseline. There was no significant change in any group Post- CO_2 inhalation.

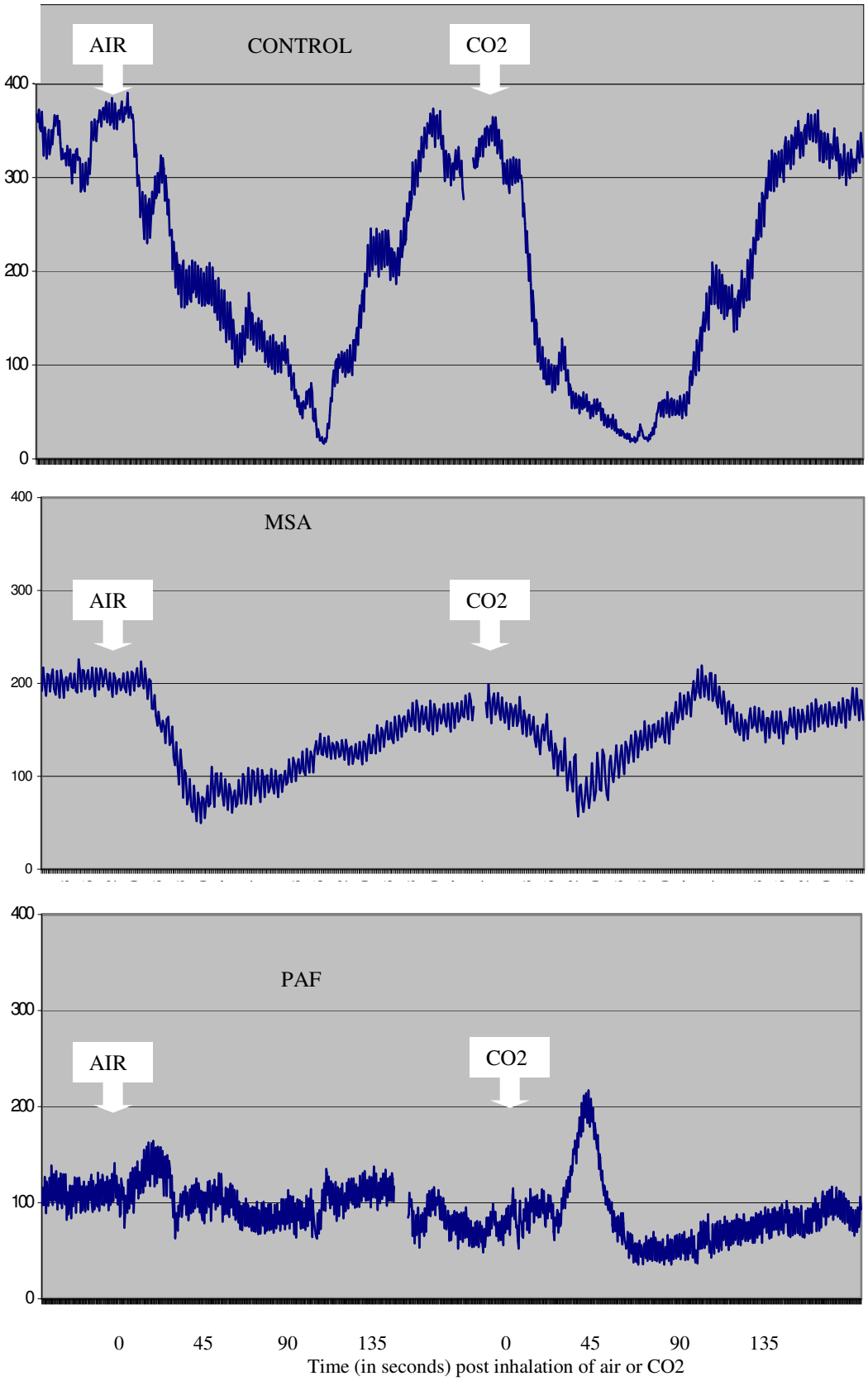

Figure 3.7:3 Skin Blood Flow changes following inhalation of air or 35% CO2 in control subject (top), PAF (middle) and MSA (lower). Y-axis shows skin blood flow in Perfusion Units.

	Baseline	% Change in SkBF after air	% Change in flow after CO_2
Controls	278.8 (18.2)	-83.8 (3.9)	- 79.0 (5.6)
MSA	143.7 (29.5)	-52.7 (8.6)	- 4.8 (15.9)
PAF	59.8 (14.6)	-31.7 (14.6)	+ 151.2 (40.2)

Table 3.7:3 Fingertip Skin Blood Flow changes in perfusion Units (+/- SE) after single breath of air or CO_2

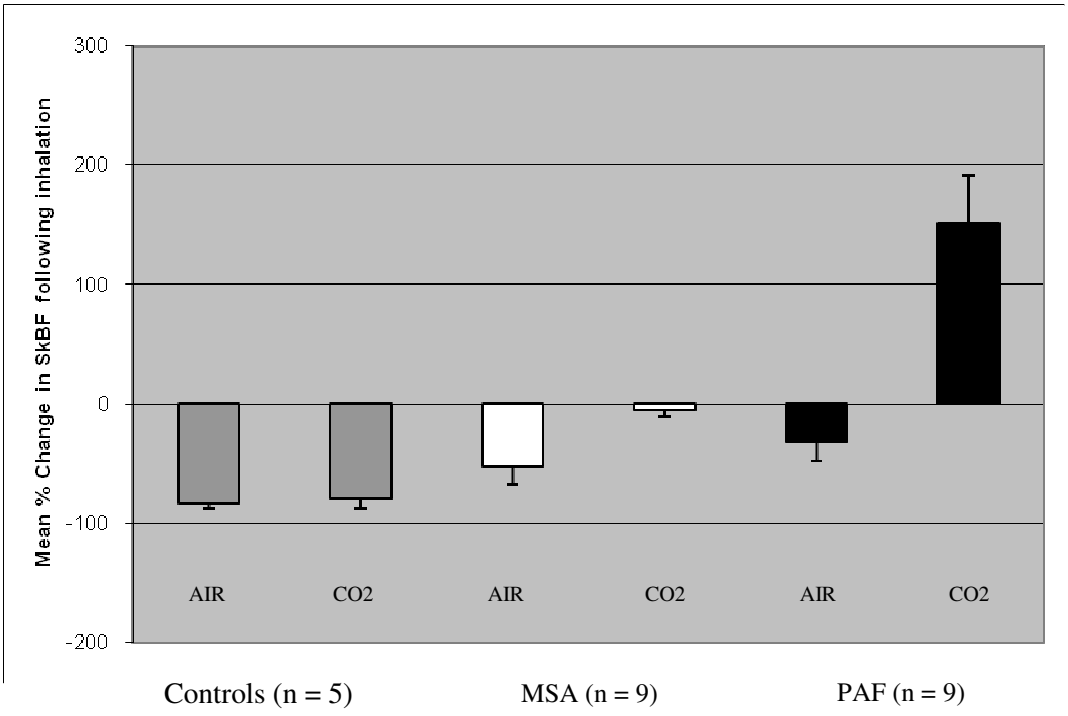

Figure 3.7:4 Fingertip Skin Blood Flow changes following inhalation of air or 35% CO_2 in control subjects (grey), MSA (white) and PAF (black). Error bars = SE. Y-axis shows % change in SkBF relative to baseline.

	Controls	MSA	PAF
SBP (mmHg) before air	124.9 (11.6)	120.4 (6.0)	140.0 (14.1)
SBP (mmHg) after air	132.0 (12.7)	121.4 (6.4)	138.0 (12.8)
ΔSBP (mmHg) after air	7.1 (4.1)	1.1 (1.9)	-2.2 (5.1)
DBP (mmHg) before air	68.3 (4.1)	69.2 (5.9)	77.7 (4.9)
DBP (mmHg) after air	69.9 (4.9)	70.1 (6.2)	78.4 (5.3)
ΔDBP (mmHg) after air	1.6 (1.4)	1.0 (1.0)	0.7 (1.8)
HR (bpm) before air	62.0 (4.1)	80.7 (6.0)	73.4 (6.5)
HR (bpm) after air	57.1 (7.0)	81.3 (6.3)	74.7 (6.2)
ΔHR (bpm) after air	- 4.9 (3.2)	1.1 (0.8)	1.3 (1.0)
SBP (mmHg) before CO2	122.5 (12.3)	132.7 (8.8)	128.8 (13.7)
SBP (mmHg) after CO2	165.9 (17.9)	135.9 (6.1)	127.7 (13.9)
ΔSBP (mmHg) after CO2	43.7 (13.6)	4.5 (8.2)	-3.3 (3.8)
DBP (mmHg) before CO2	66.9 (3.9)	76.2 (7.2)	75.7 (4.4)
DBP (mmHg) after CO2	78.2 (6.9)	79.4 (7.6)	72.9 (4.8)
ΔDBP (mmHg) after CO2	11.3 (5.2)	3.3 (3.1)	-2.9 (1.9)
HR (bpm) before CO2	60.9 (3.9)	77.1 (4.1)	66.8 (2.7)
HR (bpm) after CO2	48.4 (7.9)	80.2 (6.0)	64.6 (2.8)
ΔHR (bpm) after CO2	-12.5 (8.1)	3.2 (3.8)	1.7 (1.5)

Table 3.7:4 Portapres change in SBP, DBP and HR (+/- SE) 43-47 seconds post-inhalation of air or CO2

Discussion

We observed that the acute inhalation of air in controls, MSA and PAF, resulted in no significant changes in BP. However, following inhalation of 35% CO_2, there was a pressor response in all groups, with the magnitude and speed of onset being greater in controls than in PAF and MSA. The pressor response following identical application of 35% CO_2 has

previously been described in normal subjects, being related to activation of central sympathetic pathways [Kaye *et al* 2004]. Forearm SkBF was unchanged. However, finger SkBF responded differently between normal subjects, MSA and PAF. Whilst inhalation of air reduced SkBF in the fingertip pulp in all groups, this response was greatest in controls and least marked in PAF, with significant differences between the 3 groups. Following inhalation of CO_2 controls there was a reduction in SkBF similar to that seen after inhalation of air; in MSA the reduction in SkBF was less than in controls. However, in PAF a marked *increase* in SkBF followed inhalation of CO_2, at 45 seconds post-inhalation.

Following CO_2, the period between 43-47 seconds post-inhalation corresponded both to the midpoint of the SkBF reduction seen in controls and to the peak of increased SkBF seen in PAF. Detailed analysis of systemic haemodynamics over this time period indicated that in the controls there was a significant increase in SBP, DBP, and HR, with a non-significant increase in TPR. Neither MSA nor PAF showed significant changes in BP at this time 43-47 seconds post-CO_2, as their peak pressor effect, albeit diminished, occurred later than controls. In PAF there was a non-significant reduction in TPR and increase in CO at the 43-47 seconds time point, not seen in controls or in MSA. This suggests vasodilatation at the same time that the Laser Doppler probe was recording an increase in finger SkBF. In contrast, there was no corresponding change in SkBF measured in the forearm in any group. This is in keeping with human studies following chronic surgical sympathectomy that suggest no major disruption in vascular function in the forearm [Eisenach *et al* 2002]. There thus was evidence for selective vasodilatation of cutaneous vascular beds in PAF following CO_2 inhalation.

Plasma Noradrenaline Responses

In controls, plasma NA levels were within the normal range and significantly increased 2-minutes post-CO_2 inhalation. This is likely to reflect an increase in sympathetic nerve activity following CO_2. In MSA, baseline plasma NA levels were not significantly different to controls, in keeping with previous observations [Mathias et al 1990] and did not increase

following CO_2. In PAF, baseline plasma NA levels were lower than in controls and MSA, again consistent with the known postganglionic lesion site in PAF [Polinsky 2002], and did not change following CO_2. These results provide additional evidence for the inability of CO_2 to increase sympathetic activity in MSA and PAF. The plasma NA response to CO_2 did not differentiate MSA and PAF, confirming that both pre- and postganglionic pathways are required to elicit an increase in NA following CO_2 inhalation.

Fingertip Skin Blood Flow Responses

Following inhalation of air there was a prompt and marked reduction in SkBF in controls; this was less marked in MSA, with an even smaller reduction in PAF. With inhalation of CO2, a similar reduction in SkBF to that seen with air was observed in controls. In MSA, the mean reduction in SkBF following CO_2 was less than in healthy controls. In PAF, there was no reduction in SkBF following CO_2; instead there was a transient increase in SkBF 45 seconds post-inhalation. This transient effect thus occurred when controls were still displaying a marked reduction in SkBF following CO_2 inhalation. In controls we speculate that inhalation induced a marked cutaneous vasoconstriction to inhalation of air or CO_2. In extensive post-ganglionic sympathetic denervation, as in PAF, there is marked impairment of cutaneous vasoconstrictor response to stimuli that increase sympathetic activity [Asahina *et al* 2002; Young *et al* 2006]. In PAF the increase in arterial pCO_2 following CO_2 inhalation probably unmasked a transient cutaneous vasodilatation in PAF as it arrived in the fingertip microcirculation. In MSA, where sympathetic impairment may not have been as extreme as in PAF, these effects were not observed.

Total SkBF can vary enormously, between 200ml/min and 8 litres/min with maximal vasodilatation [Rowell 1993]. Changes in SkBF thus have the potential to change BP and TPR. However, SkBF changes were only found at the fingertip and not the forearm in our study suggesting a more selective vascular bed response.

The mechanisms by which CO_2 causes cutaneous vasodilatory properties warrants discussion, as it can cause cutaneous vasodilatation by a non-neural mechanism [Bullard 1964; Ito *et al* 1989]. In humans, topical application of CO_2 enriched water to the hands and feet leads to cutaneous vasodilatation [Irie et al 2005]. In the rat, cutaneous vasodilatation in the paw is also seen with topical CO2, and this effect is preserved following surgical sympathectomy [Nishimura *et al* 2002; Schnizer *et al* 1985; Ito *et al* 1989]. Continuous breathing of CO_2, at concentrations between 5-30%, reduces forearm blood flow in healthy volunteers, but *increases* blood flow if the ulnar, radial and median nerves are initially treated with a local anaesthetic [Blair *et al* 1959]. The circulation time of CO_2 (following inhalation of 35% CO_2 in 65% O2) is consistent with a local vascular effect of CO_2 in PAF. In normal subjects, radial artery blood gases have been measured before and after a single breath of 35% CO_2 in O_2. Pre-CO_2 levels of 40 mmHg pCO_2 rose to over 90mmHg after 20 seconds and returned to baseline levels 50 seconds after CO_2 [Ponto *et al* 2002]. Arterial blood gases sampled at greater frequency in normal subjects following a single maximal breath of 35% CO_2 in O_2, indicate that a bolus of pCO_2 (up to 120mmHg) reaches the radial artery between 20 and 35 seconds following inhalation [Ponto *et al* unpublished data].

Limitations

In PAF, the baseline finger SkBF was lower than in MSA and controls. Skin temperature was not measured but this was unlikely to account for this observation, as there was no difference in baseline forearm SkBF between PAF, MSA and controls. In addition, all measurements were performed in a temperature-controlled laboratory (24 +/- 1^0C) after 30 minutes of baseline. Whilst acute peripheral sympathetic denervation causes an increase in SkBF in humans [Lehtipalo *et al* 2000], chronic peripheral denervation (as is expected in PAF) reduces skin perfusion [Barcroft *et al* 1949; Bentzer *et al* 1997]. Laser Doppler flowmetry provides relative and not absolute measures of SkBF, and so direct comparisons of absolute levels

alone were not considered, and comparisons between the groups were made on the basis of percentage change compared with baseline.

The mean ages of our subjects differed, being 47 +/- 6.8 years in controls, 57.1 +/- 3.1 years in MSA, and 66.4 +/- 2.1 years in PAF. This reflects the age of onset of disease in these disorders, and the shortened life expectancy in MSA. A previous study suggested that SkVR to inspiratory gasp and cutaneous cold was impaired in older when compared to young healthy subjects; however, the age difference was far greater than in our study, being 68 ± 4 compared to 26 ± 5 years [Khan et al 1992]. In addition, the mean SkVR in the older subjects to gasp (51%) was greater than in our PAF of a similar age (31.7 %). Thus it seems age difference alone does not explain the lower SkVRs seen with inhalation in our PAF subjects. Similarly, it seems unlikely that the supine baseline level of blood pressure is important in the SkVR following air inhalation, as baseline BPs were similar in all 3 groups.

Conclusion

Our results indicate that there was a pressor response following CO_2 in MSA and PAF; however this was significantly smaller in magnitude and occurred later than in controls. There was no difference in the pressor responses seen in MSA or PAF. NA levels at baseline were similar to those previously reported in healthy subjects, MSA and PAF. Following CO_2 NA rose within 2 minutes in controls but showed no change in either MSA or PAF.

In Controls there was a similar reduction in fingertip SkBF following either air or CO_2. In PAF the reduction in fingertip SkBF following air was markedly attenuated, and a transient increase in SkBF was found following CO2. In controls inhalation of CO_2 may cause transient vasodilatation, but that this is not recorded because of the marked reduction in SkBF in response to inhalation itself (onset 18.8 +/- 2.7 seconds after CO_2 inhalation, lasting 87.6 +/- 12.9 seconds). Because this gasp reflex is impaired in PAF, but CO_2 dilation occurs via non-

neural mechanisms, the direct vasodilatation effect of CO_2 may be unmasked in this group. Changes in Fingertip SkBF with CO_2 may provide a useful non-invasive test to distinguish pre- from post-ganglionic lesions, especially in the early stages of MSA when the only clinical features may be those of autonomic failure.

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3.9: Conclusions

The studies performed in this thesis explored the differentiation of MSA and PAF based on the on the cardiovascular responses to various stimuli. These groups allowed comparison between a pre-ganglionic (MSA) and essentially post-ganglionic (PAF) lesion model of autonomic dysfunction. Although there is pathological evidence of some central involvement in PAF the overall evidence suggests a markedly predominant post-ganglionic pathophysiological condition. Inclusion bodies staining for α-synuclein have been identified in autonomic nerves in the epicardial fat and capsular nerves of the adrenal and parasympathetic nerves in the bladder wall, but not in the cerebral cortex or dorsal motor vagal nucleus. [Kaufmann et al 2001]. Two autopsy cases suggested moderate neuronal loss in the substantia nigra, locus ceruleus and intermediolateral cell columns of the spinal cord; however in one of these cases previous embalming cast doubt on the relevance of a perceived moderate loss of neurons in these anatomical areas [Roessmann et al 1971]. Functional imaging has shown only one out of seven PAF to have evidence of central abnormalities of the nigrostriatal dopaminergic system, with a single case report suggesting straital involvement of the caudate and putamen with SPECT [Brooks et al 1990; Compta et al 2006]. The subtle and highly localised abnormalities of cerebral grey matter appear to be a compensatory change due to the post-ganglionic lesion sites rather than a primary area of pathology [Critchley et al 2003]. Whilst some doubt thus remains about the degree of central involvement in PAF, both pathological and physiological studies strongly the support the peripheral, postganglionic dysfunction of PAF [Bradbury and Eggleston 1927; Roessmann et al 1971; Ingelghem et al 1994; Mathias 2004].

Before the studies comprising this thesis were performed it was known that both MSA and PAF demonstrated profound OH, and shared other aspects of autonomic dysfunction. It was

known that in MSA there was clear progression, with death occurring within a decade of diagnosis in most subjects [Ben-Shlomo et al 1997]. By contrast PAF subjects may well show a normal life expectancy, and yet the differentiation from MSA in the first few years of presentation is often very challenging. This not only has implications for prognostication, but increasingly for inclusion of subjects in therapeutic trials [Schroeder et al 2005; Geser et al 2005].

The first study of this thesis formed the largest study to date of the cardiovascular effects of pressor stimuli in MSA and PAF. There was no significant difference in baseline haemodynamics between the groups. Haemodynamic responses were attenuated in both groups to all pressor stimuli. In MSA HR increased following each pressor stimulus, whilst BP increased with IE and CP but not MA, which led to a fall in SBP and DBP. In PAF HR increased with CP and MA, but not with IE. There was no significant BP change with pressor stimuli in PAF subjects. Comparing groups showed a greater increase in HR following IE and CP in MSA than PAF and a greater increase in SBP in MSA following CP. SBP and DBP *decreased* to a greater extent in MSA following MA.

These results are helpful in aiding interpretation of MSA and PAF. Autonomic function tests are a routine part of the work up of autonomic failure at presentation. Whilst the results of this study do not allow clear-cut differentiation, the results of the SBP change to cold pressor stimulation and to mental arithmetic in particular may provide suggestions to the underlying diagnosis. The *reduction* in SBP with MA in MSA is somewhat suprising, and may be related to peripheral vasodilation related to differential impairment of A and NA.

As with pressor testing the intravenous clonidine test is widely used to help distinguish MSA subjects. In these subjects the preganglionic lesion site includes hypothalamic involvement resulting in a suppression of the normal increase in plasma growth hormone (GH) response with the selective α_2-adrenoceptor agonist clonidine. GH testing is not universally available

however & results can take a significant length of time after testing, unlike the haemodynamic results which are immediately obtainable. The cardiovascular response to clonidine in MSA and PAF had not been well established however. The clonidine study in this thesis is the largest published series to focus on this aspect. Clonidine infusion reduces BP and HR in both MSA and PAF, but to a greater extent in MSA. The greater vasodepressor action of clonidine in MSA suggests that there is partial preservation of brainstem sympathetic outflow pathways in MSA, and may reflect its action at sites in the brainstem and spinal cord that were in part functionally preserved in MSA. Despite similar relative degrees of NA reduction after clonidine, the vasodepressor effect of clonidine was attenuated in PAF compared with MSA. This attenuation in PAF may represent a pressor effect of clonidine mediated by vasoconstriction resulting from activation of postsynaptic peripheral α2 adrenoceptors rather than α1 adrenoceptors [Kiowskiw *et al* 1983].

We have thus shown a differing BP response to clonidine, with a vasodepressor effect occurring earlier and to a greater extent, in MSA than in PAF. This occurs despite a similar relative reduction in NA levels in both groups following clonidine. The attenuation of BP drop in PAF compared with MSA may reflect a denervation supersensitivity to clonidine in PAF, resulting in vasoconstriction via peripheral α2 adrenoceptors. The greatest differential occurred with the SBP change from baseline at 30 minutes post-clonidine. Although there was some overlap in results between MSA and PAF, our results suggested that increases in the SBP over baseline at 30 minutes were specific to PAF whilst large falls in SBP at this time point would be more in keeping with a diagnosis of MSA. Thus at this time point reductions in SBP greater than –32 mmHg, which occurred in 17/39 MSA subjects, were 100% specific for MSA but at low sensitivity (44%). At this same time point increases of SBP from baseline of ≥ 0 mmHg, which occurred in 9/19 PAF subjects, were 100% specific for PAF with a low sensitivity of 47%. Our results therefore suggest that 100% specificity is possible in just under half of our cases of MSA and PAF. As such we suggest that analysis of haemodynamic changes following this standardised test provides potentially useful information to aid the

differentiation of MSA and PAF. These BP differences may thus reflect the underlying lesion site in MSA and PAF, and the haemodynamic data following clonidine infusion may help differentiate these conditions.

This thesis has added important data to the growing knowledge about the pressor effects of oral water in autonomic failure. We confirmed the finding of a significant pressor effect after oral ingestion of 480-500ml distilled water in both MSA and PAF. The studies of this thesis have been the first to directly compare detailed haemodynamic responses to oral water in MSA and PAF. There was a more rapid onset of pressor response in PAF although ultimate magnitude of response appeared similar in both groups. In PAF the pressor effect occurred sooner (within 5minutes) unlike MSA where it occurred 13 minutes after ingestion. The pressor response to water is known to be dependant on the volume ingested, and may be the result of gastric distension increasing sympathetic nerve activity by reflex mechanisms, as has been described in normal subjects [Rossi et al 1998]. This response has been claimed to be dependant on residual sympathetic activity, and should therefore be minimal in PAF, and greater in MSA. This would be consistent with the findings that the pressor actions of yohimbine, an α_2 antagonist that is dependant on sympathetic activity, were related to the subject's pressor response of water [Jordan *et al* 2000]. However, this was not the case in our studies as the response was as great in PAF with a more rapid onset than in MSA. An alternative may be that the pressor response in PAF was related to denervation supersensitivity, which has been well documented in this group, and is greater than in MSA [Polinsky 2002]. It is possible that in PAF, even a small amount of NA released could have acted on supersensitive receptors, although the release of other vasoconstrictor substances, including factors such as endothelin, which may then exert the pressor response. These possibilities may explain the rise in peripheral resistance found in this study and the delay (minutes) between ingestion and pressor action.

For the first time the pressor response to oral water was shown to be associated with symptomatic improvement, highlighting the likely therapeutic value of oral water in MSA and PAF. As a result of these studies we suggested to both MSA and PAF subjects that cautious use of oral water could be useful in helping controlling OH. As the pressor effect may not last for much longer than an hour, and the effects of repeated dosing are unknown, we would suggest that this technique only be attempted a few times each day. Clearly much further work is required in this exciting area, not least of which includes elucidating the underlying aetiology. As suggested in the discussion of Chapter 3.4: 'Cardiovascular Responses to Water Ingestion in MSA and PAF' the implications of this study could be of considerable importance to a wider selection of the population if the tantalizing suggestions of a mild pressor response to water ingestion in older 'healthy' subjects is borne out by future larger studies.

In MSA and PAF head up tilt (HUT) is associated with significant orthostatic hypotension. On tilt reversal back to supine posture there may be an initial overshoot in the blood pressure in PAF not MSA, resulting in transiently higher supine values than those obtained prior to HUT. This had previously been suggested as a possibility in an earlier study focussing on the haemodynamics of HUT [Chandler & Mathias 2002]. The studies in this thesis confirmed the finding of a transient SBP overshoot on tilt-reversal in PAF but not MSA. Arginine Vasopressin (AVP) is a hormone produced in the hypothalamus and released from the posterior pituitary in response to head up posture and may be important in helping to maintain BP in the upright position by virtue of its vasopressor actions. As a previous study had suggested that this AVP response may be preserved in PAF but not in MSA (where central lesion sites include the hypothalamus), this study sought to ascertain if there was a difference in the BP overshoot between MSA and PAF, and if so whether this was related to the AVP response. The working hypothesis was that HUT would result in a significant AVP release in PAF but not MSA and that on subsequent return to supine, the residual AVP action would cause an exaggerated pressor response in PAF, in whom there is a known pressor

supersensitivity and the most likely mechanism of increased TPR based on Modelflow analysis of the Portapres data. Although the results showed a lower AVP level in MSA with a suggestion of a greater increase in PAF following HUT, there was not a significant correlation between AVP levels and SBP overshoot on tilt reversal. The confirmation of the existence of SBP overshoot in PAF however has important clinical implications. Given the relatively good long term prognosis in PAF, recently concern has been raised that the periods of marked hypertension (such as may occur on tilt reversal) may be associated with end organ damage [Vagaonescu et al 2000]. Thus simple steps such as gradual return to supine from standing may help reduce the peaks of SBP seen in these studies on tilt-reversal.

The Laser Doppler has been used for decades to investigate cutaneous blood flow, but its use in primary autonomic failure is relatively novel. In Chapters 3.6 (Vasomotor Responses to Sympatho-excitatory stimuli in MSA and PAF) and 3.8 (Acute Cardiovascular effects of CO_2 Inhalation in MSA and PAF) the Laser Doppler was used to explore cutaneous blood flow from the finger pulp in MSA and PAF. Healthy control subjects demonstrated marked SkVRs on the finger pulp to each of the stimuli of a magnitude similar to those seen in previous studies. In MSA SkVRs to inspiratory gasp on the finger pulp were reduced relative to controls. In PAF SkVRs were reduced relative to controls or MSA. The magnitude of SkVR response to gasp and cutaneous cold in PAF was significantly less than in healthy controls. In addition, the magnitude of the response in PAF was significantly less than MSA for inspiratory gasp. PAF showed a decreased SkVR response to all 4 stimuli, the response being significantly less than controls (for inspiratory gasp and cutaneous cold) or MSA (inspiratory gasp). The decreased responses in PAF may reflect the extensive postganglionic sympathetic denervation seen in this group. When inhaled CO2 was utilised instead of air, healthy controls showed a similar marked decrease in SkVR as with inhaled air. In MSA the SkVR responses to both air and CO2 were attenuated relative to controls. In PAF however, there was only minimal SkVR with inhaled air. Most interesting however was the response to inhaled CO2 where a marked transient cutaneous *vasodilatation*. In this thesis I have suggested that this

novel finding may reflect the fact that the inhaled CO2 may act locally in the cutaneous vascular bed as a vasodilator with the normal vasoconstriction caused by inhalation itself being attenuated by the marked post-ganglionic sympathetic denervation in PAF. If confirmed in larger studies this could provide the basis for a rapid test to help differentiate MSA and PAF. Finally the Haemodynamic actions of inhaled CO2 were also explored. Both MSA and PAF subjects showed a pressor effect following a single breath of 35% CO2, although this was less marked and later in onset than in healthy controls. It is likely that the pressor action of CO2 requires both pre- and postganglionic pathways based on the results of this study.

Overall I have aimed in this thesis to better delineate the pathophysiology of MSA and PAF based on their lesion site differences. In doing so I hope to have helped suggest improved testing for these conditions to help prognostication, clinical management and ultimately aid selection for future trials. I feel that of particular importance has been my systematic analysis of the differing responses to standard autonomic tests (such as pressor responses and intravenous Clonidine as well as the suggestion of more novel methods of distinguishing these patient groups (such as SBP overshoot on tilt reversal and with use of the Laser Doppler to assess finger pulp cutaneous blood flow). Finally I feel that the cardiovascular effects of water will be an exciting area of development over the next decade with possible implications for medicine beyond that of autonomic dysfunction.

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Appendix I: Summaries of Materials, Reagents and Suppliers

Multi-Purpose Tilt Table:

An electronically operated, hydraulic 63 x 190 cm tilt table equiped with lockable casters and stabilisers for safety in use. Maximum load is 180Kg, distributed evenly. Additional security in upright positions provided by a foot rest and body belts (2X narrow Belts, 1X Wide Belt), secured by means of Velcro strips threaded through the side chrome rails of the table. Manually operated tilt and elevation controls allow raising or lowering of the table. In addition, these controls permit tilting upright and back to horizontal (degree of tilt estimated by means of dynamic scale under bed).

Huntleigh Akron
1 Farthing Road,
Ipswich
IP1 5AP

Tel: 01473 461042
Fax: 01473 462924

Portapres Model-II:

Portpres is a class B equipment according to EN 60601-1.

Dimensions:

Main Unit:	140x90x40mm	weight 320 grams
Pump Unit:	100x90x40mm	weight 345 grams
Fronted Unit:	65x50x30mm	weight 350 grams (incuding fronted cable)
Control Unit:	145x90x35/56mm	weight 590 grams
Waist belt	1200x110x40mm	weight 270 grams
AC adapter	125x75x40mm	weight 410 grams
Finger Cuff……..		weight 25 grams

Measurement Method:	Arterial volume-clamp (Penaz) and physiocal (Wesseling) methods
Cuff Pressure:	Maximum 350mmHg

BP accuracy:	Within 1%
Data Storage:	In-built 10Mb Flash memory card (63.5 hours of recording)
Height accuracy:	Within 1%
Interbaet accuracy:	10ms
Control Unit Display:	2X16 characters LCD
Serial Port:	RS232C
Pump Unit:	Maximum pressure 400mmHg; Maximum air flow 60litres/minute
Environment limits:	0^{o}C-35^{o}C; Humidity 5-90%; Atmospheric Pressure: 700-1100 hPa

Electrical Specifications:

Power Requirements:	12 V DC, 0.2 A standby, 0.4 A measuring
Main Unit and Poump Unit fueses:	Electronic
AC adaptor:	100 to 240 V A; 47 to 65 Hz, 30 W
Protection against water contamination:	IP20

Power dissipation: In main Unit 2 W; In Pump Unit 2 W; In fronted Unit 1 W; In Finger cuff <50mW

TNO-TPD Biomedical Instrumentation
Attn. Mr. G.J. Langewouters,
Academic Medical Centre K2-228
Meibergdreerf 9
1105 AZ Amsterdam
The Netherlands

Phone: + 31 20 566 3343
Fax: + 31 20 697 6424
Email: bmi@tpd.tno.nl

Appendix II: Consensus statement on the diagnosis of multiple system atrophy

Consensus Report

Clinical Autonomic Research 1998, **8**:359–362

Consensus statement on the diagnosis of multiple system atrophy

Sid Gilman, Phillip Low, Niall Quinn, Alberto Albanese, Yoav Ben-Shlomo, Clare Fowler, Horacio Kaufmann, Thomas Klockgether, Anthony Lang, Peter Lantos, Irene Litvan, Christopher Mathias, Eugene Oliver, David Robertson, Irwin Schatz, and Gregor Wenning*

We report the results of a consensus conference on the diagnosis of multiple system atrophy (MSA). We describe the clinical features of the disease, which include four domains: autonomic failure/urinary dysfunction, parkinsonism and cerebellar ataxia, and corticospinal dysfunction. We set criteria to define the relative importance of these features. The diagnosis of possible MSA requires one criterion plus two features from separate domains. The diagnosis of probable MSA requires the criterion for autonomic failure/urinary dysfunction plus poor levodopa responsive parkinsonism or cerebellar ataxia. The diagnosis of definite MSA requires pathological confirmation.

Keywords: multiple system atrophy, parkinsonism, cerebellar ataxia, autonomic insufficiency, urinary dysfunction, glial cytoplasmic inclusions.

Address correspondence to Dr. Sid Gilman, Professor & Chair, Department of Neurology, University of Michigan Medical Center, 1500 E. Medical Center Drive/1914 TC, Ann Arbor, MI 48109-0316, USA.
Tel: (734) 936-9070; Fax: (734) 763-5059
E-mail: sgilman@umich.edu

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Multiple system atrophy (MSA), a progressive neurodegenerative disease of undetermined etiology, occurs sporadically and causes parkinsonism and cerebellar, autonomic, urinary, and pyramidal dysfunction in many combinations [1–4]. The disease affects both sexes, usually beginning in middle age and progressing over intervals of 1 to 18 years, with a median survival of 9.3 years from the first symptom [5,6]. The parkinsonian features include bradykinesia with rigidity, postural instability, hypokinetic speech, and often tremor, usually with a poor or unsustained response to chronic levodopa therapy. The cerebellar dysfunction consists of ataxia of gait, limb movements and speech, and disorders of extraocular movements [7]. Autonomic insufficiency results in orthostatic hypotension, often with an inadequate heart rate response to standing, male erectile dysfunction (MED), constipation, and decreased sweating [8,9]. Urinary symptoms include urgency, frequency, nocturia, incomplete bladder emptying, and incontinence [10]. The diagnosis of MSA requires primarily clinical assessment; however, a number of laboratory tests may help to support the diagnosis.

The neuropathological changes consist of a high density of glial cytoplasmic inclusions (GCIs) in association with degenerative changes in some or all of the following structures: putamen, caudate nucleus, globus pallidus, substantia nigra, locus ceruleus, inferior olives, pontine nuclei, cerebellar Purkinje cells, autonomic nuclei of the brainstem, and the intermediolateral cell columns and Onuf's nucleus in the spinal cord [11,12]. GCIs are ubiquitin-, tau- and a-synuclein-positive oligodendroglial inclusions [12].

Some efforts have been made to establish diagnostic criteria [4], but no consistent detailed guidelines have been developed. Accordingly, a consensus conference was convened on April 23 and 24, 1998 in Minneapolis, Minnesota, cosponsored by the American Autonomic Society and the American Academy of Neurology. The goal of the conference was to develop guidelines for the diagnosis of MSA. We achieved consensus on the items listed below and shown in Tables 1, 2, and 3. These guidelines have not yet been validated, and will almost certainly require further modification in the light of future experience.

Clinical domains

Autonomic and urinary dysfunction

Orthostatic hypotension (OH) may indicate autonomic failure and can be asymptomatic or symptomatic. When symptomatic, it typically occurs after the onset of MED and urinary symptoms. Symptoms of OH result from cerebral hypoperfusion, and syncope may occur. The consensus conference determined that the clinical diagnosis of probable MSA requires a reduction of systolic blood pressure by at least 30 mm Hg or of diastolic blood pressure by at least 15 mm Hg within 3 minutes of standing from the recumbent

* Please see "Conference Participants" section at the end of this article for a full listing of author affiliations.

Table 1. Clinical domains, features, and criteria used in the diagnosis of MSA*

I. Autonomatic and urinary dysfunction
  A) Autonomic and urinary features
    1. Orthostatic hypotension (by 20 mm Hg systolic or 10 mm Hg diastolic)
    2. Urinary incontinence or incomplete bladder emptying
  B) Criterion for autonomic failure or urinary dysfunction in MSA

Orthostatic fall in blood pressure (by 30 mm Hg systolic or 15 mm Hg diastolic) or urinary incontinence (persistent, involuntary partial or total bladder emptying, accompanied by erectile dysfunction in men) or both

II. Parkinsonism
  A) Parkinsonian features
    1. Bradykinesia (slowness of voluntary movement with progressive reduction in speed and amplitude during repetitive actions)
    2. Rigidity
    3. Postural instability (not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction)
    4. Tremor (postural, resting, or both)
  B) Criterion for parkinsonism in MSA
    Bradykinesia plus at least one of items 2 to 4

III. Cerebellar dysfunction
  A) Cerebellar features
    1. Gait ataxia (wide based stance with steps of irregular length and direction)
    2. Ataxic dysarthria
    3. Limb ataxia
    4. Sustained gaze-evoked nystagmus
  B) Criterion for cerebellar dysfunction in MSA
    Gait ataxia plus at least one of items 2 to 4

IV. Corticospinal tract dysfunction
  A) Corticospinal tract features
    1. Extensor plantar responses with hyperreflexia
  B) Corticospinal tract dysfunction in MSA: no corticospinal tract features are used in defining the diagnosis of MSA

*A feature (A) is a characteristic of the disease and a criterion (B) is a defining feature or composite of features required for diagnosis.

position. Frequently this is accompanied by an inadequate increase in heart rate (less than 10 beats per minute). We note that this is a more pronounced degree of OH than established previously [4]. MED appears early and affects virtually all male patients with MSA, but the symptom has

Table 2. Diagnostic categories of MSA*

I. Possible MSA: One criterion plus two features from separate domains. When the criterion is parkinsonism, a poor levodopa response qualifies as one feature (hence only one additional feature is required).

II. Probable MSA: Criterion for: autonomic failure/urinary dysfunction plus poor levodopa-responsive parkinsonism or cerebellar dysfunction.

III. Definite MSA: Pathologically confirmed by the presence of a high density of glial cytoplasmic inclusions in association with a combination of degenerative changes in the nigrostriatal and olivopontocerebellar pathways.

*The features and criteria for each clinical domain are shown in Table 1.

Table 3. Exclusion criteria for the diagnosis of MSA

I. History
  Symptomatic onset under 30 years of age
  Family history of a similar disorder
  Systemic diseases or other identifiable causes for features listed in Table 1
  Hallucinations unrelated to medication

II. Physical examination
  DSM criteria for dementia
  Prominent slowing of vertical saccades or vertical supranuclear gaze palsy*
  Evidence of focal cortical dysfunction such as aphasia, alien limb syndrome, and parietal dysfunction

III. Laboratory investigation
  Metabolic, molecular genetic, and imaging evidence of an alternative cause of features listed in Table 1

*In practice, MSA is most frequently confused with Parkinson's disease or progressive supranuclear palsy (PSP) [25]. Mild limitation of upward gaze alone is nonspecific, whereas a prominent (>50%) limitation of upward gaze or any limitation of downward gaze suggests PSP. Before the onset of vertical gaze limitation, a clinically obvious slowing of voluntary vertical saccades is usually easily detectable in PSP and assists in the early differentiation of those two disorders [26].

low specificity. Urinary frequency, urgency, incontinence, or incomplete bladder emptying also occur early and commonly.

Parkinsonism

The majority of MSA patients develop parkinsonian features at some stage of the disorder. All these patients have bradykinesia; rigidity, postural instability, and tremor also often occur. The tremor is usually irregular and postural, often incorporating myoclonus. A classical pill-rolling parkinsonian rest tremor is uncommon. The parkinsonism in MSA can be asymmetric. The dysarthria is mainly hypokinetic, often mixed with other components [13]. The parkinsonian features usually respond poorly to chronic levodopa therapy; however, up to 30% of patients show a clinically significant response to levodopa therapy at some time in the course, but the response is usually sustained for less than five years [5,14,15]. These are the most challenging patients for accurate diagnosis.

Cerebellar dysfunction

Ataxia of gait, the most common cerebellar feature of MSA, often occurs accompanied by dysarthria, limb ataxia, and sometimes gaze-evoked nystagmus and ocular dysmetria. A common finding is saccadic pursuit movements. The dysarthria in patients with predominantly cerebellar dysfunction is mainly ataxic, often mixed with other components [13].

Corticospinal dysfunction

Extensor plantar responses with hyperreflexia occur in about 50% of MSA patients. Corticospinal signs can contribute to the diagnosis, but are less important than abnormalities in the other domains.

Response to levodopa

Levodopa responsiveness should be tested by administering escalating doses (with a peripheral decarboxylase inhibitor) over a 3-month period up to at least 1 g per day (if necessary and if tolerated). A positive response is defined as clinically significant improvement. This should be demonstrated by objective evidence such as an improvement of 30% or more on part III (motor examination) of the Unified Parkinson's Disease Rating Scale [16].

Laboratory investigations

Autonomic function tests, sphincter electromyography (EMG), and neuroimaging may be used to support the diagnosis, and neuroimaging is helpful in excluding other conditions. The abnormalities described below have been defined principally in clinically well-established cases rather than in the early stages of the disease. In the early stages, the tests may give equivocal results. We consider it premature to incorporate laboratory results into the entirely clinical guidelines that we established, but envision the future development of "Laboratory Supported" diagnostic categories.

Assessment of autonomic function can be assisted by a comprehensive battery that evaluates the distribution and severity of sudomotor, cardiovagal, and sympathetic adrenergic deficits [17,18]. Autonomic function tests may help separate MSA from Parkinson's disease and from idiopathic cerebellar degenerations [8].

Sphincter EMG can be useful in the diagnosis of MSA. Analysis of individual motor unit potentials recorded from the external anal sphincter usually shows changes indicating chronic reinnervation, with markedly prolonged motor units [10,19].

Magnetic resonance imaging (MRI) can assist the evaluation by detecting abnormalities of striatum, cerebellum, and brainstem, but can be normal in up to 20% of cases [20]. Striatal abnormalities may include putaminal atrophy, slit-like signal change at the posterolateral putaminal margin, and hypointensity of the putamen relative to the globus pallidus [21]. Infratentorial abnormalities include cerebellar and pontine atrophy, and signal change in the pons and middle cerebellar peduncles [22]. Studies are in progress to evaluate the utility of magnetic resonance spectroscopy, positron emission tomography, and single photon emission tomography.

Diagnostic categories

We established three diagnostic categories reflecting differing levels of certainty: definite, probable, and possible. The diagnosis of definite MSA can only be made after neuropathological examination of the central nervous system revealing the characteristic density and distribution of GCIs and degenerative changes outlined above. The diagnosis of probable or possible MSA can be made using different combinations of clinical domains, criteria, and features, as indicated in Tables 1 and 2. Exclusion criteria are shown in Table 3.

Terminology

MSA is a distinct clinicopathological entity. The term should not be used to describe other neurodegenerative diseases affecting multiple systems. The use of confusing terms such as "multisystem degeneration" for MSA is inappropriate and now should be discouraged. We recommend designating patients as having MSA-P if parkinsonian features predominate or MSA-C if cerebellar features predominate [23,24]. These terms are intended to replace the striatonigral degeneration (SND) and sporadic olivopontocerebellar atrophy (sOPCA) types of MSA, respectively. The term Shy Drager syndrome has been widely misused, and is no longer useful.

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Conference participants

Sid Gilman, M.D., Department of Neurology, University of Michigan Medical Center, Ann Arbor, MI

Phillip Low, M.D., Department of Neurology, Mayo Clinic, Rochester, MN

Niall Quinn, M.D., and Clare Fowler, M.D., Department of Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London, England

Alberto Albanese, M.D., Department of Neurology, Universita Cattolica, Istituto di Neurologia, Roma, Italy

Yoav Ben-Shlomo, M.D., Department of Social Medicine, Canynge Hall, Bristol, England

Horacio Kaufmann, M.D., Department of Neurology, Mt. Sinai Medical Center, New York, NY

Thomas Klockgether, M.D., Department of Neurology, University of Bonn, Bonn, Germany

Anthony Lang, M.D., Department of Neurology, Toronto Western Hospital, Toronto, Ontario, Canada

Peter Lantos, M.D., Ph.D., Department of Neuropathology, Institute of Psychiatry, London England

Irene Litvan, M.D., National Institutes of Health, Federal Building, Bethesda, MD

Christopher Mathias, D.Phil. D.Sc., Department of Neurovascular Medicine, Imperial College School of Medicine at St. Mary's, London, England, and Department of Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London, England

David Robertson, M.D., Departments of Pharmacology and Neurology, Vanderbilt University, Nashville, TN

Irwin Schatz, M.D., Department of Medicine, University of Hawaii at Manoa, Honolulu, HI

Gregor Wenning, M.D., Universitaets-Klinik fur Neurologie, Innsbruck, Austria

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Appendix III: Consensus statement on the diagnosing MSA, PAF & orthostatic hypotension

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Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy

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1. The consensus committee [1]

A Consensus Conference was convened on November 16th, 1995, at the Ritz-Carlton Hotel, Phoenix, Arizona, with the specific aim of generating a consensus on three specific items: the definition of orthostatic hypotension, pure autonomic failure (Bradbury Eggleston syndrome, idiopathic orthostatic hypotension, progressive autonomic failure) and multiple system atrophy. The meeting was sponsored by the American Autonomic Society, and co-sponsored by the American Academy of Neurology. The following are the items on which consensus was reached:

2. Definition of orthostatic hypotension

Orthostatic hypotension (OH) is a reduction of systolic blood pressure of at least 20 mm Hg or diastolic blood pressure of at least 10 mm Hg within 3 minutes of standing. It is a physical sign and not a disease. An acceptable alternative to standing is the demonstration of a similar drop in blood pressure within 3 minutes, using a tilt table in the head-up position, at an angle of at least 60 degrees.

Confounding variables to be considered when reaching a diagnosis should include: food ingestion, time of day, state of hydration, ambient temperature, recent recumbency, postural deconditioning, hypertension, medications, gender, and age.

Orthostatic hypotension may be *symptomatic* or *asymptomatic*. Symptoms of OH are those that develop on assuming the erect posture or following head-up tilt and usually resolve on resuming the recumbent position. They may include lightheadedness, dizziness, blurred vision, weakness, fatigue, cognitive impairment, nausea, palpitations, tremulousness, headache, and neck ache. If the patient has symptoms suggestive of, but does not have documented, orthostatic hypotension, repeated measurements of blood pressure should be performed. Occasional patients may not manifest significant falls in blood pressure until they stand for at least 10 minutes.

3. Pure autonomic failure (PAF)

Pure autonomic failure is an idiopathic sporadic disorder characterized by OH usually with evidence of more widespread autonomic failure. No other neurological features are present. Some patients with the manifestations of PAF may later prove to have other disorders such as multiple system atrophy. Reduced supine plasma norepinephrine levels are characteristic of PAF.

4. Parkinson's disease with autonomic failure

A minority of patients with Parkinson's disease as defined by United Kingdom Parkinson's Disease Brain Bank criteria (Hughes et al. (1992) J. Neurol. Neurosurg. Psychiatry, 55: 181–184) may also develop autonomic failure, including OH. It is not known if these patients

[1] PARTICIPANTS: Irwin J. Schatz, M.D. (Co-Chair), Department of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA; Sir Roger Bannister (Co-Chair), Pembroke College, Oxford, UK; Roy L. Freeman, M.D., Division of Neurology, Deaconess Hospital, Boston, Massachusetts, USA; Christopher G. Goetz, M.D., Department of Neurology, Rush Medical College, Chicago, Illinois, USA; Joseph Jankovic, M.D., Department of Neurology, Baylor College of Medicine, Houston, Texas, USA; Horacio C. Kaufmann, M.D., Department of Neurology, Mount Sinai School of Medicine, New York, New York, USA; William C. Koller, M.D., Department of Neurology, University of Kansas, Kansas City, Kansas, USA; Phillip A. Low, M.D., Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA; Christopher J. Mathias, M.D., St. Mary's Hospital/Imperial College School of Medicine and the National Hospital/Institute of Neurology, Queen Square, London, UK; Ronald J. Polinsky, M.D., Sandoz Research Institute, East Hanover, New Jersey, USA; Niall P. Quinn, M.D., Institute of Neurology, University Department of Clinical Neurology, The National Hospital, London, UK; David Robertson, M.D., Autonomic Dysfunction Center, Vanderbilt University, Nashville, Tennessee, USA; David H.P. Streeten, M.D., Department of Medicine, Health Science Center, Syracuse, New York, USA.

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have a more serious prognosis than Parkinson's disease without autonomic failure.

5. Multiple system atrophy (MSA)

MSA is a sporadic, progressive, adult-onset disorder characterized by autonomic dysfunction, parkinsonism, and ataxia in any combination. The features of this disorder include:

(A) Parkinsonism (bradykinesia with rigidity or tremor or both), usually with a poor or unsustained motor response to chronic levodopa therapy.

(B) Cerebellar or corticospinal signs.

(C) Orthostatic hypotension, impotence, urinary incontinence or retention, usually preceding or within 2 years after the onset of the motor symptoms.

Characteristically, these features cannot be explained by medications or other disorders.

Parkinsonian and cerebellar features commonly occur in combination. However, certain features may predominate. When parkinsonian features predominate, the term striatonigral degeneration is often used. When cerebellar features predominate, sporadic olivopontocerebellar atrophy is often used. When autonomic failure predominates, the term Shy-Drager syndrome is often used. These manifestations may occur in various combinations and evolve with time.