HUMAN HYPERTENSION: OBSERVATIONS ON AUTONOMIC NERVOUS SYSTEM CONTROL MECHANISMS AND CLINICAL ASSOCIATIONS

by

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Abstract

Introduction: Sympathetic nerve activity (SNA) undergoes physiological modulation by respiration but it remains unclear whether this process is altered by age and hypertension.

Aims: To establish relationship between respiration and neural regulation of the cardiovascular system in aging and hypertension.

Methods: Multiunit muscle SNA, BP, respiratory parameters and heart rate were recorded at rest in young and older healthy men and hypertensive patients, then repeated in hypertensive group after acute and long-term device-guided slow deep-breathing (SDB) training.

Results: Muscle SNA was higher in older subjects but showed similar modulation by respiration in both age groups. In young acute SDB reduced SNA, with no effect on sympathetic and cardiac baroreflex sensitivity. The sympathoinhibition was not related to changes in baroreflex sensitivity, but it reflected increases in lung inflation afferent input and/or reduction in central respiratory-sympathetic coupling. Long-term SDB training inhibited muscle SNA in hypertensive patients and led to acute increase in heart rate variability and longer-term BP reduction. There were no changes in baroreflex sensitivity, cardiac structure/function or arterial stiffness in response to SDB training.

Conclusions: The study provides new mechanistic insights into sympathetic regulatory pathways in hypertension and aging, which may help to establish anti-hypertensive strategy based on respiratory modulation.

Dedications

This thesis is dedicated to

my parents Ivan and Nadzeya,

to my husband Eduard, and

to my children Eleonora and Roman

This thesis was only possible with your love,

endless support and encouragement

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List of abbreviations

ADMA - asymmetric dimethylarginine

AI – augmentation index

AU- arbitrary units

ANOVA – analysis of variance

BP – blood pressure

BMI – body mass index

CVLM - caudal ventrolateral medulla

eNOS - endothelial NOS

fMRI - functional magnetic resonance imaging

GABA -γ-amino butyric acid

HF - high frequency

HR – heart rate

IML - intermediolateral

iNOS - inducible NOS

LF - low frequency

L-NAME - N(omega)-nitro-L-arginine methyl ester

L-NMMA - NG-Monomethyl-L-arginine, monoacetate salt

LV - left ventricle

MAP – mean arterial pressure

NADPH - nicotinamide adenosine dinucleotide phosphate

nNOS - neuronal NOS

NO - nitric oxide

NOS - nitric oxide synthase

NTS - nucleus tractus solitarius

PNMT - phenylethanolamine-N-methyltransferase enzyme

pNN50% - proportion of the number of successive normal-to-normal intervals that differ by more than 50 ms

PVN -paraventricular nucleus

PWV - pulse wave velocity

rMSNA - respiratory mediated changes in muscle SNA

RMSSD - sum of successive differences in normal-to-normal interval

ROS - Reactive oxygen species

RVLM - rostral ventrolateral medulla

SD – standard deviation

SDNN - standard deviation of the normal-to-normal intervals

SNA - sympathetic nerve activity

SPSS -Statistical Package for the Social Sciences

THW - Traube–Hering arterial blood pressure waves

TP -total power

CHAPTER I. INTRODUCTION

Hypertension is a major cardiovascular risk factor. It affects approximately 1 billion people worldwide with less than half being able to achieve desirable blood pressure (BP) control with treatment.[1-5] Hypertension has a major impact on health and longevity and puts a huge strain on national healthcare services. Strikingly, each 2 mmHg increase in systolic BP translates into a 7% increase in mortality from ischemic heart disease and a 10% increase from a stroke.[6]

The sympathetic nervous system plays a key role in the maintenance of homeostasis, including the regulation of the functional status of the cardiovascular, respiratory, gastrointestinal and genitourinary systems. The sympathetic nervous system has a complex neuroanatomy and it closely interacts with, and receives inputs from, multiple other organs and systems. This reflects its status as a global regulator of bodily function. However, the chronic activation of the sympathetic nervous system can lead to a number of cardiovascular risk factors and pathologies. These disorders include ischemic heart disease, myocardial infarction, and heart failure.[7-10] Studies performed on patients with hypertension have demonstrated the relevance of sympathetic activation to the pathogenesis of hypertension starting from its early manifestations and extending to its advanced stages and development of complications. The degree of BP increase has been shown to parallel the magnitude of the sympathetic activation.[11, 12] In fact, data indicate that heightened sympathetic activation often precedes hypertension.[13] In a longitudinal observational study with a 20-year follow up, high arterial adrenaline levels strongly

predicted future occurrence of hypertension.[13] Sympathetic hyperactivity in hypertension leads to development of left ventricle (LV) hypertrophy and presence of LV hypertrophy in hypertensive patients.[14-17] Moreover high sympathetic nerve activity (SNA) is associated with LV diastolic dysfunction independently of BP levels[18, 19] Taken together, these observations indicate that the sympathetic nervous system may play a role in the initiation, development, and worsening of hypertension.

Aging is commonly associated with increased cardiovascular morbidity, higher prevalence of hypertension[20, 21] and it is reasonable to probe potential changes in sympathetic system status in older people and possible factors modulating the system's activity. Furthermore, healthy older people have raised plasma catecholamine concentrations, elevated noradrenaline spillover from the heart, brain, kidneys, and increased sympathetic neural drive to vasculature of the skeletal muscles (i.e., muscle SNA).[22, 23] These autonomic alterations have been related to abnormalities in vascular structure and function, including impairment of the elastic properties of the large arteries and vasomotor endothelial function.[24, 25] The mechanistic basis for the age-related elevation in sympathetic neural firing remains unknown.

Respiration has been long known to modulate SNA. The links between breathing and SNA are mediated by central neuronal circuits with the regulatory feedback signals from cardiorespiratory sensory afferents (e.g., lung-stretch receptors, baroreceptors, peripheral chemoreceptors).[26-33] Spontaneous shallow breathing (decreased tidal volume) has been associated with an increase in sympathetic activity in patients with heart failure, likely a consequence of diminished inhibition of SNA by pulmonary

stretch receptors[32, 34]. During normal breathing in young healthy individuals muscle SNA is inhibited during mid-inspiration, reaching a nadir when lung volume is at its highest (peak inspiration), and it peaks when lung volume is at its lowest (end-expiration).[27-30, 33] It is possible that an impairment in the normal inspiratory inhibition of muscle SNA explains the increase in the tonic level of muscle SNA and thus elevated BP with age. However, to date it is unknown whether the respiratory modulation of muscle SNA is changed as a consequence of healthy human ageing. In light of this background, the first experimental chapter (Chapter 4), aimed to establish the effect of age on respiratory related bursting of muscle SNA and on the association between the rhythmic fluctuations in muscle SNA and BP that occur with respiration in humans.

The use of breathing techniques for management of hypertension has a long history.[35, 36] Recently, the potential of the hypotensive effect of device-guided slow deep breathing have been demonstrated in several trials,[37-43] although such findings have not always been consistent.[44-47] Recently home-based device-guided training has been recommended by the American Heart Association.[48] Despite several trials investigating the effect of slow deep breathing on BP few studies assessed mechanisms of the acute and especially long-term effects of the technique. However, the physiological mechanisms leading to the BP lowering effects of device-guided slow deep breathing and the potential for associated effects on neural regulation, cardiovascular structure and function remain unclear.

It has been reported that acute slow deep breathing increases cardiovagal baroreflex sensitivity in young healthy individuals, children with obesity and patients with heart

failure and hypertension.[49-54] However, data are lacking on acute and longer-term effect of slow deep breathing on arterial baroreflex control of muscle SNA.[55] This response is important to establish because cardiovagal baroreflex sensitivity and arterial baroreflex control of muscle SNA are differentially controlled and do not always change in parallel.[55] It is known that slow deep breathing acutely inhibits increased SNA in hypertensive patients,[44, 56] but there are no published data on muscle SNA response to the acute slow deep breathing in healthy young subjects.[49, 50, 57]

Analysis of heart rate (HR) variability allows insight into cardiac parasympathetic activity, but limited knowledge is available on how this activity is influenced by device-guided slow deep breathing. Acute effect on cardiac parasympathetic fluctuations has only been tested in a group of healthy middle age volunteers with borderline elevated BP.[58] That study was not able to demonstrate a significant increase in parameters of the cardiac parasympathetic drive.[58] Also, the acute effect of device-guided slow deep breathing on HR variability in hypertensive patients was not studied, neither HR variability response was evaluated in hypertensive cohort without concomitant pathologies longer term.[59]

In this thesis, I aimed to obtain further information on influences of slow deep breathing on sympathetic and parasympathetic activity in carefully selected young healthy individuals and patients with established hypertension (Chapters 5 and 6, respectively). The hypothesis was tested that acute slow deep breathing would reduce BP and muscle SNA in young healthy and hypertensive groups, and to assess the underlying mechanisms of autonomic neural effect. I also aimed to provide a comprehensive

assessment of the long-term training effects of home-based device-guided slow deep breathing on autonomic regulation in hypertension reflected by muscle SNA, arterial baroreflex control of muscle SNA, parameters of HR variability and effect on hypertension target organ damage (e.g. heart, vessels, kidney).

CHAPTER II. LITERATURE REVIEW

2.1. Hypertension: clinical significance and epidemiology

Hypertension is a major global health problem due to the large number of patients affected and its strong association with an increased risk of coronary artery disease, myocardial infarction, stroke, renal dysfunction, and death.[60] The clinical importance of hypertension is highlighted by estimates that it contributes to a third of all cases of myocardial infarctions and strokes, and half of all cases of heart failure.[61] Hypertension is also the leading course of kidney failure.[20]

Hypertension is usually defined as systolic BP equal or above 140 mmHg or diastolic BP equal or above 90 mmHg.[20] However, in some patient populations, such as in those with complicated renal dysfunction even lower BP levels may merit treatment. It is estimated that about 1 billion people are affected by hypertension worldwide and that it affects approximately every third individual in developed countries.[1-3] Currently, hypertension is also a growing problem in the developing world with the number of affected individuals expected to rise further with improving detection and increasing urbanization.[62]

In fact, even those high numbers may underestimate the genuine rate of hypertension as it often remains undetected until the patient develops severe and often disabling complications. Identification and appropriate management of high BP are of particular clinical importance as interventions directed to BP reduction bring significant health

benefits. It has been shown that every 5 mm Hg decrease in systolic BP in the general population is associated with a 9% to 14% decrease in cardiovascular mortality.[63] Nevertheless, despite the availability of a wide range of pharmacological and non-pharmacological interventions to reduce BP, recent data show poor hypertension control in every second patient.[5] This fact partly reflects the substantial diversity and heterogeneity of pathogenic mechanisms of hypertension, which still remain insufficiently understood. Available and emerging data clearly suggest the intimate role of the sympathetic nervous system in the pathophysiology of hypertension.

In this section of the thesis, I aim to provide an overview of the structure of the autonomic nervous system, with a particular focus on the sympathetic nervous system. The methods available for the assessment of the sympathetic nervous system will be discussed along with their advantages and limitations. Following that, I will review current knowledge of the pathways involved in the sympathetic regulation of BP per se and the pathogenesis of hypertension. The section will conclude with an overview of the available therapeutic and lifestyle approaches to improve BP control via modulation of the sympathetic nervous system.

2.2. Autonomic nervous system: organization and regulation

2.2.1. Introduction

The autonomic nervous system plays a key role in the unconscious regulation of many bodily actions and is composed of sympathetic, parasympathetic and enteric divisions. The enteric nervous system, an intrinsic nervous system of the gastrointestinal tract, appears to have little role in BP control. It possesses complete reflex circuits functioning to detect homeostatic changes within gastrointestinal organs to provide outputs to keep intestinal motility, fluid exchange balance and gut circulation within the desirable range.[64] The enteric nervous system contains two nerve plexuses that continue along the entire length of the gastrointestinal tract. The system also has extensive links with other parts of the central nervous system that allow interactions with and adjustment for the state of the entire body.

Sympathetic and parasympathetic nervous systems play a crucial role in the regulation of maintenance of bodily homeostasis, including the regulation of the functional status of the cardiovascular, respiratory, gastrointestinal and genitourinary systems, but also pupillary responses and thermoregulation (Table 2.1). The autonomic nervous system has a complex structure, and it regulates a variety of parameters implicated in BP control, which will be discussed below in more detail.

Table 2.1. Overview of sympathetic and parasympathetic nervous system actions

Effector organ	**Sympathetic**	**Parasympathetic**
Heart	↑ Contractility and heart rate	↓ Contractility and heart rate
Blood vessels	Vasoconstriction	-
Pupils	Dilation	Constriction
Lungs	Bronchial dilation	Bronchial constriction
Bladder	Inhibits voiding	Promotes voiding
Gastro-intestinal tract	Inhibits digestion and secretion	Stimulates digestion and secretion
Kidneys	↑ Secretion of noradrenaline	-
Salivary and lacrimation glands	-	↑ Salvation and tear production
Liver	↑Glucose production and release	↑ Bile release

2.2.2. Central regulation

Figure 2.1 provides a simplified overview of the central regulation of sympathetic outflow. The medulla oblongata is a region of the brainstem that has a particularly important role in the control of autonomic function, and thus regulation of the respiratory, cardiovascular and gastrointestinal systems.[65] It contains several smaller specialised areas that are of importance for the generation and regulation of sympathetic outflow. The rostral ventrolateral medulla (RVLM) is a site of central sympathetic outflow, which receives both excitatory and inhibitory inputs. It is commonly recognised as the pressor area of the brain and functions as the primary regulator of the sympathetic nervous outflow to the heart and vasculature (Figure 2.1).[65] Excitatory catecholaminergic neurones project from the RVLM to the sympathetic preganglionic neurones in the intermediolateral (IML) cell column of the spinal cord via reticulospinal tract. Also, as evident from retrogradely transported tracer studies, IML preganglionic neurones receive direct input from other brain centres (e.g. hypothalamic nuclei, pontine structures, ventromedial medulla).[66] In addition inhibitory γ- amino butyric acid (GABA) receptors expressed in IML.[67, 68] The RVLM is considered to be a site at which central (e.g. pons, hypothalamus, amygdala) and peripheral (e.g. afferent inputs, including baroreflex input) receptor pathways converge. RVLM neurones possess both excitatory glutamate neurones and inhibitory GABA neurones.[69-71] The RVLM glutamatergic neurones differ neurochemically. Some of the neurones contain the phenylethanolamine-N-methyltransferase enzyme (PNMT), which is involved in the catecholamine synthesis.[72] These neurones form the C1 cell population[73, 74] and in one experiment, the retrograde labelling of C1 neurones in the IML showed that 79% of the

C1 bulbospinal neurones are glutamatergic.[75] Additionally, the C1 cells expressed substance P, enkephalin, pituitary adenylate cyclase activating polypeptide, vasoactive intestinal peptide, and neuropeptide Y.[76-78] The C1 neurones have two types of terminals, containing either glutamate[79, 80] or PNMT[81, 82] and both types of the terminals, synapse with sympathetic preganglionic neurones in the IML. Recent work by Guyenet and colleagues has provided insight into the functional role of C1 neurones using new experimental approaches. Activation of C1 cells using photostimulation evoked an increase in BP[83] while selective partial lesioning of the C1 cells diminished the pressor effects.[83] In another experiment by Guyenet and colleagues, a lentivirus expressing channelrhodopsin-2 (ChR2) under the control of the catecholaminergic neuron preferring promoter PRSx8, was used to powerfully and selectively activate C1 neurones in the RVLM. This led to a prominent sympathoactivation and a marked increase in BP.[84] Earlier work had shown that the destruction of C1 neurones by spinal microinjections of an anti-dopamine-beta-hydroxylase antibody conjugated to the ribosomal toxin, saporin (80-85% depletion)[85-87] reduced the sympathetic activation mediated by activation of the baroreflex, carotid chemoreflex, and somatic pressor reflex.[85-87] In contrast, the parasympathetic branch of the baroreflex function was not affected by C1 neuron destruction as the reduction in HR in response to an increase in BP was preserved.[87] Taken together, these experiments provide unambiguous and sophisticated evidence that C1 neurones are an important regulator of sympathetic cardiovascular function. AT(1) receptors for angiotensin II are also expressed in RVLM, as evident with quantitative in vitro autoradiography.[88] The RVLM also contains nitric oxide (NO) synthase (NOS)-producing neurones, which can be activated through sympathoexcitatory cardiac reflexes.[89] Although the region contains all 3

isoforms of the NOS (i.e., neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS)) only nNOS has been reported to mediate the cardiac sympathetic response, as evident from the attenuation of sympathoexcitatory reflexes only after specific inhibition of nNOS.[89]

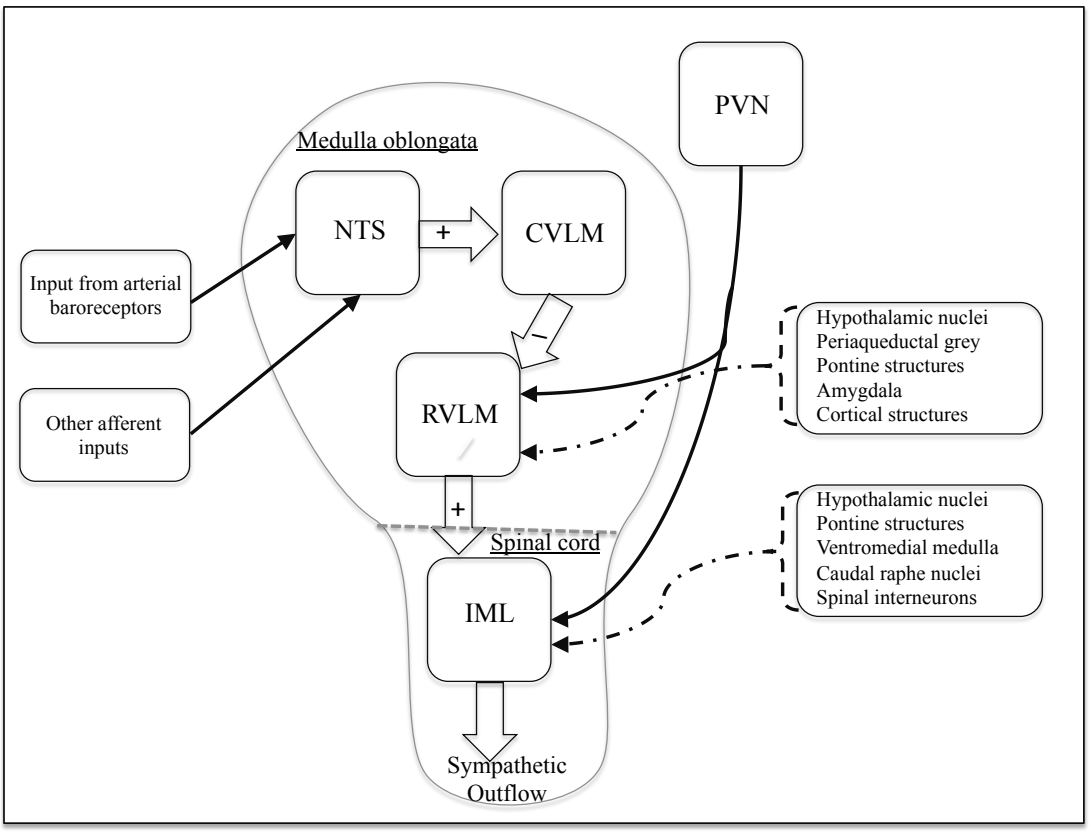

Figure 2.1. Simplified diagram showing organization of the brain regions involved in the generation and modulation of the sympathetic flow

CVLM, caudal ventrolateral medulla; IML, intermediolateral cell column; NTS, nucleus tractus solitarius; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla.

The supramedullary paraventricular nucleus (PVN) in the hypothalamus is a well-recognised area providing direct descending excitatory inputs to IML of the spinal cord and RVLM.[90] The activity of PVN neurones can be modulated by circulating angiotensin II and by renal afferent signals nerves delivered through the NTS.[91-93] Both NO and GABA are known to provide inhibitory inputs to the PVN and are thus involved in the control of sympathetic outflow.[91-93] Indeed, in rats, the microinjection of a NO donor (sodium nitroprusside) into the PVN resulted in a dose dependant reduction in renal sympathetic activity, with no response in the presence GABA blockade with bicuculline.[91] When the production of NO was inhibited with L-NAME renal sympathetic activity was increased, this excitatory effect was eliminated by activation of the GABA system with muscimol. Negative feedback of NO on the glutamate system within the PVN is responsible for the balance of the sympathetic drive both physiological and pathological states.[92]

The nucleus tractus solitarius (NTS) constitutes a vertical column of grey matter within the medulla oblongata. The NTS serves as the primary integrative centre for arterial baroreceptor and peripheral afferents and from the NTS projections travel to many other parts of the brain (e.g., preganglionic neurones, hypothalamus and thalamus).[94] In particular, NTS neurones project to the GABAergic barosensitive interneurones of the caudal ventrolateral medulla (CVLM) (as demonstrated by retrograde fluorescent tracing), which send inhibitory impulses directly to the RVLM.[95] NO amplifies glutamatergic transmission in the NTS, where blockade of NOS activity inhibits baroreflexes and cardiopulmonary reflexes.[96]

The sympathetic preganglionic neurones are the most peripheral location at which the central nervous system may modulate sympathetic outflow. The soma of the sympathetic preganglionic neurones are located in the lateral horns and central regions of the spinal cord.[97] Although the majority of the neuron bodies are placed at the thoracic and lumbar spine levels, some are situated at cervical level VIII.[98, 99] Axons of the sympathetic preganglionic neurones mainly project towards postganglionic neurones via the ventral root, but also to the adrenal medulla and accessory peripheral nerves.[100, 101] Retrograde labelling of sympathetic nerves and ganglia using Fluoro-gold was utilized to establish distribution of sympathetic preganglionic neurones in rats.[102] A large number of sympathetic preganglionic neurones (i.e., 1000-2000 neurones per ganglion) have been identified within the ganglia involved in regulation of the head or chest organs. This is in contrast to the relatively small number of sympathetic preganglionic neurones (i.e., 100-400 neurone per ganglion) in the ganglia that control abdominal and pelvic organs, including the kidneys. It has been suggested that this observation likely reflects differences in the regulatory contribution of the sympathetic preganglionic neurones to these organs.[102] The sympathetic preganglionic neurones receive modulatory inputs from afferent feedback, descending influences, and interneurones. Important advances in the understanding of the inputs to sympathetic preganglionic neurones have been made using rat experiments employing transneuronal labelling with pseudorabies virus.[103] After the injections into various sympathetic ganglia and the adrenal gland, the infection affected neurones of the RVLM, A5, raphe, and hypothalamus, which identified five brain cell clusters responsible for the regulation of the sympathetic outflow.[66, 104] On the virus expansion the label was further found in some sympathetic ganglia, mainly superior cervical and stellate ganglia. In

addition, for the first time, the virus labelling targeted spinal cord interneurones in laminae VII and X, one of the important components of the spinal cord.[66, 104] Sympathetic preganglionic neurones can also be excited by stimulation of visceral or somatic afferents.[105-108] These afferent inputs and some descending inputs are further modulated by the spinal interneurones.[109, 110] Taken together, these experiments demonstrate that sympathetic preganglionic neurones not only receive descending modulatory signals but also interact with local influences to produce functionally appropriate changes in sympathetic outflow to body tissues and organs. This view advances the previous simplistic perception of the sympathetic preganglionic neurones as relay neurones translating central influences without significant modulation by local neuronal feedback.

New information has been provided by functional magnetic resonance imaging (fMRI) regarding the central regulation of sympathetic outflow in humans. King et al. were the first to report activation in the insular and medial prefrontal cortex and the thalamus in response to changes in HR and BP caused by maximal inspiration, Valsalva manoeuvre or isometric handgrip exercise.[111] An 'activation likelihood estimation meta-analysis' of the neuroimaging experiments identified several consistently activated human brain areas involved in the autonomic regulation. These areas included left amygdala, right anterior and left posterior insula and midcingulate cortices.[112] Although the insular cortex has attracted substantial attention in animal studies, analysis of this brain area in humans initially proved challenging due to the limited spatial resolution of available neuroimaging techniques. However, work by Macey et al. has provided further insight into the role of this area in humans using newer fMRI techniques and reported that five

different areas within the insular cortex display distinct responses to various autonomic challenges.[113] Subsequently, Shoemaker and colleagues reported activation of the posterior insular cortex in response to the graded handgrip, thus indicating the role of this particular part of the insular cortex in autonomic regulation.[114, 115] These observations were supported by a human study showing that stimulation of the forearm muscles activated the posterior insular cortex but decreased the activation of the anterior insular, which might be suggestive of intra-insular connections.[115] Activation of the posterior insula was also noted during the baroreflex unloading with lower body negative pressure.[116, 117] In these studies, application of the more pronounced lower body negative pressure and higher HR response correlated with higher activity in the posterior insula, but also in the frontoparietal cortex and left cerebellum. During the same experiments, diminished activity was noted in the anterior insular cortices, right anterior cingulate, orbitofrontal cortex, amygdala, midbrain and mediodorsal nucleus of the thalamus. Deactivation of the ventral medial prefrontal cortex was evident during the handgrip challenges.[114, 115] The deactivation of the ventral medial prefrontal cortex was more prominent when participants completed a higher intensity exercise that resulted in a larger HR increase. The above studies support the role of these cortical regions in the modulation of efferent parasympathetic outflow to the heart. The consistent involvement of the insular and ventral medial prefrontal cortex in the cardiovascular reflex responses suggests a possibility that these two regions may link regulation of the sympathetic and parasympathetic systems.

2.2.3. Arterial baroreflex

The arterial baroreceptors are a key regulator of cardiovascular autonomic activity. The arterial baroreflex serves to provide a negative feedback regulation of BP and thus help to maintain circulatory homeostasis. Via the baroreflex mechanism, fluctuations in BP may be buffered, and desirable BP levels established to meet hemodynamic demand.[118] Baroreceptor afferents respond to the mechanical deformation of their receptive fields at the medial-adventitial border in the aortic arch and carotid sinuses.[119] The principle mechanism of baroreceptor activation is the opening of stretch-activated ion channels, but they can be further modulated by functional status of potassium channels and the sodium-potassium pump.[120] The magnitude by which baroreceptor activity is altered further depends on a variety of paracrine factors, such as reactive oxygen species, prostacyclin, and factors released from aggregating platelets.[120] Baroreceptor afferents travel via vagal and glossopharyngeal nerves to the NTS where the information is processed and autonomic efferent activity to the heart and vessels modulated.[94] An increase in BP and the associated increase in baroreceptor afferent activity results in a reflex-mediated decrease in SNA to the heart and the blood vessels and an increase in cardiac parasympathetic activity. Conversely, a fall in BP decreases baroreceptor afferent activity and leads to an increase in SNA and decrease in parasympathetic activity. Via this mechanism the adjustment of the sympathetic and parasympathetic activity maintain BP around an appropriate operating point level.[118]

Of note, efferent SNA outflow is dependent upon the type of baroreceptor afferent discharge. In the presence of pulse phasic afferent baroreceptor discharge, SNA remains

inhibited.[120] In contrast, continuous, nonphasic baroreceptor discharge leads to a reduction in the afferent activity and elevation of the SNA.[120] Baroreflex inhibition of the sympathetic activity is controlled by the input from the aortic arch and carotid sinuses baroreceptors.[121] But strength and occurrence of the sympathetic discharges are modulated differentially in both animal[122, 123] and human[124] investigations.

2.2.4. Peripheral chemoreflex

The peripheral chemoreceptors are situated in the aortic arch and carotid bodies and powerfully increase sympathetic activity in response to hypoxemia and hypercapnia. Carotid bodies contain glomus cells that respond to hypoxia by releasing various neurotransmitters, such as serotonin, acetylcholine, substance P and adenosine triphosphate. These neurotransmitters activate afferent neurones within the carotid sinus nerve that connect carotid bodies and NTS.[125, 126] The activation of the peripheral chemoreceptors increasing the SNA and causing arterial constriction within the renal, splanchnic and skeletal muscle circulations, and consequently increases BP.[125] The mechanism aims to compensate for a deficiency in perfusion/oxygen saturation by increasing systemic vascular tone.[127] Where the restoration of the global circulatory homeostasis cannot be achieved the mechanism facilitates the redistribution of the blood flow towards critical organs.

Sympathetic stimuli to the heart are also triggered by stimulation of chemoreceptors, which evokes both cardiac chronotropic and inotropic effects.[125] In contrast, stimulation of the chemoreceptors (a mixture of 8% O_2 and 92% N_2 given for 30 s) inhibits

sympathetic outflow to the adipose tissue, which has a biological role in counteracting hypoxia via reduction overall oxygen requirements.[128] Importantly, arterial chemoreflex activation also leads to increase in phrenic nerve activity, that enhances ventilation.[129]

The magnitude of the sympathetic response to chemoreflex activation is modified by breathing rate. Higher breathing rates increase the sympathetic response to stimulation of the chemoreceptors,[130] whereas at lower respiratory rates the scale of sympathetic response to chemoreceptor stimulation is diminished.[131] Chemoreceptor mediated sympathetic drive is also reduced when respiratory tidal volume is augmented due to an enhanced inhibitory response arising from pulmonary stretch receptors.[132]

2.2.3. Efferent pathways

Several levels of neurones mediate transmission of central sympathetic stimuli. The sympathetic preganglionic neurones are located in the thoracolumbar section of the spinal cord (from T1 to L2) with their axons in most cases spreading to paravertebral ganglia. The next level of postganglionic neurones distributes the sympathetic stimuli to the target cells through unmyelinated nerves.[64] Adrenergic receptors are the main receptors of the sympathetic system and they include alpha receptors, activation of which leads to peripheral vasoconstriction; beta 1 receptors, which increase cardiac contractility and HR, and beta 2 receptors, which activation cause relaxation of smooth muscle in peripheral vasculature, bronchi, gastrointestinal organs, and genitourinary system.[64]

2.2.4. Parasympathetic nervous system

The parasympathetic nervous system often serves as a counterpart of the sympathetic nervous system, however this is not always the case.[133] The main biological function of the system is to maintain body functions under homeostatic (resting) state as opposed to the role of the sympathetic nervous system to adapt to 'threats' by providing appropriate changes to the resting homeostasis.

Cranial nerve X (vagus) is the principle component of the parasympathetic nervous system that links the brain with peripheral organs such as the heart. The preganglionic vagal motorneurones arise from the nucleus ambiguous of the medulla oblongata. Parasympathetic activity ('vagal tone') helps to sustain stable homeostasis of cardiac, respiratory, gastrointestinal, genitourinary and endocrine systems among other functions. The parasympathetic nervous system also includes neurones in cranial nerves III (oculomotor nerve), VII (facial nerve) and IX (glossopharyngeal nerve). Preganglionic axons are mostly myelinated, and they synapse with postganglionic neurones in ganglia that are located near the end organs.[134] The parasympathetic nervous system rapidly and powerfully modulates HR. An increase in vagal tone reduces HR via inhibition of sinoatrial node discharge. The level of cardiac parasympathetic activity at a given instance is dependent upon a multitude of influences, such as excitatory central inputs from baroreceptors and the local modulatory effects of SNA. Respiration also strongly modulates HR with an inhibition of vagal activity and increase in HR observable during inspiration with the opposite dynamics seen during expiration. In the absence of direct recordings, the study of such

respiratory sinus arrhythmia (e.g., HR variability) can provide a valuable insight into cardiac parasympathetic activity in health and disease. This is discussed in detail below (2.3.5).

2.3. Assessment of autonomic nervous system

2.3.1. Introduction

Accurate quantification of the activity of the sympathetic and parasympathetic branches of the autonomic nervous system is essential to assess its impact in the pathogenesis of morbid states (e.g., hypertension) and to determine the effectiveness of therapeutical strategies targeting the autonomic nervous system. Several methods have been described, but all have limitations, which need to be considered when deciding to choose a study method.

2.3.2. Plasma catecholamine concentration

Assessment of plasma noradrenaline concentration, the principle neurotransmitter of the sympathetic nervous system, provides an accessible and well-validated method of quantifying global SNA. High-performance liquid chromatography is a relatively inexpensive and widely available technique, which can be employed in clinical settings. Advantages of the method also include simplicity of sampling (i.e., venous blood) and a possibility of postponed batched sample analysis. It has been demonstrated that forearm venous plasma noradrenaline concentration is correlated with central sympathetic outflow to the skeletal muscle vasculature in healthy individuals ($r=0.65$, $P<0.01$), aging populations and disease states (e.g., in hypertension $r=0.64$, $P<0.01$).[135-137]

Despite several benefits, the assessment of sympathetic activity using plasma noradrenaline concentration has significant limitations. For instance, plasma noradrenaline concentrations from venous samples represent only a minor portion of secreted neurotransmitter from sympathetic nerve terminals with a substantial part of the norepinephrine being taken back up by nerve terminals without spilling into plasma.[138-140] The proportion of noradrenaline reaching the peripheral circulation may vary among different individuals and within individuals, thus affecting the reproducibility of the method. It has been shown that reproducibility of venous plasma noradrenaline measurements by high-performance liquid chromatography is inferior to the reproducibility of microneurography technique (described in detail below).[141-143] It has also been suggested that the reproducibility could be improved by averaging several measurements from the same sample.[144, 145]

2.3.3. Radiolabeled noradrenaline tracers (noradrenaline spillover)

The assessment of noradrenaline spillover using radiolabelled tracers provides valuable and accurate quantitative information on SNA. The procedure involves the infusion of small doses of radiolabelled noradrenaline and the placement of a catheter for selective blood sampling from particular organs or the whole body.[138, 143, 146] The former approach allows measurement of regional sympathetic outflow to selected organs, from which direct recordings are not possible in humans (e.g., kidneys and heart). In healthy subjects, spillover of noradrenaline from the heart correlates well with muscle SNA burst frequency at rest ($r=0.70$, $P=0.03$).[147] Single unit recordings from sympathetic nerves in hypertensive patients revealed an association between firing rates of

sympathetic bursts and cardiac noradrenaline spillover.[148] The value of making regional measures of noradrenaline spillover is that a heterogeneous sympathetic activation between organs can occur, for example in obese but otherwise healthy individuals kidney noradrenaline spillover is elevated while in contrast cardiac spillover is decreased.[149]

Several studies have reported that both cardiac and renal noradrenaline spillover is increased in untreated essential hypertension.[150-153] In addition, in patients undergoing renal denervation a significant reduction of renal noradrenaline spillover rate post catheter renal denervation has been reported, that is paralleled by a 50% reduction in renin activity and an increase in renal blood flow.[154, 155] However more recent studies have reported less prominent impact of renal denervation on noradrenaline spillover with highly variable results among different patients thus challenging reliability of the renal denervation.[156]

2.3.4. Microneurography

The microneurography technique was developed in the 1960s by Hagbarth and Vallbo[157] and permitted the first direct recordings of sympathetic action potentials in awake humans[158]. This method brought multiple advantages, such as the real-time registration of SNA. Microneurography since has been widely used in a variety of experimental settings, contributing to better understanding of the role of sympathetic nervous activity in normal physiology and different disease states.[159]

Recordings can be directly obtained from sympathetic nerve efferent activity to either the skin or muscle vasculature. Those two types of nerve activity have characteristic differences, with muscle SNA having a pulse-synchronous pattern and skin SNA an arrhythmic, not cardiac linked discharge. Many studies have been focused on the investigation of the activity of sympathetic fibers supplying skeletal muscle vasculature.[143, 160] These studies provided valuable information on different aspects of regulation of cardiovascular functions, including BP control.

The direct recording of efferent muscle SNA is minimally invasive with the peroneal nerve being a common choice for such examination.[160, 161] The method involves the insertion of a unipolar tungsten microelectrode into a sympathetic fascicle at the level of fibular head and a reference electrode inserted percutaneously at a site 2-3cm distally. The raw signal undergoes amplification (x100000), band-pass filtering (bandwidth 700 – 2000 Hz), rectification and integration (0.1 s time constant). Acceptance of the multiunit muscle SNA neurogram is based on pulse rhythmicity of spontaneous signal bursts, with a signal-to-noise ratio of at least 3:1.[162] Verification of the recordings can assisted by the utilisation of an end-expiratory breath-hold or Valsalva manoeuvre.[162] The integrated neurogram contains information on frequency and activity of the sympathetic bursts. The frequency is quantified by counting the number of sympathetic bursts per one minute (burst frequency) and by a number of bursts per 100 heart beats per minute (burst incidence). The strength of the signal is determined from the amplitude or area of the sympathetic bursts.

Microneurography provides a direct measurement of central sympathetic outflow to the periphery, accurately reflecting changes in sympathetic activity due to neural (dys)function. Although there is noticeable inter-individual variability of muscle SNA, the method shows excellent reproducibility over repeated measurements.[143, 163, 164] The limitation of microneurography is that it does not provide a direct measurement of the sympathetic activity of the heart and kidney, two key organs involved in blood pressure homeostasis. Nevertheless, in healthy subjects, there is a strong association between muscle SNA from the peroneal nerve and sympathetic outflow from the heart (r=0.70, P=0.03) and kidneys (r=0.76, P<0.01).[143, 147, 165] Such an association is less well established in disease states. Since muscle SNA provides a measurement of sympathetic outflow to vascular smooth muscle cells, it is related to the level of vascular resistance.[160, 166, 167] Moreover a correlation has been established between muscle SNA and the level of plasma markers of sympathetic activity, such as plasma noradrenaline.[135, 137]

Additional parameters obtainable from the single-unit method include information on firing frequency, firing probability, the probability of multiple firing (e.g. the number of bursts per cardiac interval), recruitment (the number of active fibers) and frequency modulation.[168, 169] This approach can provide a more comprehensive evaluation of the potential for sympathoexcitation in pathological conditions.[170-173]

Direct recordings from sympathetic nerves in animal studies are usually done acutely on anesthetized models.[174] More advanced methods involve implantation of electrodes to allow longer-term recordings. These were first performed in rabbits[171, 175, 176] and now

extended to rats[177, 178] and mice.[179] This provides an opportunity for follow up data recordings in animal models of disease states. Such continuous recordings in rat have been obtained for periods longer than 3 weeks, which brings clear advantage for autonomic control research.[180]

2.3.5. Heart rate variability

HR variability provides a useful marker of cardiac autonomic control and a tool for prediction of outcomes in human studies. For example, in middle age and elderly men low HR variability predicts all cause mortality.[181, 182] In addition, post-myocardial infraction[183, 184] and chronic heart failure[185], a diminished HR variability has been shown to be an independent predictor of mortality.[183, 184] Many clinical conditions, including ageing and hypertension, are associated with reduced HR variability.[186-188] HR variability supplies indirect information on the cardiac autonomic regulation of sinus node, at baseline and in response to different physiological maneuvers. Admittedly, the precise contribution of the sympathetic and parasympathetic branches of the autonomic nervous system to HR variability has not been firmly established.[189]

Two main approaches are usually applied to evaluate HR variability: time domain methods and frequency domain methods.[190] Statistical and geometric approaches are both time domain methods. Only QRS complexes from individuals in sinus rhythm can be used to calculate successive normal-to-normal R-R intervals. One of the statistical parameters that can be calculated is the standard deviation of the normal-to-normal intervals (SDNN). SDNN is calculated as the square root of the variance in successive

normal-to-normal R-R intervals and equivalent to total power obtained from the frequency domain methods.[190] SDNN is influenced by the duration of recordings, only the same length record should be compared (5 min or 24 hours are commonly recommended), and it provides information on overall HR variability. Two other common parameters obtained from interval differences are the square root of the mean of the sum of successive differences in the normal-to-normal interval (RMSSD) and proportion of the number of successive normal-to-normal intervals that differ by more than 50 ms (pNN50%). Both RMSSD and pNN50% are considered as markers of parasympathetic activity.[190, 191]

The frequency domain method of HR variability provides information on the distribution of power (statistical variance) over the frequencies. The commonly used approach is based on fast Fourier transformation. Using short (5 min) recordings, three main frequencies determined: very low frequency range (<0.04Hz), low frequency (LF) range (0.04–0.15 Hz), high frequency (HF) range (0.15-0.4 Hz) and between (0.0-0.4 Hz) total power (TP). Interpretation of the very LF is much less robustly defined especially using 5 min recordings [190, 192] LF and HF components are described in absolute power spectral density and normalized units. Normalized units calculated as the proportion of each component to the TP minus very low frequency range. The HF component is principally a marker of parasympathetic activity,[190, 193, 194] while the LF is a marker of the interaction of sympathetic and parasympathetic activity.[191, 195, 196] There are data showing that the LF component does not relate to the spillover of noradrenaline from the heart and/or muscle SNA.[143] However some groups consider LF data to be reflective of the level of sympathetic activity.[197] In hypertensive patients, an increase in

LF and decrease in HF as compared to normotensives has been interpreted as an enhanced cardiac sympathetic activity and a reduced parasympathetic activity.[198] Beta-adrenergic blockade for 2 weeks with atenolol in hypertension resulted in a significant reduction in LF.[198] HR variability assessed using frequency domain methods have been proposed as a convenient non-invasive technique for monitoring of dynamic changes in sympatho-vagal balance in hypertension.[198] Spectral analysis of HR variability can also be used to evaluate the effect of antihypertensive agents on sympatho-vagal balance.[199]

The ratio of LF to HF is considered as an index of sympatho-vagal balance, with higher numbers indicating increased sympathetic activity and/or decreased parasympathetic activity.[200-202] It needs to be mentioned that some groups do not entirely agree with such interpretation of the ratio.[203, 204] Admittedly interpretation of results of HR variability findings could be ambiguous, partly due to the on-going dispute on the physiological meaning of changes in LF and HF.[203, 205] It is commonly agreed that the HR variability approach has important limitations to be considered as a straightforward and reliable tool for assessment of autonomic nervous status.

2.3.6. Summary

Despite several techniques being available to quantify the status of the autonomic nervous system, each approach has significant limitations and utilization of several methods can be recommended (if plausible) to provide more robust conclusions. Among the available techniques microneurography clearly benefits from being a minimally invasive method for direct assessment of sympathetic activity in real time. This method

is suitable for repeated measurement for monitoring of disease progression and response to therapeutic interventions.

2.4. Heightened sympathetic activity in disease

A number of cardiovascular disorders, and risk factors for their development have been associated with the heightened activation of the sympathetic nervous system. These conditions include myocardial infarction,[7] congestive heart failure,[8-10] metabolic syndrome,[206] and obstructive sleep apnoea.[207-209] Studies conducted on patients with essential hypertension confirmed the involvement of sympathetic system starting from the early stages of hypertension and persisting through its progression to severe and complicated forms. It was noted that there is an increase in noradrenaline spillover from the heart, kidneys, and brain in many patients with essential hypertension.[150, 151, 210-213] In addition, muscle SNA is elevated in individuals with borderline hypertension,[214] as well as in those with white coat hypertension[215] and sustained essential hypertension.[9, 140, 216-218] The degree of the sympathetic hyperactivation was related to the magnitude of the blood pressure raise.[11, 12] These observations imply pathogenic role of sympathetic system in hypertension. In fact, data indicate that heightened sympathetic activation often precedes hypertension.[13] In a longitudinal observational study with 20 year follow up, a high arterial adrenaline concentration strongly predicted future occurrence of hypertension.[13]

Evidence for the significant role of the sympathetic nervous system activation in the initiation and development of hypertension is vast.[9, 140, 214, 216-222] However, this theory is not universally accepted due to some inconsistency on the magnitude of sympathetic hyperactivity among different cohorts of hypertensive patients.[167 136, 223-225] This could partly be due limitations of the methods used for quantification of sympathetic

parameters, varying degree of imbalance of central vs. peripheral sympathetic hyperactivity. This is also due to the fact that a multitude of other factors is involved in the regulation of BP, such as cardiac output, endothelial function, neurohumoral status, salt sensitivity, and vascular adrenergic responsiveness.[226] Individual contribution of all those factors in a particular patient is often difficult to assess, and there is a complex network of excitatory and inhibitory mechanisms linking those pathogenic factors. The single unit recordings of the muscle SNA add knowledge to explain this inconsistency. Single unit recordings revealed that there is a significant increment in sympathetic activation in patients with early and mild hypertension than in severe hypertension, while increased multiunit muscle SNA was not different between hypertension stages.[173] Importance of local activity of the sympathetic system in organs implicated in BP control has been particularly highlighted in elderly hypertensive subjects who had consistently elevated muscle SNA by microneurography but did not always show elevated renal noradrenaline spillover.[146, 153, 218] This also emphasizes the importance of the direct sympathetic measurements as quantification of 'systemic' sympathetic activity might be misleading in some cases.

In summary, it is likely there is a causative association between increased SNA and hypertension (Figure 2.2).[221, 227] Good understanding of intimate details of pathways linking the heighten SNA and development of hypertension is important for establishing therapeutic targets and development of effective treatments.

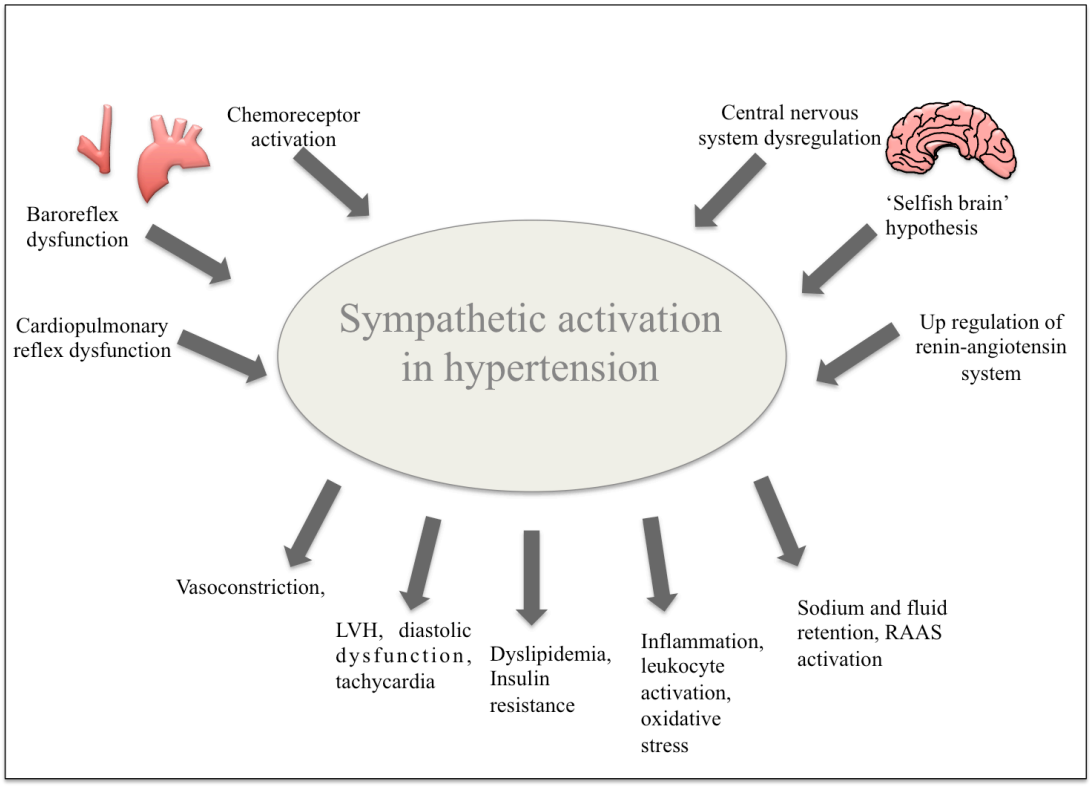

Figure 2.2. Schematic drawing of the potential mechanisms and consequences of sympathetic activation in essential hypertension

LVH, left ventricle hypertrophy; RAAS, renin-angiotensin aldosterone system.

2.5. Physiological and pathophysiological consequences of heightened sympathetic activity

2.5.1. Sympathetic nerve activity and the vasculature

Heightened vascular sympathetic nerve activity evokes complex structural and functional consequences. Chronic sympathetic activation can lead to arterial stiffening, vascular and cardiac hypertrophy and hypertension. Structural changes, such as vascular remodeling, reflect the long-term status of the SNA.[228, 229] Increased vascular smooth muscle tone secondary to high SNA activity increases arterial stiffness and aortic BP, and promotes deposition of collagen fibers.[230, 231] In addition, hypertrophy of vascular smooth muscle cells and functional and structural changes in endothelial cells occur. This results in increased wall-to-lumen ratio with unfavorable consequences to systemic hemodynamics, elevated resistance to flow, increased endothelial shear stress and ultimately atherogenesis.[24, 229]

Although high BP *per se* could mediate a catecholamine-related vascular hypertrophy, BP independent changes in the vasculature as a direct consequence of heightened SNA are now well acknowledged.[229, 232] Morphological studies conducted on animals confirmed that chronic sympathetic drive leads to structural changes of vascular smooth muscle cells (i.e., hypertrophy and proliferation) even despite maintenance of normal BP.[232] Moreover these effects could be inhibited by sympathetic denervation. Exogenous administration of norepinephrine for 2 weeks in rats increased vascular production of collagen and elastin.[233] Antagonism of endothelial A receptors promptly

and effectively reversed norepinephrine-induced aortic structural and compositional changes, suggesting a central role of endothelin in mediating this response.[233] These data indicate that SNA-related vascular effects are partly attributed to stimulation of endothelin, a potent vasopressor and regulator of multiple functional pathways within the vascular wall.

Along with changes in vascular structure, studies in humans show that sympathetic activity reduces arterial distensibility. Higher sympathetic activity in postmenopausal women was positively correlated with increased arterial stiffness assessed by augmentation index.[234] A similar association was also confirmed in a study of young men.[235] Stiffening of large elastic arteries impairs the buffering function of the arterial system and predisposes to cardiovascular disease.[236] In addition, the increased stiffness of larger arteries is associated with the age-related impairment of cardiovagal baroreflex sensitivity and arterial baroreflex control of muscle SNA in both men and women.[237] In this study a lower level of arterial baroreflex control of muscle SNA was particularly prominent in older women, which may be implicated in the higher prevalence of hypertension in this age-gender category.[237]

High carotid-femoral pulse wave velocity (PWV), an index of central arterial stiffness, is a strong independent predictor of cardiovascular morbidity and mortality. In the Framingham Heart Study, the addition of PWV to the cardiovascular risk stratification models improved risk prediction compared to conventional risk factors used alone.[236] PWV thus represents a useful and easy to obtain marker of risk of cardiovascular disease.[236] In healthy men, high muscle SNA predicted increased PWV independently

of age, BP and body mass index.[238] Of note, individuals with abnormally high PWV had significantly higher muscle SNA compared to individuals with normal PWV despite similar systolic BP.[238] There was a significant inverse association between muscle SNA and arterial stiffness in patients with systolic heart failure.[25] Furthermore, activation of sympathetic system in this population by cigarette smoking or phenylephrine significantly decreased radial artery distensibility.[25]

The acute removal of SNA, by direct anesthesia of the sympathetic nerves of upper and lower extremities in healthy subjects and pathological conditions, leads to increased arterial distensibility with no alteration in BP or HR, and no change in the properties of the contralateral control artery.[24] Clinical support of these observations comes from a study of patients undergoing hand transplant,[239] where radial artery distensibility was observed to be much higher in the transplanted and thus denervated radial artery, than in the contralateral radial artery. However, radial artery distensibility increased to match those in the contralateral hand 4 months after the surgery, once signs of reinnervation appeared.[239] These data accord with animal studies demonstrating that both surgical and pharmacological disruption of sympathetic innervation led to significant improvement in parameters of arterial distensibility.[240, 241]

Acute increases in sympathetic activity, secondary to mental stress or other triggers of sympathetic discharge (e.g., cold pressor test or lower body negative pressure) increased arterial stiffness in healthy volunteers.[242, 243] These observations are also relevant to the cardiovascular morbid states associated with increased sympathetic drive, such as heart failure.[25] However, acute effects of catecholamines (e.g., infusion of phentolamine or

application of negative pressure to lower body) on arterial stiffness appear to be less prominent in older healthy individuals.[244, 245] This likely reflects more prominent background arterial stiffness in older subjects with larger deposition of connective tissue/collagen and consequently lesser contribution of catecholamine-dependent smooth muscle tone to the overall stiffness.

In addition to the structural composition of the blood vessel, endothelial function is an important determinant of arterial stiffness. The endothelium plays a key role in the regulation of multiple biological processes related to vascular function. For example, endothelium-derived signals are involved in regulation of balance of vasodilatation vs. vasoconstriction, inflammatory and anti-inflammatory processes, pro- and antithrombotic actions. The interactions between endothelial activity and the sympathetic nervous system are often less appreciated but (patho)physiologically and clinically relevant.[246, 247] In a healthy and relatively young normotensive cohort the reactive hyperemic index (an index of endothelial function) was significantly and inversely associated with muscle SNA.[248]

Given that arterial stiffness is dependent on arterial smooth muscle tone, endothelium-dependent regulation of vascular tone has a direct implication on the arterial elastic properties. These effects are perpetuated by a vicious circle of increased vascular tone and endothelial dysfunction leading to increased arterial stiffness and increased arterial stiffness further contributing to endothelial dysfunction. The invasive assessment of arterial compliance and measurement of PWV in young healthy individuals have confirmed the capacity of NO to improve elastic characteristics of peripheral arteries.[249]

Although acute NO-dependent effects on arterial elastic properties are primarily related to changes in smooth muscle tone, impairment of endothelium-dependent function is also involved in sustained arterial stiffening.[247] For example, diminished NO availability is related to higher platelet activity that stimulates accumulation of proinflammatory leukocytes and smooth muscle cell proliferation.

The interaction of endothelial dysfunction and heightened sympathetic activation is likely to be of considerable clinical significance although direct assessment of those interactions in clinical settings is complicated by the multiple other factors involved. Vast amounts of data highlight the importance of endothelial dysfunction in pathogenesis and prognostication in cardiovascular disease, particularly in heart failure.[250-252] The magnitude of endothelial dysfunction in coronary arteries parallels the degree of left ventricle (LV) hypertrophy, a cumulative surrogate parameter of duration and severity of hypertension.[253-255] Several studies have also demonstrated the presence of endothelial dysfunction in patients with hypertension, a recognised risk factor for both ischemic heart disease and heart failure.[256-259] Of interest, presence of endothelial dysfunction of peripheral arteries often precedes occurrence of overt cardiovascular pathology.[260-262]

Another mechanism that links excessive SNA and endothelial dysfunction to atherogenesis and hypertension are the increased arterial shear stress due to vasoconstriction promoted by sympathetic hyperactivity.[247] Vasoconstriction puts an additional strain on the endothelium to provide adequate vasodilation. This may be critical at sites with naturally occurring higher shear stress, such as at arterial

bifurcations/branching points. These parts of the arterial tree are particularly prone to development of the atherosclerotic plaques. Among a group of young healthy males a higher muscle SNA was shown to be associated with more prominent NO generation.[263] It has thus been speculated that an inability to sufficiently enhance NO production in settings of sympathetic hyperactivity could be responsible for the net impairment of the vasomotor responses, resulting in abnormal peripheral resistance and hypertension.[263] Increased SNA induced by sympatho-excitatory maneuvers impairs flow mediated endothelial-dependent vasodilatation in healthy subjects[264, 265] and patients with chronic heart failure.[266] Furthermore, in vivo animal experiments show that NO can directly inhibit SNA.[267] In healthy subjects, systemic inhibition of NO production by NG-Monomethyl-L-arginine, monoacetate salt (L-NMMA) resulted in rise in peripheral resistance and a dose-dependent elevation in BP and reflector decreased in SNA and cardiac output.[268] The mediated BP elevation was particularly prominent in individuals who already had tendency towards the higher BP at baseline.

The magnitude of involvement of endothelial dysfunction in the pathogenesis of hypertension has not been firmly established. Some data indicate that endothelial dysfunction precedes developments of hypertension and may be one of the pathogenic mechanisms of essential hypertension.[269] However, a causative relationship between essential hypertension and flow-mediated dilation has not been firmly established. For example, the Framingham Heart study failed to confirm the causation.[270] This discrepancy may at least partly be due to the presence of other modulators of endothelial and overall vascular function. Heightened sympathetic tone is likely to be a major contributor to the net impact of endothelial dysfunction to the development of

hypertension in an individual. Multiple other factors are known to affect the magnitude of flow-mediated dilation, including recognised cardiovascular risk factors, such as hypertension, smoking, hypercholesterolemia.[271, 272] The multifactorial nature of endothelial dysfunction makes it difficult to firmly establish the independent pathophysiological role of endothelium in hypertension and precise impact of the sympathetic overactivation in this association.

In summary, the available data support a direct role of high SNA in arterial stiffening, pathologic remodeling, and endothelial dysfunction. These effects likely contribute to the pathogenesis of various cardiovascular disorders, including atherosclerosis, heart failure, and hypertension. However, these interactions are complex and still not fully understood.

2.5.2. Sympathetic nerve activity and the heart

Excessive SNA has been shown to affect both cardiac structure and function. In vivo studies of even sub-pressor doses of noradrenaline show direct hypertrophic effect of catecholamines on cardiac myocytes and net increase in LV thickness and mass.[273, 274] Moreover, a strong independent association has been identified between high sympathetic activity, quantified either by measurements of the plasma catecholamines or muscle SNA, and the development of LV hypertrophy in subjects with essential hypertension.[14-16] Interestingly, long-term (i.e., over 20 years) observational studies of middle age men with arterial plasma noradrenaline levels measured at baseline showed significant independent predictive value of high catecholamine levels with increased LV

mass, after accounting for systolic BP and body mass index.[275] Similar observations have been made in studies of patients with chronic kidney disease; where higher baseline muscle SNA levels were associated with higher LV mass over a 9-year follow up, despite optimal BP control.[276]

A study of normotensive siblings of hypertensive parents has provided further insight into the role of an imbalance in the autonomic system in hypertension by the demonstration that low parasympathetic activity, as well activation of sympathetic nervous system (assessed using HR variability analysis) were implicated in the progression of prehypertension into overt hypertension.[277] These findings accord with a study showing that presence of LV hypertrophy in hypertension was linked with features of inhibition of the parasympathetic system (assessed using HR variability analysis) and thus relative dominance of the sympathetic drive in regulation of cardiac processes.[17]

Collectively, these data have significant clinical implications given the well-documented role of a LV hypertrophy in prognostication. For example, higher LV mass was independently related to occurrence of cardiovascular disease, cardiovascular and all-cause mortality in the Framingham Heart Study.[278]

An abnormally high sympathetic activation can also have functional consequences for the heart. High muscle SNA is associated with LV diastolic dysfunction independently of BP levels[18, 19] and arterial baroreflex control of muscle SNA is more greatly attenuated in hypertensive patients with diastolic dysfunction than in those with normal

diastolic function.[18] In view of the negative impact of LV diastolic dysfunction on cardiovascular morbidity and mortality in hypertension, detrimental effects of catecholamine on diastolic function likely represent another pathogenic mechanism of sympathetic hyperactivity. The mechanisms responsible for the sympathetically-mediated diastolic dysfunction are not entirely clear but appear to be attributable to both increased myocardial stiffening and impaired (delayed) relaxation of cardiomyocytes.[279, 280] Increased myocardial and arterial stiffness are secondary to (i) accelerated extracellular matrix turnover with collagen deposition, (ii) increased tone of the cardiomyocytes and (iii) cardiomyocyte hypertrophy.[281] The therapeutic utility of these findings is still to be firmly established, as large-scale trials of sympatho-inhibition on outcomes specifically in patients with hypertension and predominantly catecholamine related diastolic dysfunction are lacking.

2.5.3. Sympathetic nerve activity and the kidney

The kidneys play a major role in the regulation of BP, and they serve this purpose by complex coordination of neuro-humoral pathways. In addition to the production of renin and regulation of renin-angiotensin-aldosterone system the kidneys have a rich sympathetic innervation. Sympathetic hyperactivity is evident in many hypertensive patients with renal dysfunction and successful antihypertensive treatment in these individuals seems to parallel reductions in SNA.[282] Recently the role of renal SNA in hypertension has attracted particular attention due to the initial success of renal denervation therapy.[154, 155] Development of this treatment approach was based on strong evidence for the role of the sympathetic system in elevating renal vascular resistance in

hypertension, and direct involvement of renal afferent signaling to central nuclei, sympathetic activation, and up-regulation of the renin-angiotensin-aldosterone system.[283]

The renin-aldosterone-angiotensin system is of particular importance in the pathogenesis of hypertension, and high plasma renin activity has been independently related to increase in muscle SNA.[282] The renin-angiotensin-aldosterone system has become the key target for pharmacological intervention in hypertension and the contribution of the sympathetic system to kidney related BP increase is of paramount importance. For example, high sympathetic renal activity leads to constriction of afferent renal arterioles accompanied by a reduction in glomerular perfusion; changes in electrolyte handling by the kidneys, including increases in tubular reabsorption of sodium and water balance disturbances, leading to water retention.[284, 285] All those changes invariably result in activation of the renin-angiotensin-aldosterone system and are further exacerbated by the sympathetic activation, thus completing the vicious cycle.[286] This exaggerated interaction between the renin-angiotensin-aldosterone system and the sympathetic nervous system is an important mechanism responsible for the chronicity of the BP increase (i.e., hypertension). The pro-hypertensive interactions between the sympathetic nervous system and the kidneys are further amplified by direct damage to renal tissue related to the high SNA. For example, animal experiments show that pharmacological inhibition of the central sympatholytic drive reduces occurrence of glomerulosclerosis irrespectively of BP.[287]

The clinical utilization of renal denervation therapy was hampered by the disappointing results of the randomized SYMPLICITY HTN-3 trial.[288, 289] Admittedly the trial results do not necessarily imply lack of relevance of the sympathetic inhibition in hypertensive patients but rather the need for identification of the appropriate candidates to such treatment and/or development of better treatment options (both interventional and non-interventional).[288, 290, 291]

2.5.4. Metabolic effects of sympathetic nervous system

An association between abnormal sympathetic drive and presence of metabolic disturbances has been established. Associations have been found between the sympathetic system and presence of dyslipidemia and insulin resistance.[292] Although mutual relationship between those factors are sophisticated it has been shown that high circulating levels of insulin in those with insulin resistance (e.g., in obesity) activate central sympathetic nervous system.[293]

Given the well-known role of sympathetic activation in the pathogenesis of hypertension this pathway at least partly explains close association between the components of the metabolic syndrome, such as insulin resistance/diabetes, increased body mass index and hypertension. Muscle SNA is increased in overweight individuals, even if BP remains within the normal range.[294] Activation of sympathetic nervous system in obesity is likely to have a pathogenic role in hypertension. For example, in the Framingham Heart study obesity was strongly predictive of future hypertension during 30-year follow up.[295] The hypothesis that the sympathetic nervous system links

the metabolic syndrome and hypertension is further supported by studies in healthy humans showing that insulin infusion stimulates SNA independently of insulin's vasodilatory effects.[296, 297]

It is difficult to establish the precise reciprocal associations between the sympathetic system activation and metabolic abnormalities. It is likely that their impact is mutual with a 'vicious circle' of self-perpetuation existing and predisposing to hypertension.[298] In addition to the well-documented impact of metabolic abnormalities in evoking sympathetic activation, excessive catecholamine release increases pancreatic insulin production.[299] In patients with metabolic syndrome muscle SNA promoted progression from impaired glucose tolerance to overt type two diabetes.[300]

SNA may also contribute to insulin resistance by causing chronic vasoconstriction by skeletal muscles and consequently restricting glucose clearance by the muscles, which requires more insulin to be released to overcome the effect.[298] In contrast, pharmacological vasodilation significantly improves insulin sensitivity.[301, 302] However, clinical utilization of such approach is likely to be limited by a compensatory baroreflex-mediated increase in SNA in response to the vasodilatation.

2.5.5. Interaction with the immune system

The immune system plays a pivotal role in the regulation of inflammatory process, modulation of tissue remodeling and regulation of oxidative stress. To achieve this, the immune system interacts with humoral (e.g., hormones) and nervous (e.g.,

catecholamines) systems. The complexity of those interactions allows fine adjustments of the system to meet the homeostatic needs. In this regard, the nervous control is particularly important for very fast changes as opposed to more prolonged responses mediated through humoral mechanisms.

Mutual impact of the immune and autonomic nerve systems is increasingly recognized and a mechanism of 'inflammatory reflex' has been suggested. This term describes neural circuit that includes afferent and efferent neurones of the vagus nerve that contribute to regulation of immune responses.[303]

The autonomic nervous system possesses mechanisms of sensing the presence of cytokines, bacteria and components of cell damage. Although not all details of this sensing are completely understood the receptor apparatus appears to be generally similar to that in the immune system *per se*. As such, immune cells (such as monocytes and lymphocytes) may serve as a link between the immune and neural interactions by producing cytokines and sensing catecholamines. It is also likely that the autonomic afferents can possess receptors that are sensitive to inflammatory molecules. However, the extent and variability of their expression need further investigation. Animal experiments clearly show that an up-regulation of circulating inflammatory cytokines leads to activation of the sympathetic system.[304, 305] Once activated by immune stimuli, sympathetic signals rapidly spread to central regulatory nuclei allowing for a prompt response aimed at counteracting the pathological changes (e.g., tachycardia to increase cardiac output in the setting of peripheral vasodilation seen in sepsis). These homeostatic adjustments mediated by the sympathetic system are fast as compared to

many responses produced by the immune system. They give time for the immune system to provide more specific actions. These autonomic signals from the brainstem nuclei also provide efferent outflows to the organs of the immune system, such as the spleen, lymph nodes, and reticuloendothelial system.

Catecholamines can also directly interact with specific receptors on cells of the innate and adaptive immune systems, such as monocytes/macrophages and lymphocytes. For example, lymph nodes and the spleen, reach in T-lymphocytes are have been shown to have dense innervation by nerves, and the sympathetic stimulation leads to T cell activation and prolipheration.[306-311] High sympathetic activity can stimulate the generation of inflammatory cytokines. Animal experiments indicate that catecholamine induced inflammation is pathophysiologically linked to hypertension and development of heart failure.[312, 313] In normal rats the subfornical organ of the brain has been shown to link central sympathetic activity and formation of inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β.[314]

Inflammation in hypertension can be promoted by the renin-angiotensin-aldosterone system, with the particular role of angiotensin II. Angiotensin II promotes oxidative stress by triggering production of the reactive oxygen by nicotinamide adenosine dinucleotide phosphate (NADPH) in immune cells.[315] Inflammation is also supported by angiotensin II.[310] Delivery of T cells lacking angiotensin type I receptor or transfer of functional NADPH oxidase, reduced superoxide generation, and hypertension in response to high angiotensin II infusion. These findings suggest that the development of angiotensin II-mediated hypertension may be partly explained by activation of NADPH

oxidase and increased sympathetic activity.[316] In T lymphocytes, NADPH oxidase also promotes their activation, cytokine production, and migration to tissues thus enhancing effects of sympatho-immune interactions.[317, 318]

In summary, an abnormally high sympathetic drive and diminished parasympathetic activity may result in the pro-inflammatory milieu, which serves to further stimulate SNA.

2.6. Mechanisms for increased central sympathetic activity in hypertension

2.6.1. Introduction

The central nervous system is closely involved in regulation of sympathetic activity and BP and pathogenesis of hypertension.[319] The sympathetic nervous system represents a principle mechanism mediating signals from the central nervous system and BP control. As discussed in previous sections different parts of the brain, including the brain stem, NTS, and the RVLM play a particularly important role in the regulation of BP via the sympathetic nervous system.[320] The fact that majority of patients with hypertension are classified as having essential hypertension highlights the diversity of mechanisms and pathological pathways responsible for dysbalance in BP control. Many of those pathways are mutually linked and amplify pathological effects caused by other mechanisms.

2.6.2. Role of central oxidative stress

The role of brain oxidative stress in the pathogenesis of hypertension has been suggested by experiments in spontaneously hypertensive rats.[320-322] Reactive oxygen species (ROS) in the RVLM have been shown to amplify glutamatergic-mediated excitation and reduce GABA-mediated inhibitory effects, thus enhancing overall sympathetic drive.[320] Also, in salt-sensitive hypertension rat model excessive oxidative stress in the brain increased sympathetic activity and caused hypertension via activation of reduced nicotinamide-adenine dinucleotide phosphate oxidase (NAD(P)H)).[323]

Cytochrome oxidase activity is a parameter of longer-term brain neuronal activity that reflects local metabolism and oxidative capacity.[324] Cytochrome oxidase activity is increased in spontaneously hypertensive mice, especially within the central autonomic network.[324] Of note, there is a clear heterogeneity in the cerebral distribution of cytochrome oxidase activity, with areas responsible for high-order autonomic control, such as insular cortex and the hypothalamic nuclei, being particularly affected.[324] Superoxide dismutase represents another potent endogenous antioxidant. In rats administration of tempol, a superoxide dismutase mimetic, doubled vascular superoxide dismutase production in spontaneously hypertensive rats, such that it reached levels seen in normotensive animals.[325] Accordingly, treatment with tempol reduced mean BP by 28%, HR by 16% and integrated renal SNA by 63% with the magnitude of these changes being significantly greater in hypertensive than in normotensive rats. Of note, in spontaneously hypertensive rats reductions in renal SNA during administration of superoxide dismutase mimetic, tempol, were strongly correlated with changes in mean BP ($r=0.85$, $P<0.0001$).[325]

Tempol reduces BP when delivered either intravenously or intracerebroventricularly, but the mechanisms by which tempol lowers BP differs depending upon the mode of administration.[326] For instance, tempol reduced norepinephrine secretion from the posterior hypothalamus and renal SNA when infused intracerebroventricularly but raised norepinephrine secretion from the posterior hypothalamus and increased renal SNA when infused intravenously.[326] The effects of intravenous tempol on SNA were

inhibited by sinoaortic denervation indicating involvement of oxidative stress related changes in baroreflex function and the role of superoxide dismutase in its regulation.[326]

Endogenous adrenomedullin is an antioxidant peptide that inhibits central sympathetic activation via its antioxidant properties.[327] Hyperosmotic saline-induced ROS production in the hypothalamus was more pronounced in adrenomedullin knockout mice compared to wild-type animals.[327] These data indicate presence of an intrinsic cerebral imbalance in pro- and anti-oxidative processes in spontaneously hypertensive mice and suggest the role of these abnormalities in sympathetic disturbances and BP elevation in this model.[324]

Several experimental studies suggested the therapeutic potential of antioxidants in hypertension. For example, inhibition of the oxidative stress by chronic antioxidant treatment of rats with renovascular hypertension significantly decreased sympathetic activity and BP levels which were associated with diminished expression of angiotensin II type 1 (AT1) receptor.[328] Furthermore, acute treatment with vitamin C in essential hypertension significantly reduced cardiac adrenergic activity and improved baroreflex sensitivity.[329] However, the suitability (effectiveness and safety) of long-term antioxidant treatment in hypertension needs to be tested in controlled trials.

Angiotensin system is involved in multiple pathogenic pathways in hypertension, including regulation of the ROS release. Peripheral and central oxidative stress are interacting and amplify the hypertensive effects of angiotensin.[330] High levels of angiotensin II in the PVN generation of the ROS and enhance the cardiac sympathetic

afferent reflex.[331] Angiotensin II administered into the lateral ventricle of the brain involved in the noradrenergic control of BP enhances oxidative stress, activates the sympathetic nervous system and increases BP,[332] via an AT1 receptor mechanism.[330]

In a rat model of renovascular hypertension decreases in BP by inhibition of AT1 receptors with losartan, resulted in a reduction of oxidative stress in the RVLM.[333] Deletion of ACE2 gene was shown to enhance the age-related increase in ROS and led to autonomic dysfunction and hypertension.[334] In contrast ACE2 gene therapy targeted to the PVN attenuated oxidative stress via inhibition of NADPH oxidase and improvement in cardiac autonomic function (defined by responsiveness in HR and BP to propranolol, atropine, and chlorisondamine).[334] It is speculated that clinical benefits of AT1 receptor inhibitors could be partly attributable to the reduction of brain oxidative stress.[321] Of note, angiotensin II stimulation also increases NO production that may limit angiotensin II-related sympathetic activation, thus represents an important regulatory negative-feedback mechanism.[330]

NO can act as a central neurotransmitter and can regulate SNA and consequently BP.[335] Local NO deficiency may increase SNA and promote the development of hypertension.[335] It has been demonstrated that interaction between NO and superoxide dismutase mimetic, tempol contributed to the biological effects of the drug.[336] Tempol increased the levels of nNOS in the posterior hypothalamus, PVN, and locus coeruleus when infused intracerebro-ventricularly, but it decreased the levels of nNOS when infused intravenously.[326]

The available data support the hypothesis that ROS may raise BP via sympathetic nervous system activation. This effect is modulated by central angiotensin, nNOS, and NO availability.[326] The discrepancies in responses to tempol may be attributable to the route of administration, with an inhibitory effect of the drug on SNA being evident when it is given intracerebrally, versus a vasodilatory response when given intravenously.[326]

2.6.3. Role of central nitric oxide

NO is a principle regulator of cardiovascular function and expresses its actions through a multitude of mechanisms. Peripheral vascular vasodilatory effects and antithrombotic properties have attracted particular attention. However, NO also plays a significant role in the central regulation of SNA.[96] Endogenous NO provides a significant input in maintenance of basal sympathetic tone by contracting excitatory reflex responses.[337]

NO is produced in a process involving NOS and the amino acid, L-arginine as a substrate. Several forms exist in both mammal animals and humans, which accounts for the diversity of NO functions and its activity within different organs. eNOS is localised within vascular endothelium and largely responsible for peripheral effects of the NO (e.g., vasodilation, antiplatelet and anti-inflammatory properties). nNOS is predominantly localised in brain structures with an uneven distribution and activity.[338] iNOS is not normally expressed under physiological conditions but its high levels are related to pathological states, such as sepsis, where it strongly contributes to the development of excessive oxidative stress.

The brain, particularly the medulla and hypothalamus, contains autonomic inhibitory (nitrergic) nerves, which transfer regulatory signals to the peripheral tissues, including heart and vessels.[338] These signals strongly contribute to BP control.[338] For example, stimulation of the nitrergic nerves that supply signals to the peripheral vasculature reduce arterial and venous resistance (especially targeting small calibre vessels) and lower BP.[338] NO plays a major role in the adrenergic system by enhancing the activity of neuronal uptake of norepinephrine in sympathetic nerve terminals.[339]

Cervical spinal cord transection inhibits abnormal sympathetic drive and reduces hypertension caused by NO inhibitors.[340] This supports the hypothesis that in addition to its ability to reduce BP by peripheral vasorelaxation (i.e., peripheral effects) NO also has the ability to reduce BP via its effects on the central nervous system.[340] As discussed above the central effects of NO are predominantly mediated by a reduction in sympathetic vascular tone.[340] Two decades ago it was noted that acute central administration of a NOS inhibitor increased BP in rats, and this increase could be abolished by central administration of L-arginine.[341] These data were complemented by finding that different endogenous NOS inhibitors (N(omega)-nitro-L-arginine methyl ester (L-NAME) and asymmetric dimethylarginine (ADMA)) significantly increase SNA.[342] Long-term NOS inhibition achieved by administration L-NAME resulted in sodium-sensitive hypertension with increased SNA.[343] However, brain sympathetic inhibition mediated by nNOS was in fact up-regulated in salt-sensitive hypertensive rats, which could probably precede baroreflex sympathetic inhibition in this model.[344]

Brain NO plays a principal role in inhibition of sympathetic vasoconstriction associated with baroreflex.[345] NO-dependent central SNA modulates a magnitude of baroreflex-mediated responses (i.e., in HR and BP).[346, 347] However, there appear to be some highly specific pathways directing selected functions of the baroreceptors.[346] In an anesthetized animal model basal NO release plays a significant role in the tonic BP regulation, but it does not seem to be important for the dynamic sympathetic modulation of BP or HR at least in this experimental model.[348] Administration of either L-arginine or showed that NO modulates efferent SNA via central nervous system without changing the afferent or efferent pathways of the baroreceptor reflex arc.[349] Also administration of L-arginine to healthy volunteers increased NO synthesis, reduced BP, but increased HR and SNA, thus supporting the role of NO in the tone of central sympathetic outflow in humans.[350]

Accumulating data testing relation of inhibition of brain NO synthesis with the pathogenesis of hypertension reveal a complex network of interactions with the development of hypertension being largely renal nerve dependent and mediated by the integrity of the renal nerves.[351] These findings are relevant to the recent (although somewhat controversial) success of the renal denervation therapy in patients with hypertension. Of interest, the sympathetic nervous system plays a role in the regulation of release of vascular NO thus representing an important feedback mechanism for fine tuned balance of pressor and depressor mechanisms.[352] While administration of the NOS inhibitor increases BP and decreases renal SNA, treatment with an ACE inhibitor or an AT1 receptor blocker reduced BP and increased SNA, which was associated with blunted baroreceptor reflex function.[353]

2.6.4. Changes in rostral ventrolateral medulla in hypertension

RVLM contains NOS-producing neurones, which can be activated through cardiac sympathetic excitatory reflexes.[89] nNOS-derived NO is implicated in the transmission of sympathetic baroreflex in the RVLM, which is at least partly mediated by soluble guanylate cyclase-dependent, superoxide-independent mechanism.[354] This neuronal NO-mediated suppression of tonic peripheral sympathetic drive is amplified by salt load in both salt-resistant and salt-sensitive rat models and the mechanisms are thus may be particularly relevant to salt-sensitive hypertension.[355]

In contrast to nNOS, overexpression of iNOS in the RVLM increases BP through upregulation of oxidative stress and resultant sympathetic nervous system activation.[356] Although eNOS does not directly play a role in RVLM activity, eNOS gene transfer to the RVLM to induce eNOS overexpression in rats resulted in an inhibition of SNA, bradycardia and a lowering of BP.[357, 358] These responses are mediated by an enhanced release of gamma-amino butyric acid (GABA) in the RVLM.[358] In contrast to anesthetized rats, NOS activity facilitates renal SNA in conscious rats.[359] The mechanism for the sympatho-excitatory effect of NO are not entirely clear but appear to be partly dependent on the activity of endogenous angiotensin II (in low-sodium animals only).[359] In hypertension models angiotensin II also activates AT1 receptors in RVLM, while their inhibition led to a reduction in SNA and BP.[88] Indication of that overactivity of the angiotensin system in the RVLM can be implicated in hypertension. Indeed, the bilateral injection of losartan into the RVLM in rats reduced both renal SNA and BP.[360]

The dorsomedial medulla is also involved in the pathogenesis of hypertension, but perhaps to a lesser extent than the RVLM. In animal experiments injection of glutamate into this brain area led to a hypertensive response which was paralleled by an increase in SNA.[361] Vasopressor mechanisms induced by glutamate are regulated by changes in NO levels in the dorsomedial medulla and RVLM, indicating the role of NO glutamate-activation pathways.[361] Injection of glutamate into the dorsomedial medulla and RVLM increased BP and vertebral SNA.[361] Vasopressor mechanisms induced by glutamate are regulated by changes in NO levels in the dorsomedial medulla and RVLM, indicating role of NO glutamate-activation pathways.[361]

2.6.5. Changes in nucleus tractus solitarius in hypertension

It has been shown that adenosine is involved in BP controlling baroreflex responses within the NTS with net effects of reduction of BP, HR, and renal SNA.[362] These adenosine effects in the NTS are NO and eNOS-dependent, mediated by activation of mitogen-activated protein kinase/extracellular signal-regulated kinases 1 and 2 and can be inhibited by NOS blockers.[362] In rats gene transfer induced overexpression of eNOS in the NTS inhibits SNA and reduces BP and HR.[357, 363]

2.6.6. The paraventricular nucleus

A negative feedback pathway between NO generation and glutamate-mediated system exists within the PVN, which has been implicated in regulation of the sympathetic drive

in both physiological and pathological conditions.[92] However precise contribution of the PVN (as well other nuclei discussed above) in hypertension in humans has not been firmly established and it has not become a specific target for the currently available therapeutic interventions.

2.6.7. Changes in sympathetic preganglionic neurones in the spinal cord in hypertension

High NOS levels have been demonstrated within sympathetic preganglionic neurones in the spinal cord, where NO is released in the process of the synaptic activity.[364] Excitation of renal sympathetic neurones by descending inputs from the PVN, is modulated by the inhibitory effects of NO provided through glycine interneurones at a spinal level.[364]

2.6.8. Role of arterial baroreflex in hypertension

The primary function of the arterial baroreflex has traditionally been viewed as short-term BP regulation. The role of arterial baroreflex in longer-term BP regulation is still debated, and a consensus has not been reached.[365, 366] Excessive sympathetic stimulation can sensitize the arterial baroreceptors, but the importance of this for long-term BP elevation is not clear. Both animal and human studies have consistently documented that sustained high BP impairs baroreceptor function.[367-372] Decreased baroreflex sensitivity may also occur as the result of structural changes in large arteries and baroreceptor resetting in aging, hypertension and atherosclerosis.[373, 374 375] Changes in

baroreflex sensitivity could also reflect functional changes in the reflex arc at the level of the peripheral sensory receptors and within the central nervous system.[376] Arterial baroreflex function is preserved in spontaneously hypertensive rats as compared to control rats and independent from BP level.[377]

The association between hypertension and arterial baroreflex dysfunction can also be partly due to the fact that even physiological aging is accompanied by a significant reduction in the baroreflex buffering capacity.[378] This phenomenon could be more prominent in hypertensive subjects of advanced age and affect responsiveness to antihypertensive treatment in this age category.[379] Additionally, the efficiency of the baroreflex may be influenced by female sex hormones and potentially protective hormonal effects seen in young women may be aborted post menopause.[380, 381]

It seems plausible that influences of baroreflex control on renal SNA may contribute to the pathogenesis of hypertension. Characteristics of arterial baroreflex control of renal SNA were similar in spontaneously hypertensive and normotensive rats.[382] Although angiotensin II-induced hypertension in rabbits significantly affected cardiac baroreflex sensitivity, but no noticeable change was evident in the relationship between mean BP and renal SNA.[383] Physiological baroreflex has opposing long-term effects on renal SNA to those produced by angiotensin II, but abnormalities in both systems are seen in hypertension.[384] This latter observation does not, however, prove that baroreflex abnormalities have a primary or pathogenic role in hypertension. In fact, direct experiments showed that aldosterone does impair baroreflex function in normal

volunteers and baroreflex dysfunction in hypertension could thus be secondary to activation of the renin-angiotensin-aldosterone axis.[385]

Experiments on rats with chronic intermittent hypoxia indicate that inappropriate resetting of the sympathetic and cardiac baroreflex control, rather than reduced baroreflex sensitivity can predispose to hypertension related to hypoxia, the setting commonly seen in hypertensive subjects with obstructive sleep apnea.[386]

Baroreflex inhibition of muscle SNA was found to be diminished in adolescents with a family history of hypertension before they had any evidence of hypertension thus leading to a possibility that familial predisposition to hypertension might be partly related to insufficient sympatho-inhibition secondary to inherited baroreflex dysfunction.[387]

The baroreflex plays a recognised role in the control of BP and its dysfunction may be involved in the pathogenesis of hypertension. This is important because reduced cardiovagal baroreflex sensitivity independently predicts all-cause mortality in hypertension as well as cardiac mortality after myocardial infarction, a condition associated with functional alterations in the autonomic nervous system.[388, 389] However, cardiovagal baroreflex sensitivity and arterial baroreflex control of muscle SNA are differentially controlled and do not always change in parallel.[55] This means that both arms of the baroreflex function have to be assessed in order to draw conclusions regarding the impairment or restoration of baroreflex sensitivity following a treatment/intervention.

As a summary, despite clear involvement of the arterial baroreflex in the regulation of BP and multiple lines of evidence showing impaired baroreflex function in hypertension, the causative relation between baroreflex abnormalities and long-term BP increase in essential hypertensions have not been unequivocally proven yet. Such information would be of clear benefit for the further development of new treatments for hypertension.

2.6.9. Role of chemoreflex in hypertension

Abnormalities in peripheral chemoreceptor function have been linked to several cardiovascular conditions, including hypertension.[390-393] Increased sensitivity of arterial chemoreceptors, particularly those located in carotid bodies, has been shown to increase muscle SNA, total peripheral resistance, renal vascular resistance and BP in patients with hypertension and animal hypertension models.[367, 391, 393-397] Studies of human volunteers with borderline or established hypertension, and of spontaneously hypertensive rats, have shown the presence of hyperventilation at rest with its further augmentation and increase in SNA in response to hypoxia.[393, 396]

The mechanisms by which chemoreceptor sensitivity increases in hypertension are still debated. Hypertrophy of the carotid bodies has been reported in spontaneously hypertensive rats and confirmed in patients with hypertension.[398-400] One may speculate that these changes reflect an attempt to compensate for brain hypoxia secondary to hypertension related arterial remodeling and vasoconstriction. These changes may thus be adaptive in nature and, in fact, they resemble changes seen in individuals living at high altitude.

In rats exposed to intermittent hypoxia, augmentation of basal and chemoreflex-stimulated sympathetic outflow occurs, at least in part, via activation of the renin-angiotensin system, whereas expression of neuronal nitric oxide synthase was reduced.[401] Endothelin-1 may modulate chemosensitivity by evoking a potent vasoconstriction within the carotid body vasculature thus reducing the threshold for

chemoreceptor activation and increasing the magnitude of their response to hypoxia.[402-404] However, the relevance of these findings to the management of patients with hypertension and their independent predictive value for outcomes in these settings remain unclear.

2.6.10. The 'Selfish brain' hypothesis

The ' selfish brain' hypothesis suggests that the reduced perfusion of medulla oblongata causes a reflex increase in SNA, an increase in peripheral vascular resistance and hypertension.[405] The brainstem responds to any reduction in blood flow to the cardiovascular control centers by activation of pathways (particularly sympathetic system) aiming to counteract the changes and maintain the homeostatic level of perfusion. Brainstem hypoperfusion is the key component of the Cushing's mechanism in humans, whereby sympathetic constriction of peripheral arteries occurs in response to cerebral under-perfusion. Curiously, this mechanism is physiological for growing giraffes, when gravitational brain hypotension triggers vasoconstriction and increase in BP to provide adequate blood flow to the brain.[405]

Details of the mechanism have been provided by data from animal experiments. Neurones within the RVLM and spinal cord have been found to be sensitive to hypoxia, and their resultant activation leads to sympatho-excitation.[406, 407] In patients with arterial compression of the ventrolateral medulla, an area rich in efferent sympathetic neurones, increases in muscle SNA and hypertension can be reversed by surgical decompression.[408, 409] The circulatory deficiency of the medulla oblongata can also be

secondary to concentric remodeling of the arterial wall. Increased arterial wall thickness with the reduced luminal area have been noted in the vertebral and basilar arteries of the spontaneously hypertensive rats even before they develop hypertension.[410, 411] This indirectly indicates a possibility causative link between brain hypoperfusion and an increase in peripheral resistance leading to hypertension. Similar changes in the vertebral arteries were seen in patients with hypertension and were strongly correlated with increased vascular resistance and BP.[412] These data support the hypothesis that abnormal SNA triggered by suboptimal brain circulation has an intrinsic protective role for the perfusion by the cost of peripheral perfusion, hypertension and increased risk of long-term complications.[405, 410, 411, 413]

2.6.11. Respiratory-sympathetic coupling

Appropriate respiratory-sympathetic coupling provides important regulatory inputs for the sympathetic drive. Alterations (e.g. reduction in respiratory sympathoinhibition) in respiratory-sympathetic coupling have been described in spontaneously hypertensive rats, even prior to the development of hypertension when they are at a neonatal or juvenile stage.[414-416] These observations imply that these abnormalities of respiratory-sympathetic coupling are not merely a consequence of hypertension but may be involved in pathogenesis of hypertension.[414-416]

Attenuation of the normal inspiratory sympathoinhibition has been shown to underlie the increased sympathetic outflow in chronic heart failure patients.[32] The same attenuation in inspiratory inhibition pattern of sympathetic activity was also described

in hypertensive obstructive sleep apnea patients, and was reversed by treatment with continuous positive airway pressure.[417] But these findings were not observed, by the same group in patients with hypertension without obstructive sleep apnea.[418] These discrepancies could be partly related to the difficulty with precise measurement of the rapidly changing characteristics of the sympathetic status in response to fluctuations of the respiratory output. Under physiological conditions central inspiratory drive often does not closely correlate with the centrally generated expiratory drive (e.g., post-inspiration - reflected by recordings of respiratory motor outputs to the upper airway or late-expiratory activity/pre-inspiration –reflected by respiratory motor outputs to the abdominal muscles).[419, 420] In the spontaneously hypertensive rat the respiratory pattern was found to be shifted towards domination of the enhanced respiratory motor activity with predominant control of upper airway resistance during pre- and post-inspiration phases, without any noticeable changes during the inspiration itself.[419, 420]

In normotensive subjects late inspiration/early expiration phases (i.e., phases of maximal lung expansion) are associated with sympathoinhibition. However, those inhibitory effects disappear in hypertension.[416] The phenomenon has been explained by experimental data in spontaneously hypertensive rats showing that respiratory neurones in the medulla oblongata in the settings of hypertension provide synaptic-mediated excitatory drive to RVLM pre-sympathetic neurones.[421-424] These findings explain more potent phase-related respiratory modulation of sympathetic nerves in hypertension.

However, significant gaps exist in our understanding of the influence of age and hypertension on respiratory mediated modulation of SNA in humans. Detailed

discussion on the respiratory-sympathetic modulation in healthy human ageing is provided in experimental Chapter 4. Alterations of respiratory modulations of SNA in hypertension is reviewed in Chapter 6. It would also be important to understand better the capacity of modulation of breathing patterns to improve BP control through inhibition of SNA (Chapter 5 and Chapter 6).

2.7. Therapeutic strategies

In view of the vast amount of data on the implication of chronic heightened sympathetic activation in hypertension it is only natural that several classes of inhibitors/activators of different types of adreno-receptors have been introduced. Admittedly after the introduction of inhibitors of renin–angiotensin system and new generation calcium channel blockers agents, the therapeutic role of agents working through adrenergic receptors has diminished, and they do not play a dominant therapeutic role in hypertension at present. The potential of this therapeutic target for BP control is thus currently underutilized.[425]

2.7.1. Agonists of central α2-adrenergic and imidazoline receptors

The α_2-adrenergic receptors are predominantly expressed in the medulla oblongata, and they inhibit release of noradrenaline in the brainstem and thus provide a negative feedback mechanism to reduce SNA. Centrally acting agonists of α2-adrenoreceptors, such as methyldopa and clonidine were among the first antihypertensive agents available for practice. They provide effective BP reduction, but their use is hampered by numerous and frequent side effects (Table 2.2).

Table 2.2. Pharmacological agents primarily acting through modulation of adrenergic receptors

Pharmacological target	Principle agents	Principle mechanism of BP reduction	Principle side effects
Agonists of central α2-adrenergic	Clonidine (also imidazoline receptor agonist)	Stimulates presynaptic α2-receptors in the brainstem, which reduces peripheral vascular resistance	Dizziness, drop in BP upon standing, somnolence (drowsiness; dose-dependent), dry mouth, headache, fatigue, skin reactions
Competitive inhibitor of the enzyme DOPA	Methyldopa	Inhibits conversion of L-DOPA into dopamine, which is a precursor for noradrenaline	Depression, apathy, anhedonia, anxiety, impaired attention, decreased motivation, fatigue, lethargy, agitation, cognitive and memory impairment, impaired libido, dizziness, headache, bradycardia, hyperprolactinemia, hepatotoxicity, hemolytic anemia, myelotoxicity
Agonists of	Moxonidine	Inhibits central	Dry mouth, fatigue, dizziness,

imidazoline receptors	Rilmenidine	sympathetic activity	headache, nausea, sleep disturbances intermittent facial oedema
Beta-blockers *Selective β1-blockers* *Selective β1-blocker with potentiating vasodilatory effect* *Non-selective β1/β2-blocker/α1-blocker*	Bisoprolol Metoprolol Nebivolol Carvedilol	Negative chronotropic and inotropic cardiac effect	Nausea, diarrhea, bronchospasm, exacerbation of Raynaud's syndrome, bradycardia, heart block, hypotension, heart failure, alopecia abnormal vision, fatigue, dizziness, hallucinations, nightmares, sexual dysfunction, erectile dysfunction and/or alteration of glucose and lipid metabolism.
Selective α1-adrenergic blockers	Doxazosin Prazosin Terazosin	Relaxation of arterial smooth muscle cells	Dizziness, headache, drowsiness, constipation, fatigue, nasal congestion or dry eyes

As a result methyldopa, a α2-adrenorecoptor agonist is now almost exclusively used for the management of pregnancy related hypertension. Consequently selective (I1) imidazoline receptors have been developed with two, moxonidine and rilmenidine, approved for clinical use.

The antihypertensive effects of clonidine are partly attributed to the stimulation of subtype 1 (I1) imidazoline. Imidazoline I1 receptors are found in both the RVLM pressor and ventromedial depressor areas, and their activation inhibits the activity of the sympathetic nervous system in similar to α2-adrenergic receptors.[426, 427] Administration of imidazoline I1 receptor agonists, therefore, decrease in BP. Compared to the older central-acting antihypertensives, the new selective imidazoline I1 receptor agonists have fewer side effects but protect against hypertensive target organ damage, including LV hypertrophy and kidney function.[426, 427]

The sympatho-inhibitory effects of moxonidine are partly mediated by augmented NO production in the RVLM.[428] Effects of the agent in brainstem regions other than the RVLM may also be relevant as injection of moxonidine to NTS in animals reduced SNA, BP, and HR.[429] In addition to direct BP reducing properties the beneficial effects of moxonidine include improvement in the baroreflex control of renal SNA.[430] When the baroreceptor reflex effects of selective imidazoline receptor agonist, rilmenidine was compared with clonidine in rabbits, many of the baroreflex effects of clonidine inhibition of the sympathetic component of the baroreflex was seen with rilmenidine but not with clonidine, which indicates a specific role of imidazoline receptors in the regulation of the baroreflex.[431] In addition, in a rabbit model of hypertension induced by renal artery stenosis, chronic treatment with rilmenidine normalized BP, reduced plasma renin levels and renal SNA without a reduction in renal blood flow in the kidney supplied by the stenosed artery.[432]

Selective imidazoline receptor agonists effectively reduce sympatho-activation in hypertension and have proven antihypertensive effects with reasonable side effect profile. Current guidelines indicate that they are used in patients with hypertension in whom sufficient BP control cannot be achieved with first-line antihypertensive agents.[20]

2.7.2. Beta-adrenoreceptor blockers

Inhibition of beta1-adrenergic receptors produces a negative chronotropic and cardiac inotropic effect and slows conduction velocity and automaticity in the heart. Inhibition of beta2 receptors results in smooth muscle contraction (which can, for example, exacerbate asthma) and glycogenolysis (which can affect glycaemia control), but also produce vasodilation. The principle antihypertensive action of beta-blockers is likely largely a reduction in cardiac output and if beta2-inhibition is present by some vasodilation. It may also be contributed by inhibition of renin release from the kidneys. Overall, less selective beta-blockers (i.e., inhibit both beta1 and beta2 receptors) tend to have more prominent antihypertensive effects but the cost of more side effects compared to selective beta1-blockers.

Beta-blockade was considered at one time as a first line treatment for hypertension, but this changed after the completion several randomized clinical trials. In the multicentre, prospective, controlled ASCOT trial 19,257 patients with hypertension who had at least three other cardiovascular risk factors were randomised either to amlodipine 5-10 mg adding perindopril 4-8 mg as required or atenolol 50-100 mg adding bendroflumethiazide 1.25-2.5 mg and potassium as required.[433] The study was stopped

early after a median follow up of 5.5 years as the amlodipine-based regimen prevented more major cardiovascular events and induced less diabetes than the atenolol-based regimen.

Consequently, a comprehensive meta-analysis of 13 randomized controlled trials, which involved 105,951 hypertensive patients showed that relative risk of stroke was 16% higher for beta-blockers (95% confidence interval 4-30%) than for other antihypertensive drugs.[434] When compared to placebo the beta-blockers reduced the relative risk of stroke by 19% (7-29% for different beta-blocker), which was less than expected. No difference was found regarding the risk of myocardial infarction when beta-blockers were compared vs. either other antihypertensive agents or placebo. Following the publication of these data beta-blockers have been only considered as second line agents for the management of hypertension in patients without a history of myocardial infarction or systolic heart failure. Admittedly the trials have largely utilized older beta-blockers, such as atenolol and efficacy of modern beta-blockers, such as bisoprolol have not been adequately tested in specifically designed trials. In healthy young volunteers, a double-blind, placebo-controlled, randomized cross-over study of bisoprolol has demonstrated significant improvement in cardiac baroreflex sensitivity, but it remains to be determined how this would be translated into outcome prevention in hypertensive patients.[435]

2.7.3. Selective α1-adrenergic blockers

Selective α1-adrenergic blockers include prazosin, doxazosin, terazosin. These agents

inhibit α1-adrenergic receptors on vascular smooth muscle cells. Their antihypertensive effects are based on vasodilation secondary to reduction of vascular smooth muscle tone and peripheral resistance. The agents are not considered the first-line choice for treatment of hypertension following the early termination of the doxazosin arm of the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) study due to doxazosin being less effective than diuretics and 25% excess in cardiovascular disease and about 50% excess in congestive heart failure.[436]

2.7.4. Angiotensin converting enzyme inhibitors and angiotensin receptor blockers

Angiotensin II type I receptors are expressed by sympathetic nerve terminals and cause the release of noradrenaline when activated by angiotensin II. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers have direct sympathoinhibitory effects.[437, 438] However, the sympatholytic potency of these agents is relatively mild and they reduce rather than normalize muscle SNA, and these effects have been shown to depend on age and background plasma renin activity.[282] SNA is not affected in hypertensive patients by long-term treatment with lisinopril.[439] However, chronic administration of losartan for 12 weeks in hypertensive patients inhibited muscle SNA and increased cardiac baroreflex sensitivity.[440] In a randomized, crossover trial of 12 weeks of treatment of hypertensive patients with diabetes with losartan and telmisartan reduced proteinuria, partly via inhibitory effects on SNA.[441] In a double-blind, placebo-controlled, cross-over study valsartan reduced BP and reset the baroreflex set point to the normal BP level.[442] The multicenter, prospective, randomized, open-labeled, blinded end point Valsartan Amlodipine Randomized Trial showed a significant decrease in

cardiac SNA in the valsartan group but not in the amlodipine group, which paralleled superiority of valsartan over amlodipine in reduction of LV mass index despite BP being equally well controlled in both groups.[443]

This capacity of inhibitors of the renin-angiotensin axis to inhibit sympathetic activity may contribute to their overall success in the management of hypertension and their relative superiority compared to beta-blockers.

2.7.5. Calcium channel blockers

Calcium channel blockers are vasodilating drugs and include a number of agents with very different chemical structure and significant variability in their mode of action, pharmacokinetic and pharmacodynamic properties.

The first generation dihydropyridine calcium-channel blocker, nifedipine markedly increased muscle SNA in healthy volunteers, independently of drug release formulation (standard or short release formulation). Nifedipine differentially activates cardiac and peripheral SNA depending on the pharmacokinetics of the formulations used.[444, 445] In contrast, long-term amlodipine treatment does not appear to increase SNA (measured by plasma and urinary noradrenaline) in hypertensive patients.[445]

Plasma norepinephrine concentration increases more in patients treated with amlodipine than with nifedipine delivered in gastrointestinal therapeutic system form. The evidence indicates that both these once-daily dihydropyridine calcium channel blockers

effectively lower BP with minimal effects on HR (i.e., <1 beat/min). There are small differences between the drugs in the extent to which each activates the sympathetic nervous system with an overall non-significant trend in favor of nifedipine gastrointestinal therapeutic system.[446]

In stroke-prone spontaneously hypertensive rats amlodipine decreased BP but not SNA, while oxidative stress in the brainstem, hypothalamus and cortex were reduced.[447] In patients with essential hypertension long-term treatment with amlodipine significantly increased HR and plasma norepinephrine particularly when administered at a high dose.[448, 449] In contrast, increases in muscle SNA with felodipine and lercanidipine were mainly confined to their acute administration, despite potent BP-lowering properties and increase in HR and plasma noradrenaline.[450]

The second generation dihydropyridine calcium-channel blockers have three subtypes; L type (such as amlodipine), L/T type (efonidipine) and L/N type (cilnidipine). Prolonged (6 months) treatment of hypertension with cilnidipine resulted in sympatho-inhibition (assessed using systolic BP variability analysis) and improved cardiac baroreflex sensitivity.[451] In another prospective, open-labeled, randomized, crossover study efonidipine and cilnidipine were superior to amlodipine in the reduction of cardiac SNA, as determined by HR variability analysis.[452] Cilnidipine has been shown to have more favorable effects on proteinuria progression in hypertension compared to amlodipine.[453] The antihypertensive effects of cilnidipine and ability to reduce LV hypertrophy in patients with neurovascular compression of the RVLM appear to be partly attributable to inhibition of excessive SNA.[454] Also treatment with cilnidipine

delayed development of LV fibrosis and diastolic dysfunction and was superior in the reduction of LV hypertrophy in hypertensive Dahl salt-sensitive rats compared to amlodipine. These effects of the drug are attributed to a distinct capacity to attenuate abnormally high SNA.[455]

Relative little data are available on sympathetic nervous effects of non-dihydropyridine calcium channel blockers, verapamil and diltiazem. Although slow release forms of verapamil showed a trend toward reduction in muscle SNA and plasma norepinephrine levels, it is difficult to make conclusions on the overall impact of this agent on the status of the sympathetic nervous system.[456]

In summary, the vasodilatory effects of dihydropyridine calcium channel blockers may be associated with activation of the SNA. The degree of this effect vary within the group, and it appears less for amlodipine that for nifedipine, and less for cilnidipine than for amlodipine. However evidence from large randomized clinical trials on the clinical relevance of sympathetic nervous system related effects is generally lacking (but short-acting nifedipine should be avoided post myocardial infarction as in can increases risk of death in those patients).

2.7.6. Statins

Statins have a number of pleiotropic effects, which appear to contribute to its clinical success independently of its cholesterol-lowering effects. Clinical studies suggest that statins have a subtle BP-reducing effect in hypertensive patients, which was associated

with a reduction in muscle SNA.[457] Controversial results have been reported regarding their effect on arterial baroreflex function. Although some studies showed no such effect, in studies of patients with hypertension and hypercholesterolemia simvastatin- or atorvastatin-induced reduction in muscle SNA was associated with a significant increase in arterial baroreceptor sensitivity.[458, 459] Mechanistic insights into these data comes from experiments on spontaneously hypertensive rats where statins reduced BP, renal SNA, and the activity of eNOS in NTS.[460] In addition, atorvastatin improved arterial baroreflex in stroke-prone spontaneously hypertensive rats by its anti-oxidant effects within the RVLM.[461] However, a randomized, placebo-controlled, double-blind, cross-over study of 13 hypertensive patients failed to find any significant effect atorvastatin on BP, plasma noradrenaline levels, or HR, despite a significant reduction in muscle SNA.[462] Overall statins do not have sufficient antihypertensive power to justify their use for BP control, and their possible pleiotropic benefits can be utilised in patients who need a cholesterol-lowering therapy.

2.7.7. Current management of hypertension

Current National Institute for Health and Care Excellence (NICE) guidelines in the UK for the management of hypertension recommend initial treatment with a calcium-channel blocker to people aged over 55 years and to people of African or Caribbean family origin of any age.[463] In patients younger 55 years an angiotensin-converting enzyme inhibitor or an angiotensin-II receptor blocker. If the BP remains high, the combination of calcium-channel blocker with an angiotensin-converting enzyme inhibitor or angiotensin-II receptor blocker should be used.

Thiazide-like diuretics, such as indapamide or chlorthalidone, should be the next antihypertensive group to use if the BP does not reach the target values. If the BP is still above 140/90 mmHg when three agents are used in full doses, which constitutes resistant hypertension, adding an aldosterone antagonist (spironolactone) should be considered in people with potassium level under 4.5 mmol/l. If the potassium level is above this level a higher-dose, thiazide-like diuretic treatment should be tried. Alpha-receptor blockers could be used if those diuretics are contraindicated or not tolerated. Expert advise is essential if the BP continues to be elevated despite all these medications, and providing there is adequate compliance with the treatment.

Beta-blockers are not usually used as the first line treatment of hypertension. However following the guidelines above they could be considered in some younger patients, who cannot take angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists or in those with increased sympathetic activity.

2.7.8. Lifestyle modifications

Current clinical guidelines recommend modification of lifestyle as part of the management of all patients with hypertension.[464] These recommended lifestyle components include restriction of dietary salt consumption, low-fat diet with high consumption of fruits and vegetables, maintenance of healthy weight reduction, regular physical activity and avoidance of alcohol excess and smoking.[465, 466]

Among those recommendations, moderate regular exercise and calorie restriction have the largest evidence for a beneficial effect on SNA in hypertension.[467, 468] In hypertensive patients, regular walking, was effective in lowering BP and SNA and improving exercise tolerance.[469] Exercise training (for 4 months) in never-treated hypertensive patients restored the baroreflex control of muscle SNA and HR in hypertensive patients and led to significant reduction in BP.[470] Even though BP lowering effect of endurance training can be modest in some patients it is still associated with improvement in HR variability and baroreflex sensitivity, which is of particular importance for sedentary and obese patients.[471, 472]

2.7.9. Relaxation and breathing techniques

Psychosocial stress has been shown to contribute to development of hypertension.[473, 474] Heightened activation of the sympathetic nervous system is a principle mechanism of stress-related BP increase.[473, 474] Laboratory-based stress activity (i.e. mental arithmetic) increases muscle SNA and BP [147] and individuals with more prominent reactivity to these acute stressors have a higher chance of developing hypertension in future.[475, 476] A number of studies reported BP lowering effects in hypertensive patients by using different relaxation / stress reduction techniques. [477-481] The exact mechanism, leading to BP and stress reduction, remains not completely understood.[482, 483] Partially because these techniques used several approaches, it is difficult to uniformly compare and quantify results from different methodologies.[484] Systematic reviews of the efficacy of stress reduction approaches for hypertension have shown either negative results or heterogeneity of effects on BP depending on the experimental design and selection of a

specific technique.[485-487] However, meta-analyses of stress reduction approaches indicate that the Transcendental Meditation program may be effective in reducing high BP.[482-484, 488] There is evidence that long-term practice of the above meditation program does reduce sympathetic activity in normotensive people, as judged by reduction of catecholamine levels in plasma[489] and urine,[490] although these studies have not assessed muscle SNA. Manipulation of the breathing using yoga techniques leads to a BP reduction in hypertensive patients.[491, 492] In one study that evaluated the effect after completion of the trial, BP lowering effect was still present at 1 year [35] and 4-year follow up.[36] Although methodological approaches were not consistent, due to the different types of yoga available, resent summaries of the studies suggested consistent BP lowering effect among them.[493]

Several tools have been developed to help facilitate slow breathing training. In 1974 Leuner invented a device that guided a slow breathing rate by producing sound or light signals and providing feedback to the user.[494] Gavish has described and patented a device able to detect the biorhythmic activity (breathing), based on continuous analysis of this activity (including the feedback monitoring, working on the principal of the closed-loop operation).[495] The device included a circuit to produce the parameter signals to be translated to the user as guiding tones. This approach helped to achieve and maintain the desirable breathing rate during the training session.[495] Later Gavish modified the device to detect and monitor (including the feedback monitoring) the breathing pattern.[496] The main principle of the device was the individualised lowering of the breathing rate by a relatively greater prolongation of the exhalation duration and thus a reduction in the inhalation-to-exhalation duration ratio. The device produces two

distinct guiding tones, one for inhalation and another for exhalation.[497] The reduction of the breathing rate by prolongation of the exhalation is purported to be beneficial by virtue of a reduction in the development of an inspiratory muscle fatigue (as the perfusion of the breathing muscles mostly occurs during expiration).[498] This is particularly relevant as the inspiratory muscle fatigue results in muscle SNA activation[27, 499] and a breath-hold per se (either on inspiration or expiration) can profoundly increase muscle SNA.[500] To simplify slow deep breathing training the InterCure Inc., New York company developed the RESPeRATE device (www.resperate.com/MD) based on the design of the Gavish device. The device interactively guides the patient to reduce their breathing rate, while monitoring their performance (providing feedback on performance).[497] The thresholds for the BP lowering effect was suggested as a cut-off of 65% of the actual slow breathing time spent in synchronization of inhalation and exhalation to the guiding tones.[497] Slow deep breathing (e.g., assisted by the commercially available RESPeRATE device) has been recommended by the American Heart Association with no side-effects reported.[501] Several studies involving hypertension patients are summarized in Table 2.3. In a recent meta-analysis, based on 13 original studies that included 608 participants, the benefits of the home-based use of the device was confirmed, with a lowering of systolic and diastolic BP by 4 mmHg and 3 mmHg indicated, after accounting for BP changes in a placebo group.[48] However, the physiological changes that lead to such BP lowering remain unclear. In hypertensive patients, acute device-guided slow deep breathing for 10 min was shown to reduce muscle SNA.[44, 56] The muscle SNA response to the long-term slow deep breathing training was studied only in one study, performed on untreated hypertensive patients.[44] At the baseline visit, 15 min of acute device-guided

slow breathing resulted in sympathoinhibition but no reduction in BP. Long-term, 8 week training of the above group of patients failed to reduce both muscle SNA and ambulatory BP.[44] In prospective studies the device-guided slow deep breathing intervention reduced BP more effectively in older patients and those with higher baseline BP values, thus encompassing subjects with higher vascular resistance.[40, 42]

Table 2.3. Longer-term studies examining the effect of the RESPeRATE device-guided slow deep breathing on BP

Study	Design	Overall duration	Daily duration of use	Population	n Intervention / controls	Age Intervention / controls	Primary Outcome	Results (mmHg) intervention vs. control
Altena et al. (2009)[45]	Randomized	9 weeks	15 min	HTN, no DM	15/15	60/59	Office BP Home BP QOL	↓ 4.2 (95% CI -12.4 to 3.9) ↓ 2.6 (95% CI -8.4 to 3.3) ✖
Anderson et al. (2010)[46]	Randomized	4 weeks	15 min	Pre-HTN, mild HTN	20/20	53/53	24hr BP Office BP	✖ ↓ SBP and DBP,
Elliot et al. (2004)[37]	Randomized	8 weeks	15 min	Uncontrolled HTN	79 (43 high use, 33 low use)/57	60/59	Office SBP	↓ 15.0 high use vs. 7.3 low use ↓ 15.0 high use vs. 9.2 in controls

Grossman et al. (2001)[38]	Randomized	8 weeks	10 min	Uncontrolled HTN	18/15	52/50	Office BP Home BP	↓ SBP (7.5 vs. 2.9) ✖ DBP (4.0 vs. 1.5) ✖ SBP (5.0 vs. 1.2, p=0.07) ↓ DBP (2.7 vs. +0.9)
Logtenberg et al. (2007)[47]	Randomized	8 weeks	15 min	DM2 + ≥1 anti-HTN	15/15	63/61	BP QOL	✖ SBP (7.5 [95% CI 12.7-2.3] vs. 12.2 [95% CI 17.4-7.0) ✖ DBP (1.0 [95% CI 5.5-+3.6) vs. 5.5 [95% CI 9.7-1.4] ✖
Meles et al. (2004)[39]	Open label	8 weeks	15 min	Mild HTN	47/26	57/49	Office BP Home BP	✖ SBP 5.5 ↓ DBP 3.6 (also p<0.05 intervention vs. controls) ↓ SBP 5.4 ↓ DBP 3.2 (also p<0.05 intervention vs. controls)

Rosenthal et al. (2001)[40]	Open label	8 weeks	15 min	HTN	13/0	51/0	24hr BP Office BP Home BP	↓ SBP 7.2; ✖ DBP 2.3 ↓ SBP 7.2; ✖ DBP 3.4 ↓ SBP 5.8; ✖ DBP 3.0
Schein et al. (2001)[41]	Randomized 6 moths after	8 weeks	10 min	HTN	32/29 22/21	58/57	Office BP	↓ SBP 15; ↓ DBP 10 Intervention vs. controls: *End of treatment* ✖ SBP: 95% CI 2.7-+10.6 ↓ DBP: 95% CI 1.1-7.6 *6 month follow up* ↓ SBP 95% CI 3.5-14.6 ↓ DBP 95% CI 2.7-12.9
Viskoper et al. (2003)[42]	Open label	8 weeks	15 min	Resistant HTN	17/0	67/0	Office BP Home BP	↓ SBP 12.9; ↓ DBP 6.9 ↓ SBP 6.4;↓ DBP 2.6

Schein et al. (2009)[43]	Randomized	8 weeks	15 min	DM2 + Uncontrolled HTN	33/33	62/63	Office BP	↓ SBP 10.0; ↓ DBP 3.6 Intervention vs. controls: ↓ SBP 10 vs. +1.6 ✖ DBP 3.6 vs. 1.0 (p=0.08)
Bertisch et al. (2011)[502]	Open label	8 weeks	30 min	HTN + OSA	21/0	55/0	Office BP	↓ SBP 9.6; ✖ DBP 2.5
Landman et al. (2013)[503]	Randomized	8 weeks	15 min	DM2 + HTN	21/24	64/65	Office BP Home BP	✖ SBP 2.35 (95% CI 6.50-+11.20) ✖ DBP 2.25 (95% CI 6.67-+2.16) ✖ SBP 3.02 (95% CI 13.22-+7.17) ✖ DBP +0.10 (95% CI 6.88-+7.08)

Howorka et al. (2013)[59]	Randomized	8 weeks	12 min	DM1 or DM2 + HTN	16/16	50/49	24hr BP HRV(min)	↓ SBP 2.9 ↓ Pulse pressure 2.3 Intervention vs. controls: ↓ Pulse pressure 2.3 vs.+0.2 ↓LF
Hering et al. (2013)	Randomized	8 weeks	40min/week	Untreated HTN	10/12	37/matched	Office BP 24hr BP Muscle SNA	↓ SBP 18.0;↓ DBP 7.0 ✖ SBP; ✖ DBP ✖ -1 burst/min

BP, blood pressure; CI, confidence interval; DBP, diastolic blood pressure; DM1, diabetes mellitus type 1; DM2, diabetes mellitus type 2; HRV, heart rate variability; HTN, hypertension; LF, low frequency; MSNA, muscle sympathetic nerve activity; OSA, obstructive sleep apnea; QOL, quality of life; SBP, systolic blood pressure.

↓ Significant reduction in the parameter tested; ✖ no difference in the parameter tested.

Despite several trials investigating the effect of device-guided slow deep breathing on BP few studies assessed underlying BP-lowering effect mechanisms of the acute and especially long-term effect of the technique. Furthermore, controversial findings have been reported, likely a reflection of the intervention duration and population studied. Acute (10 min) slow deep breathing was shown to reduce BP, muscle SNA and improve cardiac baroreflex sensitivity in untreated or washout patients with hypertension.[54, 56] However, another study failed to show any acute effect of the technique on BP in untreated hypertension, despite reductions in muscle SNA.[44] Neither BP nor muscle SNA changed in this study during longer-term training.[44] A study of patients with both hypertension and diabetes showed a reduction in BP and improvements in HR variability parameters with slow deep breathing, but the study did not assess SNA, this limiting pathophysiological insight.[59]

2.7.10. Surgical: renal denervation, carotid denervation

The carotid baroreceptors have been viewed as a therapeutic target in hypertension when optimal BP control cannot be achieved using medications.[504] In patients with resistant hypertension insertion of implantable arterial barostimulator, to provide continuous electrical stimulation of the carotid sinus, reduced BP.[505-507] This hypotensive effect was paralleled by a reduction in muscle SNA and this confirms the therapeutic relevance of interruption of abnormal SNA. The procedure appears to be safe and well tolerated. Nevertheless, the definite proof is required from prognostic trials to show that “baropacing” of the carotid sinus is superior to intensified medical treatment.[508]

Carotid body denervation prominently reduced renal SNA and improved sympatho-respiratory coupling in pacing-induced congestive heart failure rabbits.[509] These effects were followed by an improvement in cardiac function and breathing stability. Carotid sinus nerve denervation in spontaneously hypertensive rats was effective for both prevention of hypertension and reduction of BP in animals with already present hypertension.[391, 510] Carotid sinus nerve denervation in spontaneously hypertensive rats provided sustained inhibition of renal SNA and reduction in BP. The improvement in BP was shown to be related to a resetting of the renal SNA-baroreflex function and recovery in sensitization of the cardiac baroreflex.[510] Translation of these promising findings to humans is warranted.[511]

Experimental models of hypertension consistently show that renal sympathectomy significantly improves BP control.[155] Catheter-based renal denervation has been suggested as an effective and safe method of BP control in resistant human hypertension.[512] Initial reports were very promising and showed prominent and sustained (i.e., over 1-year) BP reduction following the procedure.[146, 513-515] Renal denervation in humans and animal model of hypertension progressively increase cardiac and sympathetic baroreflex function.[514] However, a recently completed trial examining renal denervation in severe resistant hypertension, the prospective, single-blind Symplicity HTN-3 trial of 535 patients randomized to undergo renal denervation or a sham procedure (in a 2:1 ratio)[289] failed to show any benefits of the procedure. These results challenged utility of the procedure and it is not currently recommended for routine clinical practice.[516]

2.8. Summary

Hypertension exerts a number of unfavorable effects on cardiac myocardium such as LV hypertrophy, concentric remodeling, diastolic dysfunction and ultimately increase in LV filling pressure (which is commonly associated with symptoms of HF). Susceptibility of individual patients to these changes as a result of hypertension varies and factors responsible for this are poorly understood.

The sympathetic nervous system plays a major role in the pathogenesis of hypertension through its vascular, cardiac, renal, central and metabolic effects. Detrimental consequences of sympathetic activation include vasospasm, activation of the renin-angiotensin-aldosterone system, promotion of oxidative stress and diastolic dysfunction among others. However, the reasons for the heightened sympathetic activation commonly observed in patients with hypertension, or indeed in healthy older individuals, remain unclear.

Several classes of antihypertensive agents target different units of the sympathetic system as their primary mode of action. Some of these agents were among the very first antihypertensive medications and they have a very long track record in the field. However, these medications have some significant drawbacks, particularly due to rather a large number of side effects. This is perhaps not surprising given the multitude of sympathetic nervous system functions and the complexity of its actions in the regulation of different organs. Additionally, controlled clinical trials suggest relatively modest BP lowering effects of some of these agents. The SNA is also relevant to other

antihypertensive medications, particularly those with vasodilatory properties, which have a potential to cause undesirable activation of the sympathetic system.

Overall the therapeutic agents acting through various adrenergic receptors are currently considered second-line choices for management of hypertension. Initially very promising procedures of renal denervation are now not recommended for routine use due to lack of longer-term efficacy. All this does not necessarily mean that the sympathetic system does not have future as a therapeutic target in hypertension. In fact, evidence supporting the role of the sympathetic system in the pathogenesis of hypertension and its complications is ample.

Limitations of the available treatment options highlight still incomplete knowledge of the complex mechanisms involved in activation and regulation of the sympathetic system leading to sustained BP increase. The potential of respiratory control, for example, delivered by the RESPeRATE device for BP reduction has been recognized recently. However, details of the physiological mechanisms leading to the BP lowering effects of the approach and factors modulating sympathetic-respiratory coupling remain insufficiently understood. Further research is essential in this direction.

CHAPTER III. METHODS

3.1. Methods of data acquisition

3.1.1. Introduction

This chapter provides a description of the study methods: 1) the instruments used to collect and quantify the cardiovascular and respiratory parameters in the 3 experimental chapters contained within this thesis and 2) the methods and techniques used to assess and quantify cardiovascular and respiratory parameters.

3.1.2. Heart rate

While subjects rested in a supine position a lead II ECG was obtained. The electrodes were placed on left and right anterior aspects of shoulders and on a chest in a triangular pattern, to detect repolarization and depolarization periods of the heart. The quality of the obtained signal was assessed to ensure that the R wave peak was the most prominent peak on recorded ECG. HR was calculated based on the number of R waves per minute.

3.1.3. Blood pressure

Beat–to-beat arterial BP was recorded from the middle finger using photo-plethysmography (Finometer Midi, Finapres Medical Systems BV, Arnhem, the Netherlands). The main principle used in this device is a volume clamp method

developed by Penaz.[517] The system contained a source of infrared light and sensors inside the inflatable finger cuff. It detects changes in blood volume (photo-plethysmography approach). These changes in volume were used as indicators of BP. The finger cuff was designed to constantly process plethysmographic data and adjust the pressure in a cuff in order to maintain the diameter of measured artery constant. To compensate for differences in position of the finger to the heart level, hydrostatic height correction system was used. The absolute BP values recorded using photo-plethysmography were adjusted according to brachial artery BP measures obtained in triplicate using a standard automated sphygmomanometer (Omron UK, East Sussex, UK). This approach was used for data presented in Chapters 5 and 6.

3.1.4. Muscle SNA

Standard microneurography techniques were used to record efferent postganglionic multiunit muscle SNA.[518, 519] The advantages and limitations of the techniques were discussed in details in Chapter 2.3.4. Among the available techniques microneurography clearly benefits from being a minimally invasive method for direct assessment of the sympathetic activity in real time. This method is suitable for repeated measurements for monitoring of disease progression and response to therapeutic interventions.[143, 163, 164]

The subject's leg was supported in a relaxed position with foam cushions. In order to locate peroneal nerve, palpation around the fibula head was followed by provision of small electrical stimuli (for 0.2 ms, ranging from 2.4 mA to 12.4 mA in this study) and observation for small muscle contractions. The anatomical location of the nerve was

further confirmed by the ultrasound scanning using LOGIQ-e ultrasound machine with 12-MHz probe (General Electric Healthcare, Tokyo, Japan) (Figure 3.1).

Direct muscle SNA recordings from the peroneal nerve were obtained using unipolar tungsten microelectrodes. A recording microelectrode was placed into the peroneal nerve at a fibular head, and a reference electrode was inserted 2-3 cm distally. The raw signals were amplified (×100k), filtered (bandwidth 700 – 2000 Hz), rectified and integrated (time constant 100 ms) to obtain a mean voltage neurogram. The muscle SNA neurogram was confirmed by (i) pulse-synchronous bursts pattern, (ii) signal-to-noise ratio of >3:1, (iii) excitation during an end-expiratory breath-hold or Valsalva manoeuvre, and (iv) lack of changes in response to an unexpected loud noise or skin stroking.[162] After obtaining a muscle SNA signal participants rested for at least 10 min to confirm recording stability, following which all study measurements were obtained.

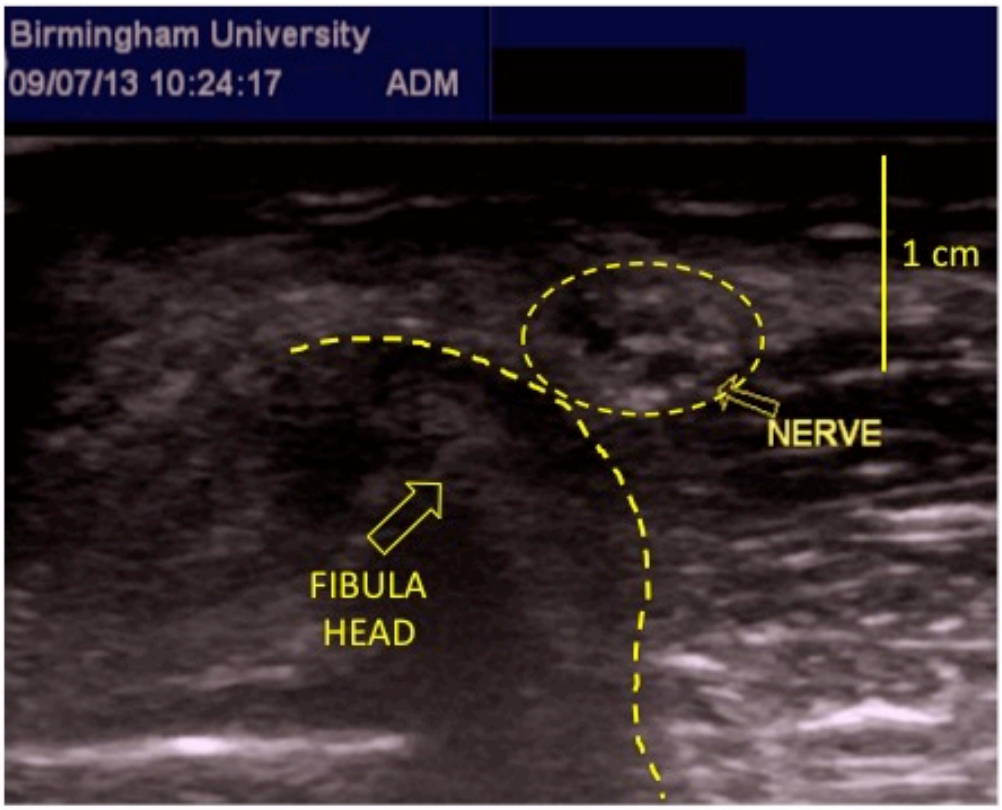

Figure 3.1. B-mode image of the peroneal nerve

3.1.5. Respiratory parameters

3.1.5.1. Thoracic circumference

Respiration related changes in thoracic circumference were measured using strain gauge pneumobelt placed securely around the upper abdomen (Pneumotrace, UFI, Morro Bay, CA, USA). This approach for respiration monitoring was chosen as it is unobtrusive for participants and avoids the need for the participants to breathe through a mouthpiece, which may alter breathing pattern.[520, 521]

3.1.5.2. Respiratory volumes and end-tidal gases

Participants were fitted with an oro-nasal mask and two-way valve (Hans Rudolph, Kansas City, KS, USA) connected to a heated pneumotachometer (Hans Rudolph, Kansas City, KS, USA). Prior to data collection, the pneumotachometer was calibrated with a known sample volume (3 litres). Using the Spirometer settings of the LabChart software (ADInstruments), assessments of tidal volume, respiratory frequency, and minute ventilation were made on a breath-by-breath basis. A sample tube connected to a side port on the oro-nasal mask permitted the sampling of the expired air and determination of the partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) using a side stream capnograph (Nonin Medical, Plymouth, MN, USA).

3.1.6. Echocardiography

Two-dimensional echocardiography was used to assess cardiac function and structure. Images were acquired with a LOGIQ-e ultrasound machine with 2-MHz probe (General Electric Healthcare, Tokyo, Japan) in accordance with international guidelines.[522-524] Off-line software (Xcelera, Phillips Ultrasound Quantification Module, USA) was used for quantification of cardiac parameters. Left ventricular systolic and diastolic volumes and left ventricle ejection fraction were measured using the modified Simpson's biplane method. To assess left ventricular diastolic function pulsed Doppler was used to detect transmitral inflow velocity (E and A) in the apical 4-chamber view. Early diastolic mitral annular velocity (e') was measured by tissue Doppler imaging with average septal E/ e' ratio calculated as a parameter of the diastolic function. Left atrial volume, interventricular septal and posterior wall thicknesses were measured. Left ventricular mass index as well as left atrial volume index were calculated.

3.1.7. Arterial stiffness

Aortic augmentation (AI) index was measured using Sphygmocor device (Sphygmocor, AtCor Medical, Sydney, Australia). The AI is influenced by HR and it is a standard practice to adjust it for a HR of 75 beats per minute, and this done in the present study. A high-fidelity hand-held applanation tonometer was used to record radial arterial waveforms non-invasively for over 10 seconds. These data were used to calculate AI, which is an index of wave reflection and is influenced by arterial stiffness.[525] Intra-

observer coefficient of variation (CV) for the aortic augmentation index was 12.0%. Inter-observer CV for the aortic augmentation index was 5.29% and 11.12% for the two subjects, with average CV 8.21%.

3.2. Data analysis

3.2.1. Introduction

The raw ECG, BP, and respiratory signals underwent analogue-to-digital conversion at 10 kHz (Powerlab and Chart v7, AD Instruments, Bella Vista, NSW, Australia) and were stored for offline analysis. HR was calculated on a beat-to-beat basis from the ECG. Beat-to-beat systolic and diastolic BP were obtained from the arterial BP waveform and mean arterial pressure (MAP) obtained by integration of the arterial BP waveform over the entire cardiac cycle. Peak inspiration was defined as the highest point of the pneumobelt waveform (Chapter 4) or of the tidal volume waveform (Chapters 5 and 6) and respiratory rate was calculated from the inspiratory peaks. Tidal volume and minute ventilation were calculated using the Spirometry module from LabChart (ADInstruments, Dunedin, New Zealand). $P_{ET}CO_2$ level was derived from the relative percentage of expired CO_2 waveform as the maximum value.

3.2.2. Steady-state muscle SNA

A custom written interactive scoring program (Spike 2, Cambridge Electronic Design, Cambridge, UK) was used for the muscle SNA bursts identification. Sympathetic

neurograms were shifted in time to account for conduction delays, calculated according to subject height.[526] The baseline level of neurogram was set to a zero value and the amplitude of the highest SNA burst spontaneously occurring during the baseline period was assigned a value of 100 arbitrary units (AU). The amplitudes of all other bursts within a recording session were normalized to this.[527, 528]

All bursts were inspected and scored as 'burst' or 'no burst'. Muscle SNA was quantified as burst incidence (bursts·100 heart beats^{-1}), burst frequency (bursts·min^{-1}), burst amplitude (i.e., strength) and total activity (product of burst frequency and mean burst amplitude). The location of each burst within the respiratory cycle was determined, (for data presented in Chapters 4 and 5) and burst incidence, burst frequency, burst amplitude and total activity were calculated for each 10% time interval of the breath from the peak of inspiration (i.e., peak inspiration = time point 0).

Inter-observer CV was determined for calculations of the burst frequency (bursts·min^{-1}) and burst incidence (bursts·100 heart beats^{-1}) with another postgraduate student from the same laboratory. Independent scoring was performed on 5 study participants with CV 7.8% for burst frequency and 8.5% for burst incidence (Table 3.1).

Table 3.1. Measurements of muscle SNA parameters for reproducibility analysis

	Operator 1		Operator 2	
	Frequency	Incidence	Frequency	Incidence
Subject 1	17.24	31.85	23.25	43.42
Subject 2	26.22	50.00	29.71	56.6
Subject 3	27.58	51.82	27.68	52.37
Subject 4	26.46	52.29	27.76	55.64
Subject 5	29.32	57.59	31.79	63.56

SNA, sympathetic nerve activity

3.2.3. Interaction between respiration and SNA (for Chapter 4)

In order to examine to examine the relationship between respiratory mediated changes in muscle SNA (rMSNA) and Traube–Hering wave (THW) amplitude a novel time domain analysis was employed using a custom written interactive analysis programme (Spike 2) (Figure 3.2). Respiration triggered averaging of the sympathetic neurogram and beat-to-beat MAP time series (obtained by the beat-to-beat integration of the arterial BP waveform over a cardiac cycle) was undertaken to identify temporal relationships between the THW amplitude and rMSNA. Regions of interest were set around the THW nadir, around the THW peak, and around rMSNA. The rMSNA was quantified as the area under the sympathetic activity curve during the inspiratory period plus 50% of this duration, which thus extended into the post-inspiratory period. For each subject, this approach was used to generate a breath-by-breath respiratory amplitude, THW amplitude and rMSNA time series allowing the relationships between

these variables to be examined. For this analysis of the association between neural events and the THW, sympathetic neurograms were neither time-shifted nor normalised. This is in line with previous studies that have examined the associations between human SNA and the ensuing vascular or BP response.[528]

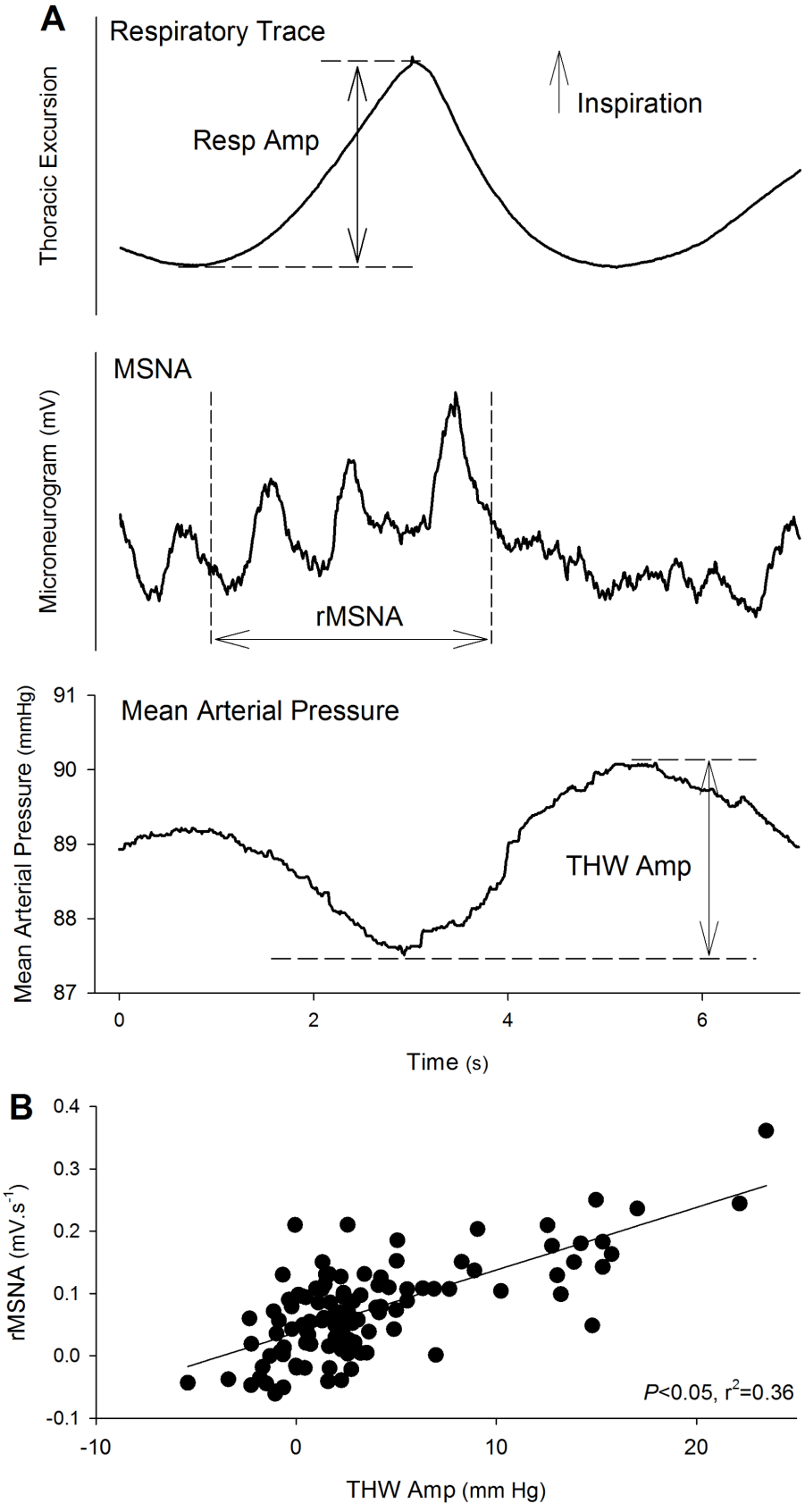
A
Respiratory Trace
Thoracic Excursion
Resp Amp
Inspiration
MSNA
Microneurogram (mV)
rMSNA
Mean Arterial Pressure
Mean Arterial Pressure (mmHg)
THW Amp
91
90
89
88
87
0
2
4
6
Time (s)
B
rMSNA (mV.s-1)
0.4
0.3
0.2
0.1
0.0
-0.1
-10
0
10
20
THW Amp (mm Hg)
P<0.05, r2=0.36

Figure 3.2. Respiratory-triggered waveform averaging of the raw respiratory, blood pressure and muscle SNA signals (panel A), and sample rMSNA vs. Traube-Hering wave amplitude (THW Amp) relationships in one older subject (panel B).

Following respiratory-triggered averaging, regions of interest were set around the Traube–Hering wave (THW) nadir, around the Traube–Hering wave peak, and around the muscle SNA associated with respiration (rMSNA). The rMSNA was quantified as the area under the sympathetic activity curve during the inspiratory period plus 50% of this duration, which thus extended into the post-inspiratory period (panel A). For each subject this approach was used to generate a breath-by-breath respiratory amplitude, THW Amp and rMSNA time series, and the association between these variables examined (panel B). As the aim of this analysis was to assess the association between sympathetic neural events and Traube-Hering waves no time shifting of the sympathetic neurogram was undertaken. Note that with a shift to account for conduction delays in muscle SNA (1.38 s in this subjects) a sympathetic inhibition is apparent from approximately peak inspiration to end-inspiration.

3.2.4. Arterial baroreflex sensitivity

The sequence technique was used to determine spontaneous cardiac baroreflex sensitivity.[529, 530] This approach was chosen as a good non-invasive estimate of the baroreflex sensitivity that can be used during the breathing manoeuvres performed by participants, and it is known to correlate with modified Oxford measures of resting cardiac baroreflex function. [530-532] Analysis of the spontaneous fluctuations of the systolic BP and R-R interval or HR was performed using a customized program (Spike

2) to identify sequences of three or more consecutive beats where systolic BP and R–R interval changed in the same direction (by >1 mmHg and 1 ms, respectively) and where systolic BP and HR changed in the opposite direction. Linear regression analysis was applied to identify acceptable sequences with an r^2>0.85. The slope of the systolic BP–R-R interval and the systolic BP–HR regression line represents an index of cardiac baroreflex sensitivity that correlates with the modified Oxford method measurements of resting cardiac baroreflex function.[530-532]

Calculations of arterial baroreflex control of muscle SNA were obtained from the relationships between diastolic BP vs. burst incidence and total muscle SNA by weighted linear regression analysis.[23, 124, 527] Based on 10 min recordings each diastolic BP value was grouped into 3 mmHg bins. Then for each diastolic BP bin, the percentage of cardiac cycles in which a burst occurred (burst incidence) and total burst amplitude divided by the number of cardiac cycles (total muscle SNA; expressed as AU·beat^{-1}) were determined.

3.2.5. Heart rate variability

Time and frequency domain indices of HR variability were evaluated using short (5 min) recordings by commercially available software (Kubios HRV Software, Biomedical Signal Analysis Group, University of Kuopio, Finland) in accordance with current guidelines.[190]

The time domain indices included: RMSSD, SDNN, pNN50%. Frequency analysis of HR variability (fast Fourier transformation) was considered at a HF range (0.15-0.4 Hz), a LF range (0.04–0.15 Hz) and TP between 0.0-0.4 Hz. Absolute power spectral density and normalized power spectral density were identified at each frequency range. Normalized units calculated as the proportion of each component to the total power minus very low frequency range.

For the purposes of this thesis, the HR variability indices of interest are RMSSD, SDNN, pNN50% and HF, as they have been generally implied as indicators of cardiac parasympathetic activity.[190, 191, 195, 196] The influence of parasympathetic and sympathetic blockade (with glycopyrrolate and propranolol, respectively) in conscious dogs on HR variability has been investigated by Akselrod et al.[533] Parasympathetic blockade abolished the HF power, while the sympathetic blockade had very little effect. These findings confirmed that HF power is an indicator of parasympathetic activity. Further studies in unrestrained rats have shown significant reduction of LF power with propranolol, but not a complete abolition.[534] Following the parasympathetic blockade with atropine, HF power was completely abolished, but also LF power was markedly reduced.[534, 535] As discussed in more details in Chapter 2.3.5 interpretation of results of HR variability findings could be ambiguous. In hypertensive patients, an increase in LF and decrease in HF, RMSSD and pNN50%, as compared to normotensives, has been interpreted as an enhanced cardiac sympathetic activity and a reduced parasympathetic activity.[198]

CHAPTER IV. INFLUENCE OF AGE ON RESPIRATORY MODULATION OF MUSCLE SYMPATHETIC NERVE ACTIVITY, BLOOD PRESSURE AND BAROREFLEX FUNCTION IN HUMANS

4.1. Introduction

Healthy ageing is associated with elevated plasma catecholamine concentrations, increased noradrenaline spillover from the heart, brain, kidneys, and greater SNA directed to the skeletal muscle vasculature.[22, 23] Such heightened SNA has been linked to structural and functional abnormalities of the peripheral vasculature (e.g., increased arterial stiffness, impaired endothelial function) in several chronic disease states[24, 25] and in elderly individuals.[265] Alongside increases in tonic muscle SNA, α-adrenergic receptor sensitivity and vascular responsiveness are reportedly diminished with increased age.[536-538] The mechanistic basis for the age-related elevation in sympathetic neural firing remains unclear.

It has been known since the earliest direct recordings that SNA shows respiratory modulation.[539] This is generated in large part by central neural circuits[26] and upon which is superimposed modulatory feedback signals from cardiorespiratory afferents that include lung-stretch receptors, baroreceptors, central and peripheral chemoreceptors.[27] During normal breathing in young healthy individuals muscle SNA is inhibited during mid-inspiration, reaching a nadir when lung volume is at its highest (peak inspiration), and peaking when lung volume is at its lowest (end-expiration).[27-30, 33] Patients with chronic heart failure show increased muscle SNA linked to an

attenuation of the normal inspiratory sympathoinhibition.[32] Given the increase in the tonic level of muscle SNA with age this may predict a reduction in the inspiratory inhibition of muscle SNA, however there is a paucity of information on the effect of aging on respiratory-sympathetic coupling. Fatouleh and Macefield[418] reported a similar pattern of respiratory modulation of muscle SNA in young (29±2 years) and middle-aged groups (50±3 years) using cross-correlation histograms constructed between sympathetic spikes and respiratory-related chest excursions. However, the respiratory modulation of muscle SNA was not assessed in terms of sympathetic burst occurrence (i.e., incidence) or strength (i.e., amplitude),[122, 123, 540, 541] thus whether ageing affects the within-breath modulation of these distinct parameters of SNA is unclear.

The respiratory modulation of vasomotor sympathetic outflow causing phasic changes in arteriolar smooth muscle tone generates Traube–Hering arterial BP waves (THW). Importantly, Simms *et al*. (2009) demonstrated that spontaneously hypertensive rats exhibited augmented respiratory–sympathetic coupling and larger THW that contributed more to vascular resistance than in normotensive Wistar–Kyoto control rats at all ages. [415] However, the contribution of the sympathetic nervous system to the regulation of THW in humans remains controversial, perhaps as a consequence of the multiple mechanisms implicated and the methodological approaches previously used to investigate this (e.g., complete pharmacological autonomic blockade, individuals with brain death).[542, 543] In a recent preliminary investigation employing a novel time domain analysis, Towie *et al.* (2012) revealed a significant positive correlation between respiratory mediated changes in muscle SNA and the following THW in a group of young individuals (age 21-30 years). It was suggested that this finding supported the

hypothesis that THW are partly a consequence of central respiratory-sympathetic coupling in humans.[544]

However, it is incompletely understood whether there is an alteration in respiratory coupling of muscle SNA with healthy human ageing; whether this could account for the increased muscle SNA seen with aging; and if this sympathetic flow still gives rise to THW.

4.2. Aims and hypotheses

The aim of this study was to determine the influence of age on respiratory related bursting of muscle SNA and on the association between the rhythmic fluctuations in muscle SNA and THW that occur with respiration in humans.

The following hypothesis were tested:

1. The increase muscle SNA in healthy ageing would be due to diminished inspiratory inhibition of muscle SNA.
2. The sympathetic contribution to respiratory mediated fluctuations in BP (THW) would be attenuated in older individuals.

4.3. Methods

4.3.1. Study subjects

Ten young (22±2 years old, mean±SD) and ten older (58±6 years) males participated in the study, which was approved by the local ethical review committee and conducted in accordance with the Declaration of Helsinki (2000). All participants provided written informed consent before they took part in any experiments. Participants were healthy with no significant medical history and were not taking any prescription or over-the-counter medications. All subjects were asked to abstain from caffeine use for at least 12 h and from alcohol intake and strenuous physical activity at least 24 h prior to the participation. All study measurements were made in a temperature controlled room (20-22ºC).

4.3.2. Experimental protocol

The experimental protocol consisted of 10 min of uncontrolled spontaneous breathing at a normal resting rate and depth.

4.3.3. Experimental measurements

While subjects rested in a supine position the following recordings were simultaneously obtained and stored: muscle SNA, beat-to-beat arterial BP, electrocardiogram, respiratory rate, as described in section 3.1 (Figure 4.1).

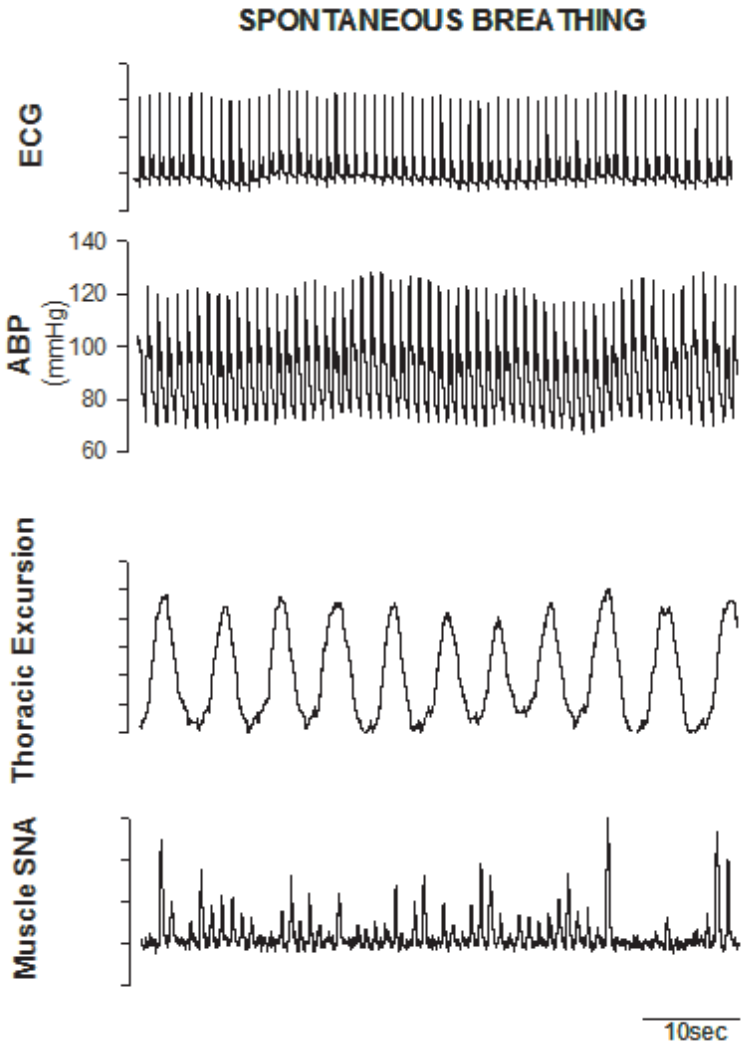

Figure 4.1. A raw trace of an original recording

ABP, arterial blood pressure; ECG, electrocardiogram; SNA, sympathetic nerve activity.

4.3.4. Experimental data analyses

The analyses of HR, BP, steady-state muscle SNA, respiration related muscle SNA, arterial baroreflex sensitivity, HR variability were performed as described in section 3.2. Briefly HR variability was calculated from the ECG signal, as described in section 3.2.5. Calculations of the BP were obtained from the arterial BP waveform as described in section 3.2.1. Respiratory rate was calculated form thoracic excursion as described in section 3.2.1. The raw muscle SNA signal (Figure 4.1.) was used to calculate steady-state muscle SNA as burst incidence (bursts·100 heart beats^{-1}), burst frequency (bursts·min^{-1}), burst amplitude (i.e., strength) and total activity (product of burst frequency and mean burst amplitude). The location of each burst within the respiratory cycle was also determined, and burst incidence, burst frequency, burst amplitude and total activity were calculated for each 10% time interval of the breath from the peak of inspiration (i.e., peak inspiration = time point 0) The same raw muscle SNA, as well as arterial BP, and thoracic excursion waveforms were used for calculation of interaction between respiration and SNA as described in section 3.2.3.

4.4. Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 19.0 for Windows (IBM Inc, Chicago, IL). Data were tested for normality by Kolmogorov-Smirnov test. Baseline subject characteristics were compared using an independent sample t-test. Mixed between-within subjects ANOVA analysis, adjusted using the Greenhouse-Geiser correction, was used to examine the

main effects of respiratory phase, age group and their interaction. Spearman correlation analysis was used to evaluate relationships between breath-by-breath values of respiratory waveform amplitude (e.g., index of breath depth), rMSNA and THW amplitude (Chapter 3, Figure 3.2, panel A). Chi-square analysis was employed for comparisons of categorical data. Data are expressed as mean ± SD, or median (interquartile range) in text and tables and as median ± SE in figures. A *P* value of <0.05 was considered statistically significant.

4.5. Results

4.5.1. Subject characteristics

The mean age difference between young and older groups was 36 years (Table 4.1). Young and older participants were matched for body mass index (BMI 25 ± 3.5 vs. 26 ± 4.0 $kg \cdot m^2$, respectively, P=0.82). MAP was significantly higher in the older group (95 ± 8.9 mm Hg) than in the young group (86 ± 8.2 mm Hg, P=0.04), whereas HR (60 ± 7.9 $beats \cdot min^{-1}$ in the young group vs. 62 ± 17.2 $beats \cdot min^{-1}$ in the older group, P=0.81) and respiratory rate (15.3 ± 1.8 $breaths \cdot min^{-1}$ in the young group vs. 13.4 ± 2.4 $breaths \cdot min^{-1}$ in the older group, P=0.06) were similar. THW amplitude was also similar between groups (2.0 ± 0.9 mm Hg in the young group vs. 2.7 ± 1.3 mm Hg in the older group, P=0.19), but as anticipated muscle SNA burst incidence (22.7 ± 9.2 vs. 42.2 ± 13.7 $bursts \cdot 100\ heart\ beats^{-1}$, P=0.002) and burst frequency (13.5 ± 6.0 vs. 25.0 ± 7.6 $bursts \cdot min^{-1}$, P=0.001) were higher in the older group. Spontaneous cardiac baroreflex sensitivity was lower in the older group compared with the younger group (P<0.001), while arterial baroreflex control of muscle SNA was similar between groups (Figure 4.2.).

Table 4.1. Subject characteristics

	Young	Older	*P* value
Age, years	22±2	58±6	<0.001
Weight, kg	79±11	81±18	0.75
Height, cm	177±5	177±9	0.97
SBP, mm Hg	123±14.0	136±14.3	0.046
DBP, mm Hg	68±5.6	74±6.3	0.04

Data presented as mean±SD. DBP, diastolic blood pressure; SBP, systolic blood pressure.

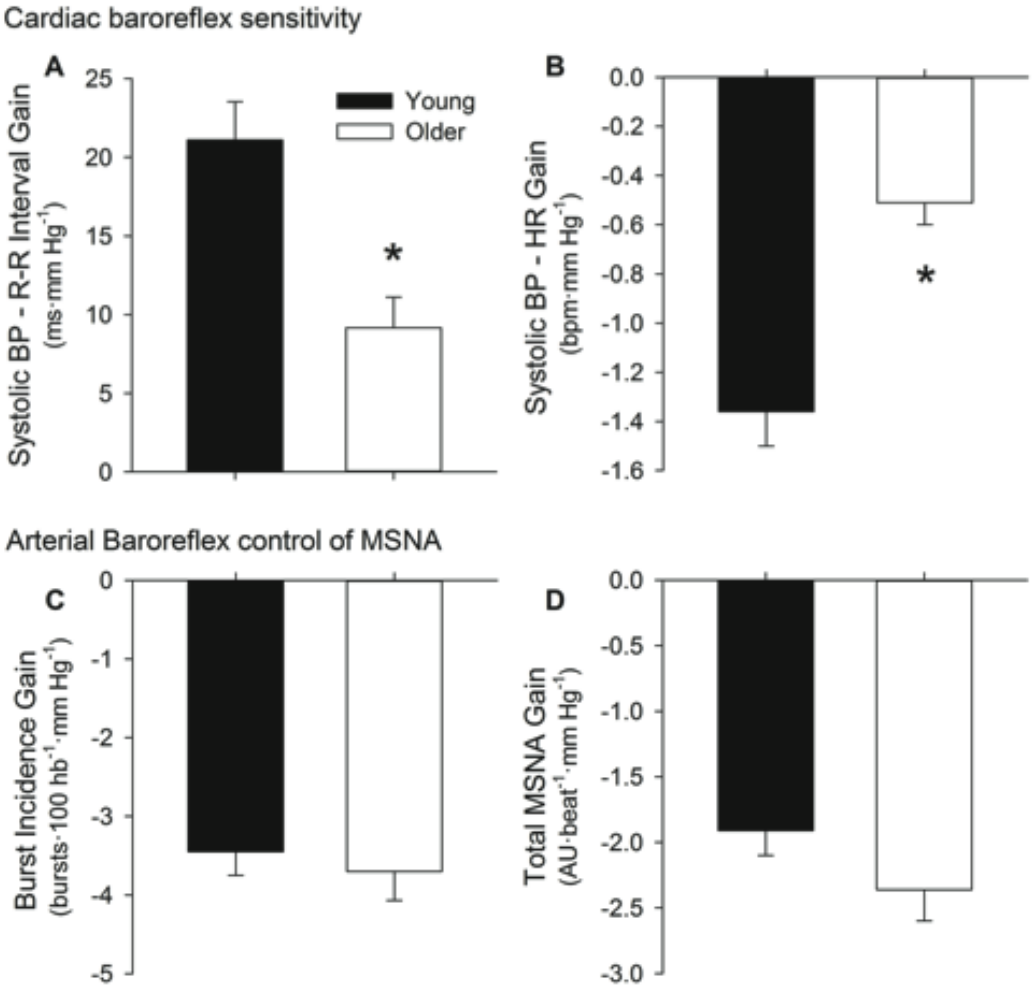

Figure 4.2. Spontaneous measures of cardiac baroreflex sensitivity (upper panels) and arterial baroreflex control of muscle sympathetic nerve activity (lower panels) in young and older groups.

Data presented as mean±SE; * represents $P<0.05$ vs. young; AU, arbitrary units; BP, blood pressure; HR, heart rate; MSNA, muscle sympathetic nerve activity.

4.5.2. Respiratory-sympathetic coupling

The influence of respiration on muscle SNA parameters in young and older participants is summarised in Figures 4.3 and 4.4. All parameters of muscle SNA examined (burst incidence, burst frequency, burst amplitude and total activity) were significantly higher in the older group in all respiratory phases ($P<0.05$, ANOVA main effect of age). Muscle SNA was modulated by respiration, such that muscle SNA burst incidence, burst frequency, burst amplitude and total activity were lowest around the mid-inspiratory to post-inspiratory period and highest during mid-to-late expiration ($P<0.05$, ANOVA main effect of respiratory phase) (Figure 4.4). Importantly, the magnitude of the respiratory modulation of all parameters of muscle SNA was similar in both groups (i.e., no significant statistical interactions were observed between age group and respiratory phase [ANOVA]). More specifically, muscle SNA burst incidence, burst frequency, burst amplitude and total activity were significantly higher in the older group compared to the younger group during both the mid-to-late expiratory period and the inspiratory to post-inspiratory period (Figure 4.4). However, a similar degree of inspiratory attenuation of muscle SNA was observed in both groups (i.e., a significant main effect of respiratory phase was observed, but a significant interaction between age and phase was not), such that muscle SNA was significantly lower during the inspiratory to post-inspiratory period compared to the mid-to-late expiratory period (Figure 4.4).

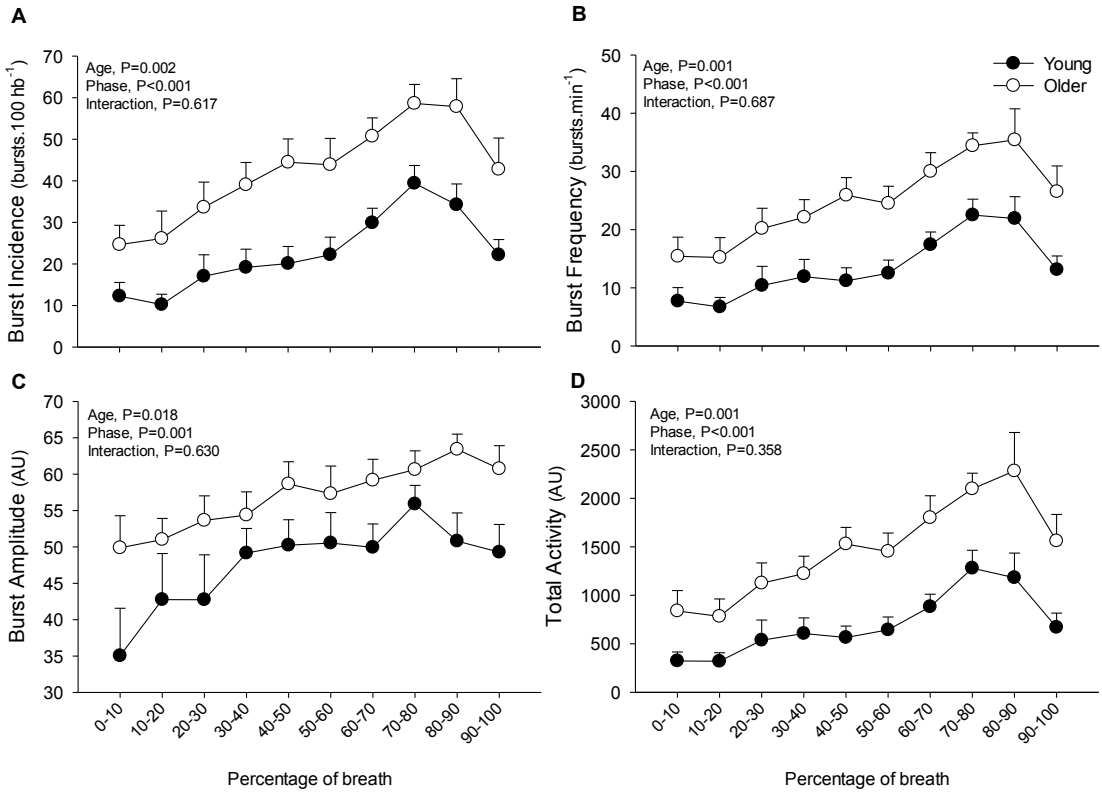

Figure 4.3. Respiratory modulation of muscle sympathetic nerve activity burst incidence (panel A), frequency (panel B), amplitude (panel C) and total activity (panel D) in young and older groups

Peak of inspiration at 0 mid-to-late expiration was denoted as 30-79% of the breath, while inspiratory to post-inspiratory period was taken as the remaining period of the respiratory cycle. Data presented as mean±SE; AU, arbitrary units.

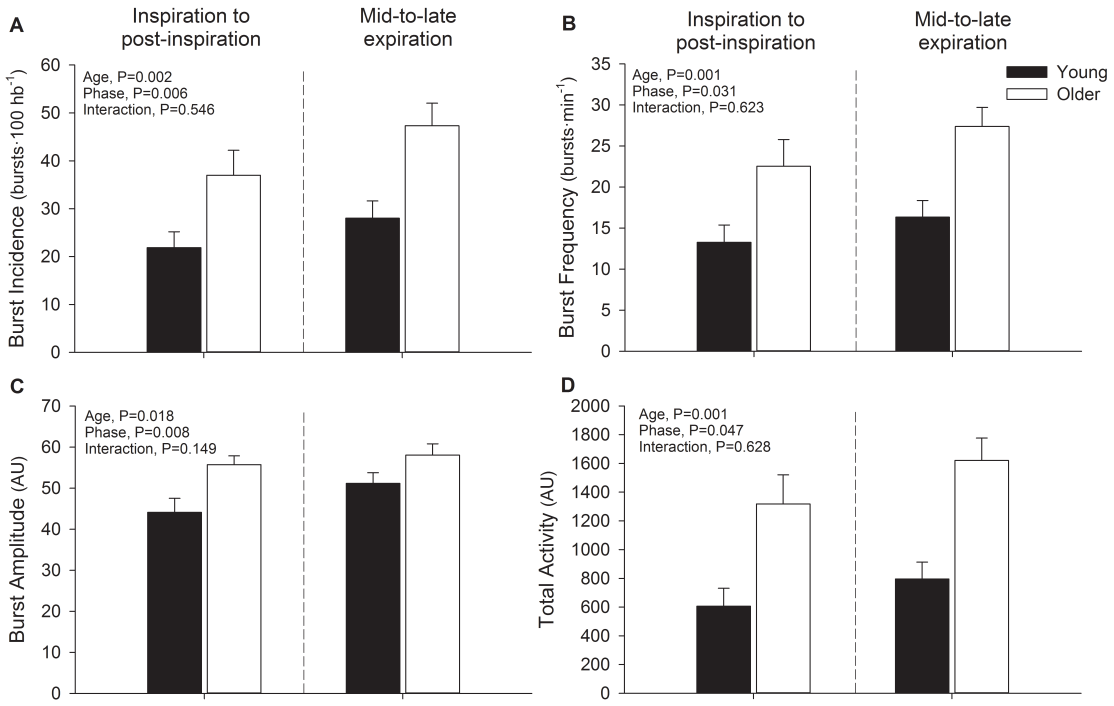

Figure 4.4. Respiratory modulation of muscle sympathetic nerve activity burst incidence (panel A), frequency (panel B), amplitude (panel C) and total activity (panel D) during the inspiratory to post-inspiratory period and mid-to-late expiration in the study groups

Note that sympathetic neurograms were shifted in time to account for conduction delays calculated according to subject height. Mid-to-late expiration was taken as 30-79% of the breath and the inspiratory to post-inspiratory period taken as 0-29% and 80-100% of breath as presented in Figure 4.3. Data presented as mean±SE; AU, arbitrary units.

RMSSD, SDNN, pNN50%, HF, LF and TP were significantly lower in older group compared to the younger group, while LF/HF was higher in the older group (Table 4.2).

Table 4.2. Time and frequency domain measures of HR variability in young and older subjects

	Young	Older	*P* value
RMSSD (ms)	49.3 (37.2-74.4)	20.2 (16.8-36.3)	0.004
SDNN (ms)	64.2±18.7	42.6±16.1	0.013
pNN50 (%)	29.2 (16.7-48.8)	2.2 (1.4-10.9)	0.003
HF (ms2)	981 (633-1593)	152 (112-234)	0.002
LF (ms2)	869 (475-1817)	407 (211-681)	0.02
TP (ms2)	3288 (2624-5848)	1218 (857-3381)	0.02
HF (n.u.)	50.1±12.4	34.7±19.5	0.05
LF (n.u.)	49.9 ± 2.4	65.3±19.5	0.05
LF/HF	1.1 (0.6-1.4)	2.2 (1.5-3.8)	0.02

Data presented as median (interquartile range) or mean±SD; RMSSD, square root of the mean of the sum of successive differences in R-R interval; SDNN, standard deviation of normal to normal R-R interval; pNN50%, proportion of successive R-R interval which vary by >50 ms; TP, total power (0.0-0.4 Hz); HF, high-frequency power (0.15-0.4 Hz); LF, low-frequency power (0.04-0.15 Hz).

4.5.3. Respiratory related muscle SNA-BP coupling

The proportion of participants in whom significant correlations between parameters of rMSNA-BP coupling (THW amplitude, rMSNA, respiratory waveform amplitude [i.e., breath depth]) is summarised in Table 4.3. rMSNA positively and significantly predicted the magnitude of the following THW (lag 0) in 100% of young (Chapter 3, Figure 3.2, panel B) and 80% of older subjects (P=0.14 young vs. older groups). This positive correlation was not observed with the magnitude of the THW amplitude associated with the next breath (lag +1), indeed this showed a negative correlation in 80% of young and 70% of older subjects (P=0.606 young vs. older groups). In some individuals (30% of young and 50% of older subjects, P=0.36) a significant correlation was noted between THW amplitude and the following rMSNA, however in the majority, this relationship was positive (counter to the expected relationship). This test of reverse causation (i.e., whether the rMSNA related to the magnitude of the preceding THW through the engagement of the arterial baroreflex) was thus not proven and was less common than the proportion of individuals in which a correlation between rMSNA and the following THW was observed. In 60% of the young group and 50% of the older group, the respiration waveform amplitude was significantly and positively correlated with the following THW amplitude, which was not different between groups (P=0.65 young vs. older groups).

Table 4.3. Proportion of participants in whom significant correlations between parameters of respiratory related muscle SNA-BP coupling were observed

	Young (n=10)	Older (n=10)	*P* value
Respiratory amp vs. THWamp Direction of correlation, r	60% positive 0.28± 0.07	50% positive 0.22±0.05	0.65
rMSNA vs. THWamp Direction of correlation, r	100% positive 0.45± 0.07	80% positive 0.48± 0.19	0.14
rMSNA vs. THWamp (lag +1) Direction of correlation, r	80% negative -0.31± 0.09	70% negative -0.34± 0.09	0.61
Previous THWamp vs. rMSNA Directions of correlation, r	30% 1 negative -0.19 2 positive 0.20±0.02	50% 1 negative -0.18 4 positive 0.20± 0.04	0.36
Respiratory amp vs. rMSNA Directions of correlation, r	50% positive 0.34± 0.11	30% 1 positive 0.60 2 negative -0.24± 0.04	0.36

Data presented as mean±SD; Respiratory amp, amplitude of the respiratory waveform excursion (e.g. index of breath depth); THWamp, amplitude of the Traube-Hering wave; rMSNA, respiratory linked MSNA; r, correlation coefficient.

4.6. Discussion

The major novel findings of this investigation are twofold. First, in contrast to the initial hypothesis, the strength of the respiratory modulation of the muscle SNA parameters (e.g., burst incidence, frequency, amplitude and total activity) was preserved in healthy older individuals. Second, a significant association between the rMSNA and THW amplitude was identified and that this was similar in healthy young and older groups. Collectively, these findings suggest that a potential attenuation of inspiratory-linked inhibition of muscle SNA does not appear to explain the elevated resting muscle SNA in older individuals, and that central respiratory-sympathetic coupling is a component of the THW in both young and older humans.

A respiratory modulation of SNA is evident in recordings from rats[415], cats[545] and humans[27-30, 33, 546], with the exact pattern of respiratory modulation of SNA being species and target organ specific. In adult rats muscle vasoconstrictor-type sympathetic neurones are typically inhibited during early-inspiration, with a peak of activity observed during the mid-inspiratory to post-inspiratory phase (i.e., first part of expiratory interval) and sometimes a smaller peak in late expiration.[415] Importantly, this pattern of respiratory-sympathetic coupling is altered in several rat models of hypertension, such that sympathetic activity becomes particularly enhanced during inspiration, and appears to be a causative factor in the increased vascular resistance, BP and potentially end organ damage in these animals.[415, 424, 547, 548] Increased sympathetic activity in patients with chronic heart failure has also been linked to alterations in respiratory-sympathetic coupling such that the muscle SNA is highest in those patients

in whom the normal inspiratory-linked inhibition of muscle SNA is most diminished.[32] An attenuation of the direct inhibitory effect of pulmonary stretch receptors on sympathetic activity in response to lung inflation could potentially explain this observation,[549, 550] although an enhanced central-respiratory coupling that elevates muscle SNA during inspiration remains a possibility. Given the well-established increase in SNA in older individuals[22] it was expected to observe a reduced inspiratory inhibition of muscle SNA in older compared with young participants. However, a clear inspiratory attenuation of muscle SNA was evident and no statistical interaction between age and respiratory cycle phase was observed. This indicates that a diminished inspiratory-linked inhibition of muscle SNA in the older group does not contribute to the elevated muscle SNA in the older individuals. Nevertheless, it is important to note that all indices of muscle SNA studied were significantly higher in the older group compared to the younger group at all respiratory phases examined, which may represent an enhancement of respiratory drive to muscle SNA across respiratory cycle phase, although the temporal pattern of the modulation is similar. Given the similar arterial baroreflex regulation of muscle SNA in young and older individuals observed in the present study and reported before[551] this also appears to be an unlikely explanation. However, impaired cardiopulmonary baroreflex buffering of muscle SNA,[552] elevated brain noradrenaline activity[553] and or enhanced peripheral afferent drive from, for example, the heart,[554] kidney[555] or carotid body[510] remain as potential mechanisms for the elevated muscle SNA observed in the older group.

Using cross-correlation histograms between the sympathetic spikes and respiratory-related chest excursion signals Fatouleh and Macefield[418] reported a similar respiratory

modulation of muscle SNA in groups of young (29 ± 2 years) and middle-aged individuals (~20 years older), although no respiratory mediated fluctuations in BP were detected. The findings of the present study partly support these observations and extend them by determining whether sympathetic burst occurrence (i.e., incidence) and strength (i.e., amplitude) are differentially modulated within a breath. Both animal[122, 123] and human [124] investigations have identified that the arterial baroreflex differentially modulates sympathetic burst incidence and amplitude. Previous work examining respiratory modulation of muscle SNA bursts in humans has focused on the evaluation of muscle SNA in terms of total activity.[27-31, 33] In this work it has been observed that all indices of muscle SNA parameters examined (e.g., burst incidence, burst frequency, amplitude and total activity) were significantly modulated by respiration and, despite an age-related elevation in all parameters, no significant interactions between age and respiratory phase were observed.

The study utilises a novel methodological approach to examine the relationships between respiratory mediated changes in muscle SNA and arterial BP.[544] Whilst animal experiments support the contention that respiratory modulation of vasomotor sympathetic outflow causes phasic changes in arteriolar smooth muscle tone thus generating THW,[415] the results of human work are more equivocal.[542, 543] In several recent studies the ability of spontaneously occurring muscle SNA bursts to evoke a beat-to-beat change in peripheral vascular resistance and BP has been carefully described.[528, 536] In accordance with these reports there was significant association between the rMSNA and the THW amplitude of the following breath in all of the young individuals and the large majority of older individuals studied. This relationship was

evident in a similar proportion of young and older individuals. This was somewhat surprising given the reported age-related reduction in a-adrenergic responsiveness,[537, 538] and may be related to the reported down regulation of uptake mechanisms and/or down regulation of degrading enzymes for noradrenaline,[538] or indeed it may be the case that despite a reduction in a-adrenergic responsiveness there is a sufficient safety margin for transmission at the neurovascular junction to maintain effective sympathetic signalling. Notably, the changes in rMSNA were not robustly associated with the previous THW amplitude, supporting the contention that respiratory modulation of muscle SNA is independent of fluctuations in BP.[29, 31]

These data support the contention that respiratory modulation of vasomotor sympathetic outflow causes phasic changes in arteriolar smooth muscle tone thus generating THW. However, it is important to appreciate that a number of complex feedforward and feedback mechanisms have also been implicated.[543, 556] Indeed, Tan and Taylor[556] demonstrated that respiratory fluctuations in heart period cause arterial BP fluctuations especially in young healthy individuals, rather than buffering such pressure fluctuations. In the present investigation a clear reduction in the respiratory linked fluctuations of heart period was noted in the older group, however as in a previous investigation[557] respiratory-linked fluctuations in BP were not significantly different in the young and older groups. Nevertheless, further studies are required to determine how age changes the relative contribution of the many mechanisms implicated in the generation of THW.

In the present study muscle SNA was examined due to its well-established importance in BP regulation. The inability to record directly from the sympathetic nerves supplying

the renal or splanchnic vascular beds in humans means that the potential contribution from these regions to THW amplitude was not ascertained. Furthermore, a definitive explanation for the age-related elevation in BP remains elusive. Aside from adrenergic mechanisms, a number of other factors remain as possible contributors including increased arterial stiffness,[558] up-regulation of endothelin-1 mediated vasoconstriction,[559] reductions in endothelial nitric oxide bioavailability[560] and alterations in renin-angiotensin-aldosterone pathways.[561] Of note, the higher BP with a concomitant preservation in the sensitivity of arterial baroreflex control of muscle SNA observed in the older group, may be explained by the resetting (or shift of the set-point) around which muscle SNA is regulated. However, a limitation of the method employed to assess arterial baroreflex function in the present study was that a complete assessment of the full stimulus–response relationship is not provided and for this a more direct method is required (e.g., modified Oxford technique). Furthermore, while the 'spontaneous' index of arterial baroreflex function used has been considered to provide a useful indicator of sensitivity around the prevailing BP (i.e., operating point of the full baroreflex function curve),[562] they are poorly associated with sensitivity measures derived using the modified Oxford technique.[563]

As in many human studies examining respiratory modulation of SNA, respiration was assessed using a strain-guage pneumobelt.[520, 521] The advantage of this approach is that it is unobtrusive and avoids participants having to breathe through a mouthpiece, which almost inevitably tends to alter breathing pattern.[520, 521] The potential disadvantage of this approach is that the time-delay between the occurrence of respiratory related events within the central nervous system and changes in thoracic circumference (as well as

delays between respiratory central pattern generators and muscle SNA) is not accounted for, and I have assumed that this is a constant between young and older groups. It should also be noted that as in several other cross-sectional studies of respiratory sympathetic coupling[418, 564] respiratory rate and depth were not controlled. However, importantly, it has been reported that muscle SNA is no different during uncontrolled spontaneous breathing and controlled breathing at 12 breaths per minute.[28] Given that the absolute amplitude of a sympathetic burst is related to the proximity of the recording microelectrode to the sympathetic fibres, as is conventional in human sympathetic microneurography studies burst amplitude have been expressed in normalized units.[415, 416]

4.7. Conclusion

In conclusion, the study suggests that despite an age-related elevation in muscle SNA the strength of the respiratory modulation of muscle SNA is similar in young and older individuals. Indeed, the normal inspiratory-linked inhibition of muscle SNA is preserved in older individuals. This suggests that the elevation in muscle SNA found in older individuals is unrelated to a diminished respiratory-sympathetic coupling. In addition, rMSNA changes appear to be a significant component of THW amplitude in both young and older groups.

Contribution

For this experimental chapter, I processed the raw data for extraction, and undertook the data analysis, statistical analysis, data interpretation, and writing of the thesis chapter.

CHAPTER V. INFLUENCE OF DEVICE-GUIDED SLOW DEEP BREATHING ON RESPIRATORY AND ARTERIAL BAROREFLEX CONTROL OF MUSCLE SYMPATHETIC NERVE ACTIVITY IN HUMANS

5.1. Introduction

Interest in the potential BP lowering effects achieved by the volitional control of breathing has a long history.[35, 36] Recent trials of devices that guide slow deep breathing have reported a hypotensive effect from their chronic (e.g., 10-15 min/day for 8 weeks) [37-43] or acute (10 min) use,[56, 58] although such findings have not been uniform.[44-47] The mechanism by which slow deep breathing may lower BP is complex and incompletely understood,[48] but in light of studies reporting coincident reductions in total peripheral resistance,[56] the manipulation of autonomic activity to the peripheral vasculature likely plays a key role.

In a cross sectional study a positive association between spontaneous breathing rate and vasoconstrictor SNA to the skeletal muscle vasculature was identified in young group of healthy men.[130] Moreover, acute device-guided slow deep breathing has been reported to reduce muscle SNA in treated mild hypertensives,[56] in untreated hypertensives,[44] and in patients with chronic heart failure.[565] Although such observations were not confirmed in a mixed group of participants, half of whom were healthy and half had metabolic syndrome.[518] To date there has been no assessment of muscle SNA in young healthy participants performed in studies investigating acute effect of slow deep breathing on cardiovascular parameters.[49, 50, 57]

There are multiple mechanisms whereby slow deep breathing might be expected to evoke a sympatho-inhibitory effect. These include the pulmonary-stretch inflation reflex (Hering-Breuer), the peripheral chemoreflex and baroreflex mechanisms. It has long been established that SNA undergoes respiratory modulation in humans.[539] In young healthy individuals, during normal breathing sympathetic outflow to the skeletal muscle vasculature reaches a nadir at peak inspiration and shows maximal activity during expiration.[27-33] The interaction of central cardiovascular-respiratory circuits along with modulatory feedback signals from cardiorespiratory sensory afferents account for these cyclical sympathetic and cardiac parasympathetic flow fluctuations.[26-33] The sympatho-inhibitory effects of pulmonary-stretch inflation reflex are the primary mechanism of within-breath variation of muscle SNA in humans at higher lung tidal volumes.[29, 31] Spontaneous shallow breathing (decreased tidal volume) has been related to sympathetic hyperactivity in patients with heart failure, likely on account of a reduced inhibition of sympathetic activity by pulmonary stretch receptors.[32, 34] It is presently unclear whether slow deep breathing in young health individuals enhances the inspiratory inhibition of muscle SNA and thus lowers BP.

The acute effect of device-guided slow deep breathing on cardiac parasympathetic activity mediated HR variability has to date been examined in a single study.[58] In a group of healthy middle age participants (with borderline elevated BP) slow deep breathing evoked a significant increase in low-frequency power spectral density, but no effect on high-frequency power spectral density.[58] It has been reported that slow deep breathing increases cardiovagal baroreflex sensitivity in young healthy individuals,[49, 50] obese children,[51] chronic heart failure patients[52, 53] and hypertensive patients.[54] This

improvement in cardiovagal baroreflex sensitivity was attributed to an increase in tidal volume, a reduction in sympathetic overactivity and a restoration of autonomic cardiovascular balance.[53, 54] However, to date it remains unknown whether the slow deep breathing would affect arterial baroreflex control of muscle SNA. This is important to establish because cardiovagal baroreflex sensitivity and arterial baroreflex control of muscle SNA are differentially controlled and do not always change in parallel.[55] To date no studies evaluated the acute effect of slow deep breathing on muscle SNA in young healthy subjects without cardiovascular risk factors.

5.2. Aims and hypotheses

The aim of this study was to investigate whether slow deep breathing reduces BP and muscle SNA in young healthy individuals, and to investigate the underlying autonomic neural control mechanisms. To achieve this, microneurographic recordings of muscle SNA were obtained in young healthy men during spontaneous breathing and acute device-guided slow deep breathing. Offline calculations were made of arterial baroreflex control of muscle SNA, and indices of cardiac parasympathetic regulation derived using HR variability analyses.

The following hypotheses were tested:

1. Slow deep breathing will reduce muscle SNA and BP in young healthy individuals.
2. Slow deep breathing will enhance the inspiratory inhibition of muscle SNA.
3. Slow deep breathing will enhance HR variability and increase the sensitivity of arterial baroreflex control of muscle SNA and HR.

5.3. Methods

5.3.1. Study participants

Ten young males (27±5 years old, body mass index 24±2 kg/m^2, mean±SD) were recruited into the study. All participants were healthy, non-smokers and were not taking any prescription or over-the-counter medications. All participants were asked to abstain from caffeine for at least 12 hours and from alcohol and strenuous physical activity for at least 24 hours prior to the study. All study measurements were made in a temperature controlled room (20-22ºC). Whilst participants were seated office BP were measured from the right arm, prior to any experimental measurements were performed.

The study was approved by local research ethics committee (National Research and Ethics Committee West Midlands-Edgbaston, 11/WM/0368) and conducted in accordance with the Declaration of Helsinki (2008) with written informed consent provided by all participants.

5.3.2. Experimental protocol

The experimental protocol consisted of 10 min of uncontrolled spontaneous breathing followed by 10 min of slow deep breathing guided by the RESPeRATE device (InterCure [UK] Limited, London, UK), which generates melodic tones to assist the individual in slowing their respiratory rate below 10 breaths per minute or less.

5.3.3. Experimental measurements

5.3.3.1. Blood sampling

Fasting venous blood samples were taken prior to any other tests after 15-20 min rest in the supine position.

5.3.3.2. Cardiovascular measures

While participants rested in a supine position muscle SNA, beat-to-beat arterial BP, electrocardiogram, respiratory parameters, $P_{ET}CO_2$, echocardiography and arterial stiffness were obtained and stored as described in section 3.1.

5.3.4. Experimental data analyses

The analyses of HR, BP, steady-state muscle SNA, arterial baroreflex sensitivity, HR variability were performed as described in section 3.2.

5.4. Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 21.0 for Windows (IBM Inc, Chicago, IL). Data were tested for normality by Kolmogorov-Smirnov test. Effect of device-guided slow deep breathing on cardiorespiratory and autonomic parameters was assessed using paired sample t-test for parametric data and Wilcoxon test for non-parametric data. Mixed between-within subject ANOVA analysis, adjusted using the Greenhouse-Geiser

correction, was used to examine the main effects of respiratory cycle phase, condition (spontaneous breathing/slow deep breathing) and their interaction with respiratory modulation of muscle SNA. Data are expressed as mean ± SD, or median (interquartile range) in text and tables and as median ± SE in figures. A *P* value of <0.05 was considered statistically significant.

5.5. Results

5.5.1. Subject characteristics

Demographic characteristics of the study participants are summarised in Table 5.1. Blood biochemistry and arterial stiffness were within the normal range for this age group. Any underlining abnormalities in cardiac structure or left ventricular systolic and diastolic function were excluded by echocardiography (Table 5.2).

Table 5.1. Demographic and clinical characteristics of the study groups

Age (years)	27±5.4
Weight (kg)	78±8.1
Height (cm)	180±3.2
Body mass index (kg/m^2)	24±2.0
Aortic augmentation index HR@75	-4.1±10.1
Total cholesterol (mmol/l)	4.1±0.5
HDL cholesterol (mmol/l)	1.3±0.2
Triglycerides (mmol/l)	0.9±0.4
Glucose (mmol/l)	4.1±0.7
Creatinine (µmol/l)	78.5±8.8
Estimated glomerular filtration rate ($mL/min/1.73\ m^2$)	89.2±2.5
Haemoglobin (g/l)	145.1±8.7
Lymphocytes ($x10^3$ per µl)	1.70±0.72
Monocytes ($x10^3$ per µl)	0.60±0.19

Data presented as mean±SD. HDL, high-density lipoprotein

Table 5.2. Echocardiographic characteristics of the study group

Left ventricular mass index (g/m^2)	68.2±8.8
Left ventricular ejection fraction (%)	61.3±1.8
Systolic annular septal velocity (cm/sec)	7.1±0.8
Systolic annular lateral velocity (cm/sec)	9.4±2.1
Cardiac output (L/min)	4.0±0.6
Mitral valve inflow (E/A) ratio	2.1±0.6
E/e' septal ratio	6.5±1.6
Lateral E/e' ratio	4.7±1.0
Left atrial volume index (ml/m_2)	18.1±4.5

Data presented as mean±SD

5.5.2. Acute device-guided slow deep breathing

5.5.2.1. Cardiorespiratory parameters and steady–state muscle SNA

Device-guided slow deep breathing resulted in a 2-fold decrease in respiratory rate (P<0.001) and ≈2-fold compensatory increase in tidal volume, such that minute volume and $P_{ET}CO_2$ were unchanged (Table 5.3). Muscle SNA was reduced with slow deep breathing (burst incidence, 23 (19-34) vs. 20 (17-29) bursts·100 heart beats^{-1}, P=0.03; burst frequency, 14 (12-19) vs. 13.5 (9-17) bursts^{-1}, P=0.04; total activity, 772 (521-858) vs. 598 (492-746) AU, P=0.04) with no effect on burst amplitude, 49±7 vs. 46±10 AU, P=0.25 (Figure 5.1, Figure 5.2). However, systolic BP, diastolic BP, mean BP, pulse pressure and HR were not significantly changed with device-guided slow deep breathing.

Table 5.3. Influence of device-guided slow deep breathing on cardiorespiratory parameters

	Spontaneous breathing	Slow deep breathing	*P* value
Respiratory rate (breaths/min)	12.4±0.8	6.2±0.7	0.001
Tidal volume (L)	0.8±0.2	1.4±0.3	0.001
Minute ventilation (L/min)	8.9±1.6	8.2±1.7	0.20
$P_{ET}CO_2$ (mmHg)	40.7±3.1	40.3±3.2	0.63
Systolic BP (mmHg)	125±9.3	124±9.9	0.62
Diastolic BP (mmHg)	88±6.3	86±7.3	0.38
Mean BP (mmHg)	69±6.7	68±8.2	0.29
Pulse pressure (mmHg)	53±10.0	56±8.5	0.28
Heart rate (beats/min)	61±3.0	62±3.2	0.61
R-R interval (s)	1.013±0.176	1.005±0.179	0.8

Data presented as mean±SD; BP, blood pressure; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide.

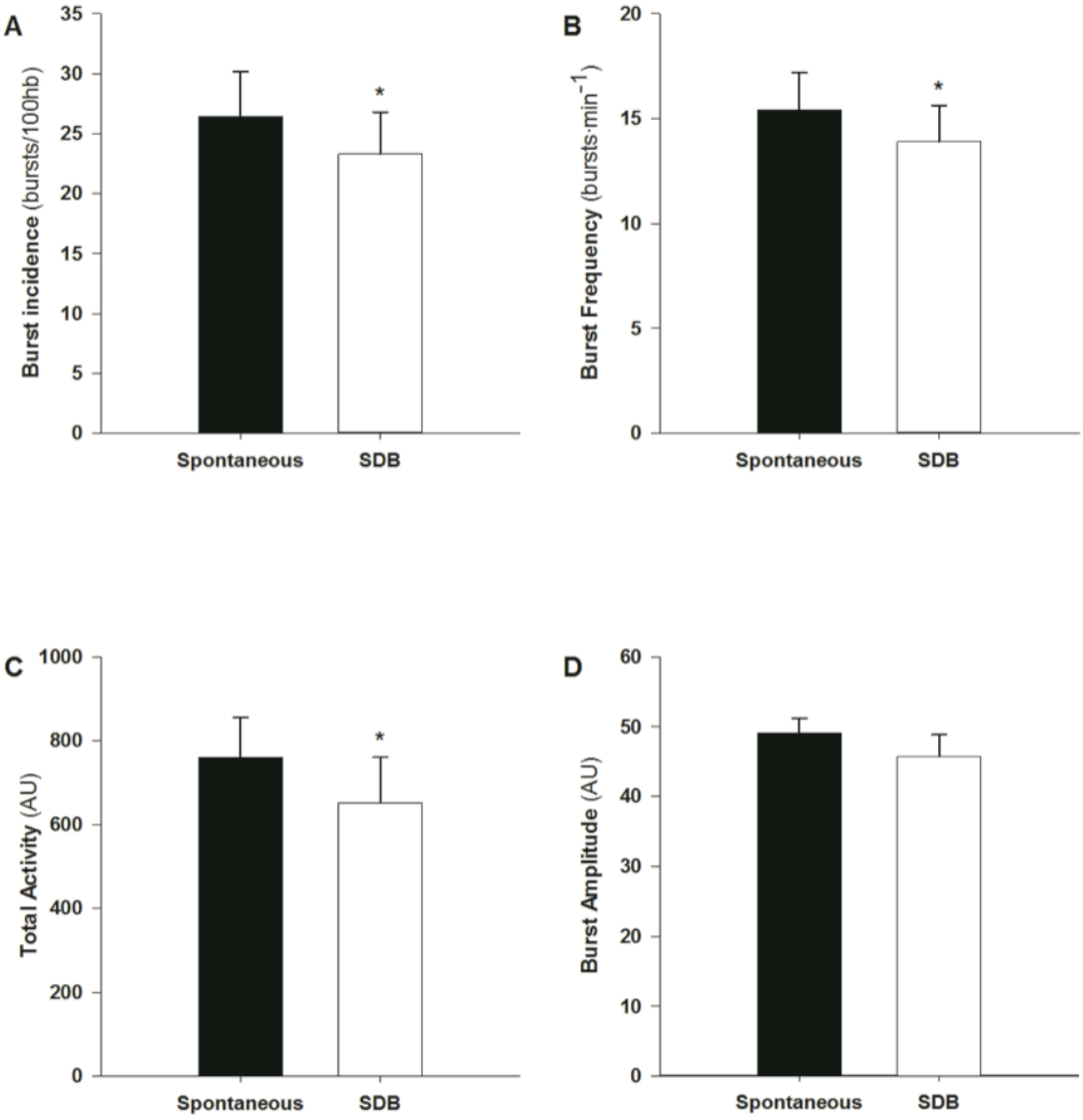

Figure 5.1. Influence of acute device-guided slow deep breathing (SDB, 10 min) on muscle SNA burst incidence (panel A), frequency (panel B), total activity (panel C) and amplitude (panel D)

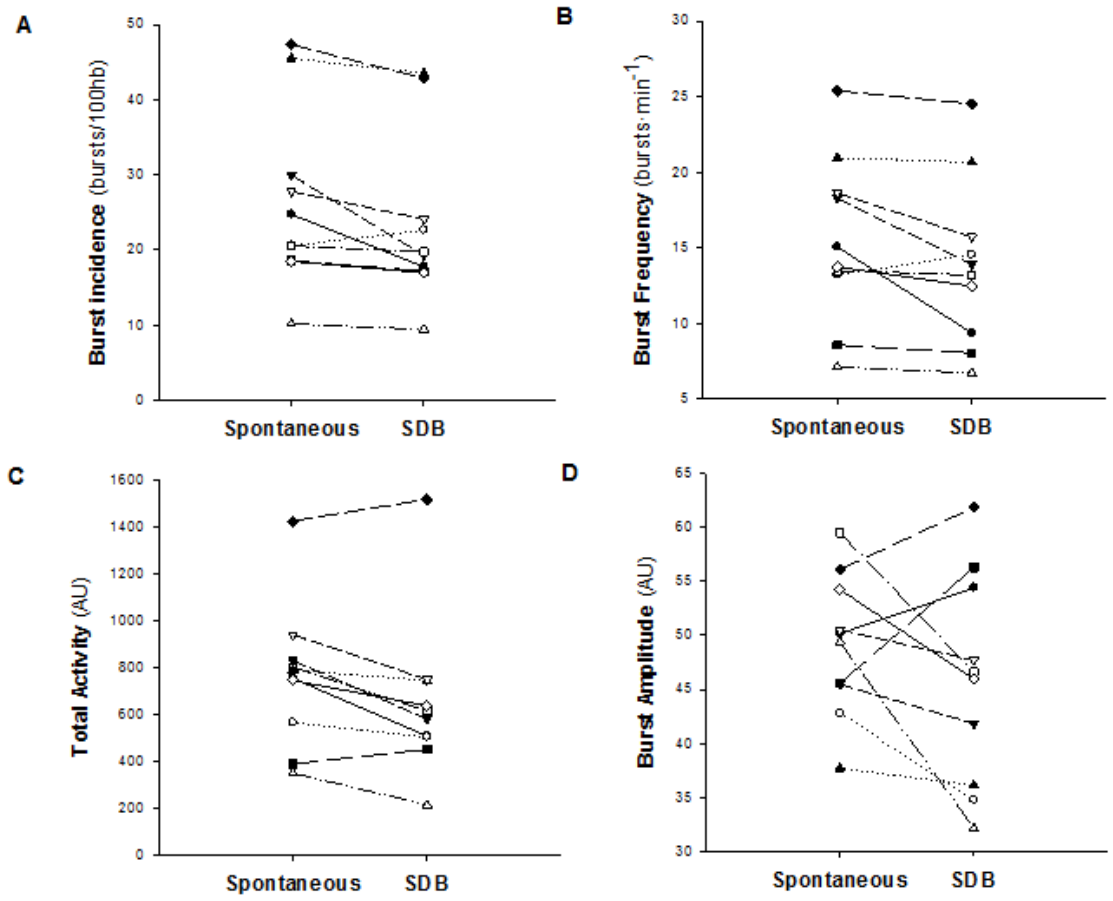

Figure 5.2. Individual influences of acute device-guided slow deep breathing (SDB, 10min) on muscle SNA burst incidence (panel A), frequency (panel B), total activity (panel C) and amplitude (panel D)

5.5.2.2. Baroreflex sensitivity and HR variability

Device-guided slow deep breathing did not significantly alter either cardiac baroreflex sensitivity (systolic BP–RR interval, 13.6±3.4 vs. 14.7±2.1, *P*=0.21) or arterial baroreflex control of muscle SNA (burst incidence gain, -3.96 (-4.86 – -2.55) vs. -3.52 (-4.69 – -2.64), *P*=0.88; total muscle SNA gain, -17.2 (-26.4 – -11.1) vs. -14.4 (-17.9 – -9.01), *P*=0.39; (Figure 5.3). Time domain indices of HR variability (RMSSD, SDNN and pNN50%) were not changed with device-guided slow deep breathing (Table 5.4, Figure 5.4). However, device-guided slow deep breathing significantly decreased HF power spectral density, while LF power spectral density and LF:HF ratio were significantly increased and total power remained unchanged.

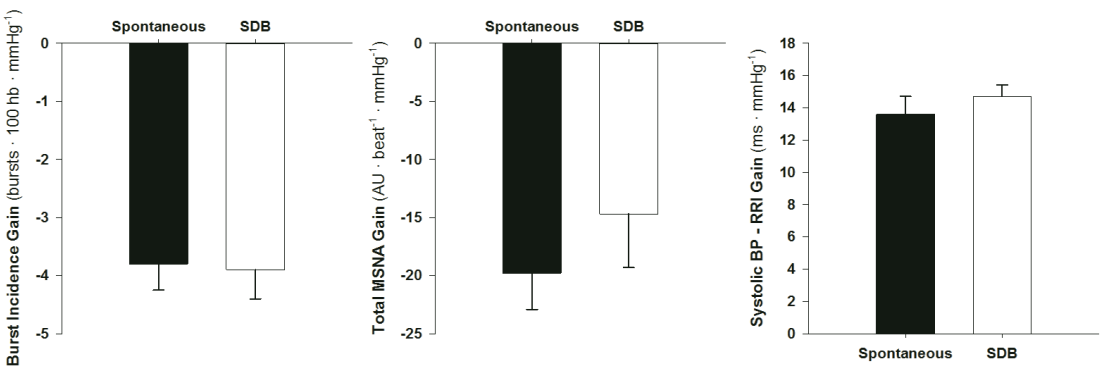

Figure 5.3. Influence of acute device-guided slow deep breathing (SDB, 10 min) on indices of spontaneous cardiac and sympathetic baroreflex sensitivity

Data presented as mean±SE; BP, blood pressure; MSNA, muscle sympathetic nerve activity.

Table 5.4. Influence of device-guided slow deep breathing on heart rate variability parameters

	Spontaneous breathing	Slow deep breathing	*P* value
RMSSD (ms)	54.4 (36.0-77.5)	46.8 (33.8-66.1)	0.96
SDNN (ms)	67.8±22.5	76.7±20.8	0.21
pNN50 (%)	34.5 (14.8-51.4)	23.8 (12.0-46.2)	0.29
HF (ms^2)	1098 (708-2426)	331 (204-939)	0.005
LF (ms^2)	1092 (562-1993)	3724 (2344-5741)	0.01
TP (ms^2)	3479 (1839-5715)	4581 (3182-7370)	0.11
HF (n.u.)	58.4 (42.0-66.0)	7.9 (6.7-15.4)	0.005
LF (n.u.)	46.3±15.0	88.7±8.0	0.001
LF/HF	0.7 (0.5-1.4)	11.6 (5.5-13.9)	0.005

Data presented as median (interquartile range) or mean±SD; RMSSD, square root of the mean of the sum of successive differences in R-R interval; SDNN, standard deviation of normal to normal R-R interval; pNN50%, proportion of successive R-R interval which vary by >50 ms; TP, total power (0.0-0.4 Hz); HF, high-frequency power (0.15-0.4 Hz); LF, low-frequency power (0.04-0.15 Hz).

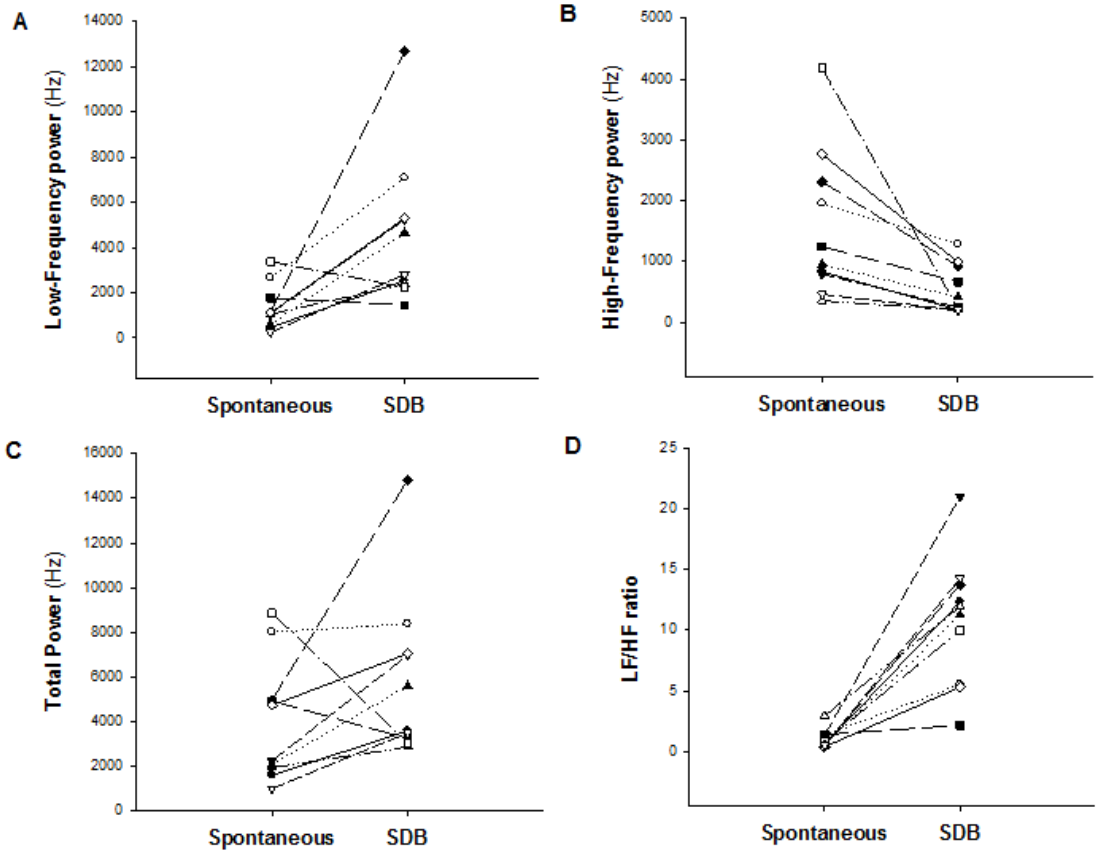

Figure 5.4. Individual influences of acute device-guided slow deep breathing (SDB, 10min) on heart rate variability parameters, low-frequency (LF) (panel A), high-frequency (HF) (panel B), total power (panel C) and LF/HF ratio (panel D)

5.5.2.3. Effects on respiration related muscle SNA

Figure 5.5 provides an original record from a representative subject illustrating the effect of device-guided slow deep breathing on the measured cardiorespiratory and autonomic parameters. With the decreased respiratory frequency and increased tidal volume a more pronounced inhibition in muscle SNA during the inspiratory to post-inspiratory period was observable. Figure 5.6 shows distribution of muscle SNA over the breathing cycle (the inspiratory peak was marked as 0). Mixed between-within subject ANOVA analysis revealed a significant interaction between respiratory cycle phase and the study condition (spontaneous breathing vs. slow deep breathing) for parameters of muscle SNA (burst incidence, burst frequency, total activity) ($P<0.001$). More specifically, device-guided slow deep breathing enhanced the inhibition of the muscle SNA (incidence, frequency and total activity) during inspiration, whereas during expiration muscle SNA was elevated. In contrast, muscle SNA burst amplitude was not significantly different at any phase of the respiratory cycle during either the spontaneous breathing or slow deep breathing conditions.

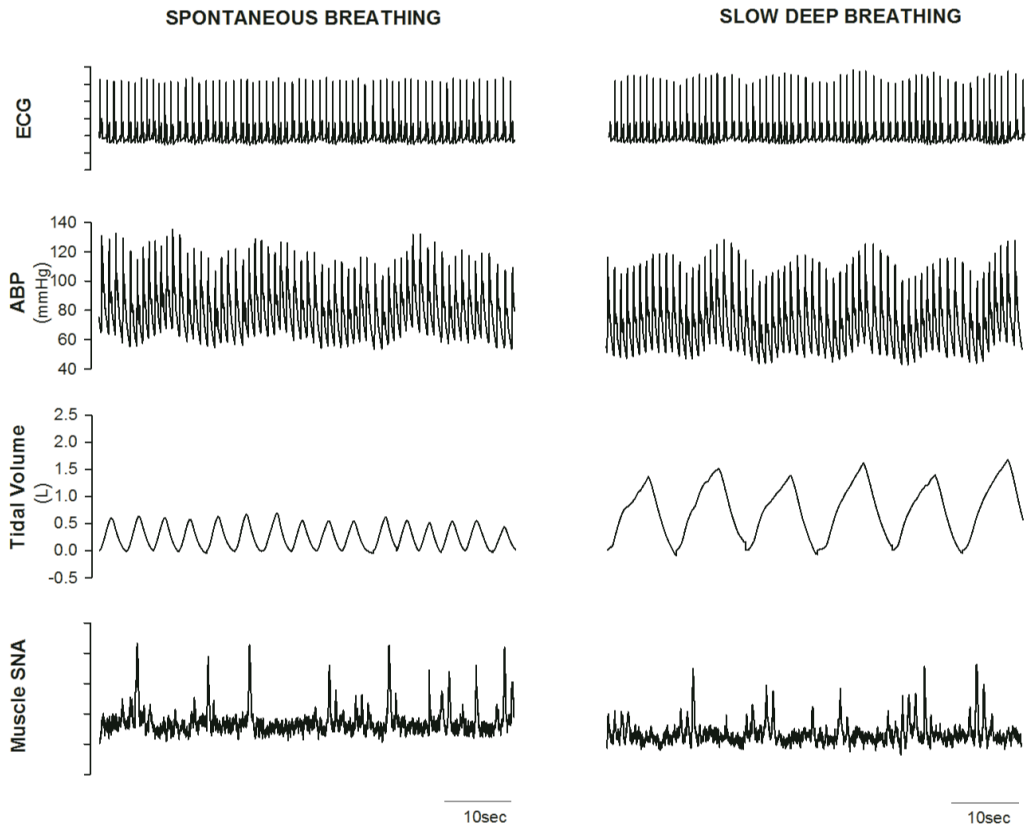

Figure 5.5. Original record showing cardiorespiratory responses to slow deep breathing (see more details in text)

ABP, arterial blood pressure; ECG, electrocardiogram; SNA, sympathetic nerve activity.

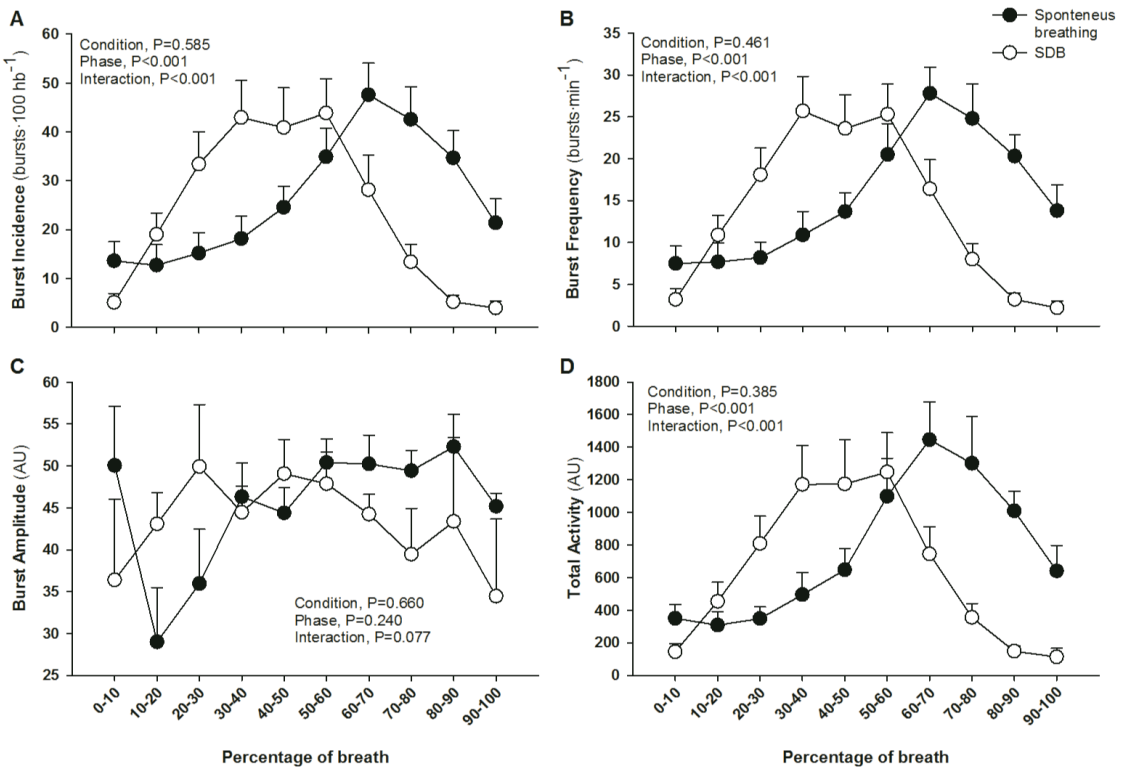

Figure 5.6. Influences of acute device-guided slow deep breathing (SDB, 10 min) on respiratory modulation of muscle SNA burst incidence (panel A), frequency (panel B), amplitude (panel C) and total activity (panel D)

Data presented as mean±SE; peak of inspiration at 0 mid-to-late expiration was denoted as 30-79% of the breath, while inspiratory to post-inspiratory period was taken as the remaining period of the respiratory cycle.

5.6. Discussion

The main finding of the present study is that acute device-guided slow deep breathing reduces muscle SNA (burst incidence, burst frequency and total activity) but not BP, arterial baroreflex control of muscle SNA or cardiovagal baroreflex regulation in young healthy males. These observations indicate that device-guided slow deep breathing reductions in central sympathetic outflow are not accompanied by an increase in baroreflex sensitivity (cardiac or muscle SNA), but may be attributable to enhanced sympatho-inhibitory effects of lung inflation reflex and/or changes in central respiratory-sympathetic coupling.

No changes in BP were seen in healthy volunteers in this study. This is in agreement with previous observations that young healthy individuals with normal BP pressure are unlikely to exhibit reductions in BP in response to the slow deep breathing, potentially due to the fact that their vascular resistance is not elevated.[49, 50, 57] In contrast, the majority of studies on hypertensive patients show an acute reduction in BP with slow deep breathing.[54, 56] In prospective studies, device-guided slow deep breathing training reduced BP more effectively in older patients and in those with higher baseline BP values, two populations known to typically exhibit a higher vascular resistance.[40, 42]

This study evaluated for the first time the acute effect of the RESPeRATE device on directly recorded muscle SNA in a young healthy cohort, showing a small but statistically significant reduction in skeletal muscle SNA. A slower spontaneous breathing rate has previously been associated with lower levels of sympathetic

activation. For instance, Narkiewicz at al. reported that respiratory rate in a group of healthy males was the only independent predictor of muscle SNA, and that individuals with slower spontaneous respiratory rates have reduced chemoreflex responses to hypoxia and hypercapnia.[130] Furthermore, acute slow deep breathing reduces chemoreflex sensitivity within an individual.[50, 58, 566] This study results are consistent with these findings as the device-guided slow deep breathing mediated reduction in respiratory rate led to a reduction in muscle SNA. The majority of studies of hypertensive patients show acute reduction in muscle SNA and improvement in cardiac baroreflex sensitivity in response to the slow deep breathing intervention.[54, 56] Previous data from Hering at al. from untreated relatively young (mean age 37 years) hypertensive patients showed no acute reduction in BP or HR but significant reduction in muscle SNA following the acute use of this device.[44] The present study extends these findings by determining whether acute device-guided slow deep breathing modulates arterial baroreflex regulation of heart and muscle SNA. It is important to emphasise that both arms of the baroreflex do not always change in parallel, and both functions need to be assessed in order to comprehensively evaluate baroreflex function.[55, 567] In contrast to the original hypothesis, no significant increase in the arterial baroreflex control of muscle SNA measured by both incidence gain and total muscle SNA gain was observed. Similarly, device-guided slow deep breathing did not change cardiac baroreflex sensitivity. Reports examining the acute cardiac baroreflex sensitivity responses to slow deep breathing in young healthy subjects have not been uniform, with studies showing increases[49, 50] or no effect on sensitivity.[57] This study on carefully selected healthy volunteers free from any cardiovascular system pathologies does not show any improvement in cardiac baroreflex sensitivity.

At a higher lung tidal volume the pulmonary-stretch inflation reflex is the main sympatho-inhibitory mechanism, however afferent feedback from arterial baroreflexes, chemoreflexes and central respiratory motor output also play a modulatory role.[26-33, 539, 549, 568-572] The original record provided (Figure 5.5) shows clearly that the pattern of the within-breath inhibition during the inspiratory to post-inspiratory period was more pronounced during the device-guided slow deep breathing. Group analysis shows the within breath modulation of muscle SNA burst incidence, burst frequency and total activity during the spontaneous breathing and slow deep breathing.

In this study, acute device-guided slow deep breathing (mean frequency 0.1Hz) led to a significant increase in LF (0.04-0.15 Hz) power spectral density, LF/HF ratio and reduction of HF (0.15-0.4 Hz) power spectral density of HR variability. This result is consistent with the data from previous studies, showing the effect of slowing of breathing rate (non-device guided).[573] This effect was specifically attributed to the significant increase in LF power spectral density, LF/HF ratio in young healthy men and women participants. The acute effect of the RESPeRATE device has not been studied before in a young healthy cohort and only a single study tested this in a healthy older group with borderline elevated BP.[58] That latter study found an increase in LF power spectral density, which is consistent with this study, but no effect on the HF power (time domain indices were not reported). An increase in LF power spectral density have been also noted during yoga and meditation. Furthermore, this increase in LF power spectral density was correlated with changes in frequency of respiratory sinus arrhythmia and increased coherence in cardiopulmonary coupling (i.e., between HR and breathing rate).[574, 575] Respiratory sinus arrhythmia depends principally on

parasympathetic tone.[569, 576] Thus in the settings of reduced breathing rate, an increase in the power in LF band corresponds to increase in the parasympathetic tone. Bernardi et al. pointed out that when the HR variability is assessed by frequency analysis and the breathing pattern is altered, the mechanistic implication of the finding should be interpreted with care.[577] As specified before in Chapter 2.3.5 traditional indicators of increased cardiac parasympathetic tone is reduced LF power spectral density and increased HF power alone with higher time domain parameters (RMSSD, SDNN, pNN50%).[190 21] Admittedly interpretation of results of HR variability findings could be ambiguous, partly due to the on-going dispute on physiological meaning of changes in LF and HF.[578, 579]

5.7. Limitations

The relatively small sample size is a potential limitation of the present study however this did not prevent detection of a significant reduction in muscle SNA in response to acute device-guided slow deep breathing with RESPeRATE device.

5.8. Conclusion

The study findings indicate that although slow deep breathing does not affect BP or arterial baroreflex control of muscle SNA in young healthy individuals it does reduce muscle SNA burst incidence, frequency and total activity. The reduction in muscle SNA with device-guided slow deep breathing in young healthy participants appear not to be mediated by a change in baroreflex sensitivity, but may reflect an increase in lung

inflation afferent input and/or a reduction in central respiratory-sympathetic coupling. Undoubtedly, complexity of regulatory mechanisms implicated in the processes studies leaves a possibility for alternative contributing factors, and certainly further research is essential to understand intimate details of the phenomenon examined in this study.

Contribution

For this experimental chapter, I contributed to the study design. I undertook identification of the study participants, their screening, recruitment, consent, examination, data acquisition (except for the microneurography, where positioning of the electrodes into the peroneal nerve was performed by Dr J. Fisher). I performed data analysis, data interpretation and wrote the thesis chapter.

CHAPTER VI. THE INFLUENCE OF HOME-BASED, SLOW DEEP BREATHING TRAINING ON CENTRAL SYMPATHETIC OUTFLOW AND BAROREFLEX SENSITIVITY IN ESSENTIAL HYPERTENSION

6.1. Introduction

Hypertension is a key cardiovascular risk factor and affects around 1 billion people worldwide, as emphasized in section 2.2.1.[1-4] More than half fail to achieve optimal BP control.[1, 5] This has major clinical and social consequences because with each 2 mmHg increase in systolic BP there is a 7% and 10% increase in mortality from coronary disease and stroke, respectively.[6] In parallel with pharmacological management, non-pharmacological approaches can help to achieve desirable BP control. The potential for BP lowering by the volitional control of breathing has long been purported[35, 36] and recently home-based device-guided slow deep breathing training (e.g., RESPeRATE® device) has been recommended by the American Heart Association.[48] In a recent meta-analysis based on 13 original studies, such home-based slow deep breathing training was concluded to reduce systolic and diastolic BP by 4 and 3 mmHg respectively, after accounting for BP in placebo group.[48] However, the physiological mechanisms leading to the BP lowering effects of device-guided slow deep breathing, and the potential for associated effects on neural regulation, cardiovascular structure and function remain unclear.

The respiratory-related modulation of the SNA has been recognized since the earliest direct recordings and is substantively generated by central neuronal circuits and the

modulatory influence of feedback signals from cardiorespiratory sensory afferents (e.g., lung-stretch receptors, baroreceptors, peripheral chemoreceptors).[26-33] Activation of baroreflex afferents by BP changes stimulates central neuronal circuits and modulates efferent parasympathetic and sympathetic activities to the sinus node, resulting in characteristic fluctuations in HR.[28, 31, 549, 568-572] Increased SNA and reduced parasympathetic tone is believed to be a strong contributor to development of hypertension.[9, 12, 28, 140, 214, 216-222, 568, 569] Excessive sympathetic outflow in hypertension leads to development of LV hypertrophy[14-16] and presence of LV hypertrophy in hypertensive patients was linked to reduced HR variability.[17] Furthermore high muscle SNA is associated with LV diastolic dysfunction independently of BP levels[18, 19] Hypertensive patients have increased arterial stiffness.[238, 580, 581] It has been shown that heightened SNA directly affects vascular smooth muscle cell proliferation and hypertrophy independently from BP levels thus contributing to target organ damage in these patients.[229, 232, 238] Emerging evidence suggests that device-guided slow deep breathing may favorably modify the profile of autonomic cardiovascular control, but it is not known whether it could diminish the target organ damage.

In a study of middle age community volunteers, 15 min slow deep breathing produced a significant reduction in systolic BP, $P_{ET}CO_2$ and increase in low-frequency HR variability. However, HF power spectral density of HR variability was not altered and muscle SNA was not studied.[58] Slow deep breathing for 10 min in hypertensive patients is reported to reduce BP,[54, 56] increase cardiac baroreflex sensitivity[54] and reduce muscle SNA.[44, 56] There has been only one investigation of the muscle SNA response to the long-term slow deep breathing training, performed on untreated hypertensive patients.[44]

At the baseline visit, 15 min of acute device-guided slow breathing resulted in sympathoinhibition but no reduction in BP. Long-term follow up for 8 weeks of the above group of patients[44] failed to show a reduction in BP or muscle SNA. In patients with hypertension and diabetes, 8 weeks of home-based device-guided slow deep breathing training reduced BP and increased LF power spectral density HR variability, with no effect on HF and total power. However, the study did not assess SNA, thus limiting the pathophysiological insights provided.[59]

Despite several trials investigating the effect of device-guided slow deep breathing on BP few studies have assessed the mechanisms of the acute and especially long-term effects of the technique. Indeed, the acute and longer-term effects of slow deep breathing on arterial baroreflex have not been considered. Of note, arterial baroreflex control of muscle SNA is modulated by different mechanisms than cardiac baroreflex and they do not correlate.[55] The acute effect of device-guided slow deep breathing on HR variability in hypertensive patients has not been studied, and neither has the effect of longer term slow deep breathing training on HR variability been evaluated in a hypertensive cohort without diabetes. Furthermore, controversial findings have been reported regarding the BP and muscle SNA responses to longer-term slow deep breathing training, likely reflecting duration of the intervention and populations studied. Finally, the effects of home-based device-guided slow deep breathing training on cardiovascular function and structure have not been evaluated.

6.2. Aims and hypotheses

The aim of this study was to comprehensively evaluate the acute and long-term training effects of device-guided slow deep breathing on autonomic regulation in hypertension and effects on target organ damage (e.g. heart, vessels, kidney). To achieve this, cardiorespiratory parameters, muscle SNA, HR variability and arterial baroreflex function were measured at rest and in response to acute slow deep breathing (10 min), both before and after home-based long-term device-guided slow deep breathing training (8 weeks). The influence of this training regime on cardiac (echocardiography), vascular (arterial stiffness) and kidney function was also determined.

The following hypotheses were tested:

1. BP will be reduced in response to acute and longer-term device-guided slow deep breathing training in patients with hypertension.
2. BP reductions will be accompanied by a reduction in muscle SNA, and enhanced HR variability and arterial baroreflex control of the heart and muscle SNA.
3. Home-based device-guided slow deep breathing training in patients with hypertension will improve cardiac and vascular function.

6.3. Methods

6.3.1. Study design

Ethical approval was granted by local research ethics committee (National Research and Ethics Committee West Midlands-Edgbaston, 11/WM/0368) and conducted in accordance with the Declaration of Helsinki (2008). Prior to any study measurements all participants provided written informed consent for participation.

The study contained both cross-sectional and longitudinal components. In the cross-sectional study patients with essential hypertension were compared with matched healthy control participants. In the longitudinal study hypertensive patients underwent home-based training with the RESPeRATE device. This involved patients spending at least 10 min breathing at ≤10 breath/min ('therapeutic zone') on a minimum of 4 days per week for 8 consecutive weeks.[41] To ensure adherence, weekly telephone calls were made to participants and usage data from the last 30 days of the training was stored in the microprocessor of the RESPeRATE device for quantification of adherence to intervention and average synchronization of the breath (i.e., how well duration of inhalation and exhalation synchronized with guiding tones).

In total 21 hypertensive patients were recruited, however 1 experienced multiple cardiac rhythm ectopics meaning that their data was not suitable for analysis. Therefore, in the cross-sectional comparison 20 hypertensive patients were compared with 19 matched

controls. In the longitudinal study 19 from 20 completed follow up and were included in the analysis.

6.3.2. Study participants

In the cross-sectional study twenty patients with essential hypertension were compared with 19 age (mean±SD, 54±12 vs. 54±15 years old, respectively, *P*=0.9), sex (13 vs. 12 males, *P*=0.5) and body mass index (28±3 kg/m^2 vs. 26±3 kg/m^2, *P*=0.07) matched normotensive healthy controls. All hypertensive patients were clinically stable and treated (≥3 months, median time since diagnosis 48 months, range 12-169 months). Control participants were not taking prescription or over-the-counter medications and deemed healthy by careful medical history, clinical examination, baseline blood tests, ECG and transthoracic echocardiography. Participants were free from coronary artery disease, significant valvular heart disease; recent (<6 months) primary angioplasty, stroke; atrial fibrillation; active infections and/or a history of inflammatory or connective tissue disorders; chronic and systemic illnesses (e.g. respiratory diseases, renal or liver failure, diabetes mellitus, malignancy); were not on hormone replacement therapy and were not pregnant.

All study participants were asked to abstain from caffeine use for at least 12 hours and from alcohol intake and strenuous physical activity for at least 24 hours prior to the study tests. All participants were non-smokers. Hypertensive subjects refrained from taking their medications on the study day. All study measurements were performed in the morning, in a quiet and temperature controlled room (20-22ºC). Office BP

measurements were performed prior to any testing from the right arm whilst participants were seated.

6.3.3. Experimental protocol

The experimental protocol consisted of 10 min of uncontrolled spontaneous breathing followed by 10 min of slow deep breathing guided by the RESPeRATE device (InterCure (UK) Limited, London, UK), which uses melodic tones to slow the respiratory rate below 10 breaths per minute. All measurements were obtained while participants were in a supine position. The same protocol was repeated in hypertensive patients who completed their 8 weeks follow up training.

6.3.4. Experimental measurements

6.3.4.1. Blood sampling

Baseline venous blood sampling was performed prior to any other tests after 15-20 minute rest in the supine position. The samples were used for clinical biochemistry and full blood counts analysis.

6.3.4.1. Cardiovascular measures

HR, beat-to-beat arterial BP, muscle SNA, respiratory parameters, $P_{ET}CO_2$, echocardiography and arterial stiffness were obtained and stored as described in section 3.1.

6.3.5. Experimental data analyses

The analyses of HR, BP, steady-state muscle SNA, arterial baroreflex sensitivity, HR variability were performed as described in section 3.2.

6.4. Statistical analysis and power calculation

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 21.0 (IBM Inc, Chicago, IL). Data were tested for normality by Kolmogorov-Smirnov test. Data are expressed as mean ± SD, or median (interquartile range) in text and tables. Chi-square analysis used for comparisons of categorical data. In the cross-sectional analysis the groups were compared using unpaired sample t-test for parametric data and Mann-Whitney test for non-parametric data. Comparisons of the acute effects of slow deep breathing were made using calculated differences (deltas) between values before and after the test in each group.

In the longitudinal study, the effect of device-guided slow deep breathing on study parameters was assessed using paired sample t-tests for parametric data and Wilcoxon tests for non-parametric data. The analysis also included comparison of the acute effects of slow deep breathing at the beginning and the end of the study. The acute effects were quantified as differences (deltas) between values after and before the test performed at each time point of the study.

In the longitudinal study Spearman correlation analysis was used to assess associations between number of successful training sessions over the last 30 days of follow up period and diastolic BP at follow up visit. A *P* value of ≤0.05 (two tailed) was considered statistically significant.

On the basis of the data of Schein et al.,[43] demonstrating the BP lowering effects of device-guided slow deep breathing in patients with hypertension, a sample size of 20 will allow the minimal detectable difference in systolic BP pre and post training of 8% (with *P*=0.05, power = 80% and assuming that the patients have a similar systolic BP of 156.6 ± 14.0 mmHg).

6.5. Results

6.5.1. Cross-sectional study of healthy controls and patients with hypertension

6.5.1.1. Subject characteristics

Patients with hypertension were closely matched by age, sex and body mass index to the healthy control participants (Table 6.1). Three patients did not receive antihypertensive treatment and 17 were treated with medications. Medication use (60% calcium channel blockers, 45% angiotensin-converting-enzyme inhibitor or angiotensin receptor blockers, 40% thiazide diuretics, 20% beta-blockers and 25% statins) was stable for at least 3 months before the study. As expected hypertensive group had significantly higher office BP compared with healthy group (P<0.001). There was no difference in parameters of clinical biochemistry (Table 6.2) or AI corrected for HR of 75 bpm (Table 6.3) between the study groups. Echocardiography ruled out any abnormalities in cardiac structure and left ventricular systolic and diastolic function in healthy controls (Table 6.3). Compared to healthy controls hypertensive patients had a significantly higher left ventricle mass index and E/e' septal ratio, while all other parameters examined were not different.

Cardiorespiratory parameters for the two groups are provided in Table 6.4. Muscle SNA burst frequency was higher in the hypertensive group (31.3 ± 7.7 vs. 24.0 ± 7.9 bursts/min in healthy controls, P=0.04) with no significant difference in the burst incidence found. Reductions in spontaneous cardiac baroreflex sensitivity and arterial

baroreflex control of muscle SNA were observed in the hypertensive group ($P \leq 0.05$). Parameters of HR variability (SDNN, LF and TP) were higher in healthy controls ($P \leq 0.05$) value (Table 6.5).

Table 6.1. Baseline characteristics in healthy controls and patients with hypertension

	Healthy Controls	Hypertensive Patients	*P* value
Age (years)	53.7±14.8	54.4±12.4	0.88
Males (n, %)	13 (72)	12 (60)	0.51
Weight (kg)	78±10.5	81±13.3	0.45
Height (cm)	173±10.4	170±8.2	0.35
BMI (kg/m^2)	25.9±2.7	27.6±2.8	0.07
Office systolic BP (mmHg)	122±9.7	147±17.7	<0.001
Office diastolic BP (mmHg)	71±6.4	85±9.8	<0.001

Data presented as mean±SD. BMI, body mass index; BP, blood pressure.

Table 6.2. Blood biochemistry at rest in healthy controls and patients with hypertension

	Healthy Controls	Hypertensive Patients	*P* value
Total cholesterol (mmol/l)	4.9±1.0	5.3±1.1	0.64
HDL (mmol/l)	1.4±0.3	1.3±0.4	0.19
Triglycerides (mmol/l)	4.6 (4.1-5.8)	5.2 (4.8-6.3)	0.32
Glucose (mmol/l)	4.9±0.8	5.0±0.4	0.65
Creatinine (μmol/l)	83.3±21.6	84.4±22.7	0.49
Estimated glomerular filtration rate (mL/min/1.73 m^2)	76.2±13.2	75.4±14.1	0.60
Lymphocytes (x10^3 per μl)	1.8±0.6	1.9±0.6	0.37
Monocytes (x10^3 per μl)	0.48 (0.33-0.59)	0.56 (0.37-0.63)	0.44

Data presented as mean±SD or median (interquartile range). HDL, high-density lipoprotein.

Table 6.3. Echocardiography parameters and arterial stiffness at rest in healthy controls and patients with hypertension

	Healthy Controls	Hypertensive Patients	*P* value
Left ventricular mass index (g/m^2)	66.3±8.6	82.3±20.1	0.004
Left ventricular ejection fraction (%)	60.9±3.3	61.6±2.9	0.53
Systolic annular septal velocity (cm/s)	6.3±0.8	6.9±1.6	0.22
Systolic annular lateral velocity (cm/s)	7.8±1.4	7.2±2.4	0.42
Stroke volume (ml)	65.8±11.6	62.2±12.5	0.38
Cardiac output (L/min)	3.7±0.4	3.9±1.0	0.37
Mitral valve inflow (E/A) ratio	1.4±0.4	1.1±0.4	0.02
E/e' septal ratio	7.8±2.0	10.8±3.2	0.002
Left atrial volume index (ml/m^2)	16.9±4.2	18.0±4.4	0.48
Aortic Augmentation Index HR@75	18.0 (1.0-27.0)	26.5 (12.3-29.8)	0.14

Data presented as mean±SD or median (interquartile range). HR, heart rate.

Table 6.4. Baseline cardiorespiratory parameters in healthy controls and patients with hypertension

	Healthy Controls	Hypertensive Patients	*P* value
Respiratory frequency (b/min)	11.6±2.6	12.3±3.5	0.49
Tidal volume (L)	0.6 (0.5-0.7)	0.4 (0.4-0.9)	0.19
Minute ventilation (L/min)	6.6±1.6	5.9±1.9	0.23
$P_{ET}CO_2$ (mmHg)	41.0±2.5	39.9±2.3	0.16
Heart rate (beats/min)	57±8.6	61±9.1	0.14
R-R interval (s)	1.09±0.17	1.01±0.15	0.13
Burst incidence (bursts/100hb^{-1})	42.5±14.9	53.1±17.8	0.14
Burst frequency (bursts/min)	24.0±7.9	31.3±7.7	0.04
Total activity (AU)	1309.1±515.9	1658.4±436.6	0.09
Burst incidence gain (bursts/100hb/mmHg)	-4.6 (-6.4– -3.8)	- 2.8 (-5.0 – -2.2)	0.03
Total muscle SNA gain (AU/beats/mmHg)	-28.1 (-40.5 – -18.1)	- 13.2 (-27.8 – -1.8)	0.05
Systolic BP-RRI gain (ms/mmHg)	12.3 (10.7-16.6)	9.4 (6.9-14.8)	0.03

Data presented as mean±SD or median (interquartile range). BP, blood pressure; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; SNA, sympathetic nerve activity.

Table 6.5. Baseline HR variability indices in healthy controls and patients with hypertension

	Healthy Controls	Hypertensive Patients	*P* value
RMSSD (ms)	40.6 (28.6-51.2)	30.0 (26.2 –38.2)	0.07
SDNN (ms)	53.7 (50.8-63.2)	42.2 (35.6-63.0)	0.04
pNN50 (%)	18.6 (6.4-34.5)	7.4 (1.6–18.4)	0.06
HF (ms^2)	602.2 (228.2-1058.1)	330.8 (130.1-612.7)	0.22
LF (ms^2)	944.4 (622.5-1398.1)	335.0 (230.1–1058.6)	0.009
TP (ms^2)	2704.0 (2183.4-3883.6)	1692.4 (1039.1-2743.9)	0.02
HF (n.u.)	43.2 (22.1-60.1)	45.2 (24.3-65.2)	0.41
LF (n.u.)	56.8 (39.9-77.9)	54.8 (34.8-74.7)	0.41
LF/HF	1.3 (0.7-4.0)	1.2 (0.5-3.2)	0.41

Data presented as median (interquartile range). RMSSD, square root of the mean of the sum of successive differences in R-R interval; SDNN, standard deviation of normal to normal R-R interval; pNN50%, proportion of successive R-R interval which vary by >50 ms; TP, total power (0.0-0.4 Hz); HF, high-frequency power (0.15-0.4 Hz); LF, low-frequency power (0.04-0.15 Hz).

6.5.1.2. Effect of acute device-guided slow deep breathing on cardiorespiratory parameters in healthy controls and patients with hypertension

The magnitude of the device-guided slow deep breathing evoked change in respiratory parameters was not significantly different between the two groups (Table 6.6). Acute device-guided slow deep breathing led to a significantly greater reduction in systolic, diastolic and mean BP in the hypertensive group (P=0.05 for systolic BP, P=0.02 for diastolic BP, and P=0.01 for mean BP). The change in muscle SNA and baroreflex sensitivity was not different between two groups. The HR variability responses to the acute slow deep breathing was more pronounced in the healthy group, in particularly there was a significant difference in change in TP of HR variability (2520 (723–3980) ms^2 in the healthy group vs. to 334 (-508–946) ms^2 in the hypertensive group (P=0.002) as well as LF power P=0.003, RMSSD, P=0.004, SDNN, P=0.006, in a favor to the healthy group (Table 6.7). HF power and LF/HF were unchanged

Table 6.6. Acute effects of device-guided slow deep breathing on cardiorespiratory parameters in healthy controls and patients with hypertension

	Healthy Controls	Hypertensive Patients	*P* value
Δ Respiratory frequency (b/min)	-5.4 (-7.5 – -2.6)	-6.2 (-8.2 – -3.3)	0.39
Δ Tidal volume (L)	0.4 (0.2 – 0.7)	0.4 (0.1 – 0.8)	0.87
Δ Minute ventilation (L/min)	-0.5 (-1.2 – 1.3)	0.3 (-1.6 – 1.9)	0.73
Δ $P_{ET}CO_2$ (mmHg)	-1.1 (-4.2 – 0.3)	0.1 (-3.9 – 2.1)	0.33
Δ Supine systolic BP (mmHg)	-1.0 (-4.3 – 2.0)	-4.5 (-7.8 – -2.3)	0.05
Δ Supine diastolic BP (mmHg)	-0.5 (-1.3 – 2.0)	-2.0 (-4.5 – -0.3)	0.02
Δ Supine mean BP (mmHg)	0.0 (-2.0 – 1.0)	-3.0 (-4.8 – -1.3)	0.01
Δ Supine PP (mmHg)	-0.5 (-3.5 – 1.0)	-2.0 (-7.0 – 0.0)	0.15
Δ Heart rate (beats/min)	1.1 (-0.5 – 3.4)	2.2 (0.6 – 3.9)	0.41
Δ Burst incidence (bursts/100hb^{-1})	-4.5 (-5.3 – -0.9)	-3.7 (-7.7 – 2.5)	0.67
Δ Burst frequency (bursts/min)	-0.6 (-3.3 – -0.2)	-1.9 (-3.8 – 0.8)	1.0
Δ Total activity (AU)	-43.7 (-135.2 – 41.7)	3.3 (-186.0 – 428.9)	0.36

Δ Burst incidence gain (bursts/100hb/mmHg)	0.04 (-1.1 – 2.0)	0.9 (-0.4 – 1.8)	0.45
Δ Total muscle SNA gain (AU/beats/mmHg)	2.3 (-6.1 – 11.2)	-1.7 (-4.3 – 7.1)	0.92
Δ Systolic BP-RRI gain (ms/mmHg)	-1.3 (-3.1 – 1.60	-1.5 (-3.0 – 0.4)	0.80

Data presented as median (interquartile range). BP, blood pressure; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; SNA, sympathetic nerve activity.

Table 6.7. Acute effects of device-guided slow deep breathing on indices of HR variability in healthy controls and patients with hypertension

	Healthy Controls	Hypertensive Patients	*P* value
Δ RMSSD (ms)	10.5 (- 3.0– 15.7)	- 4.8 (- 8.7– 0.5)	0.004
Δ SDNN (ms)	20.3 (3.9– 27.8)	1.5 (- 4.0– 9.1)	0.006
Δ pNN50 (%)	2.9 (- 6.3– 11.3)	- 2.3 (- 5.9– -0.03)	0.07
Δ HF (ms^2)	- 133.2 (- 558.0– 93.6)	- 146.9 (- 434.6– -54.2)	0.73
Δ LF (ms^2)	1914.8(1077.1–4060.4)	277.5 (57.1–1177.9)	0.003
Δ TP (ms^2)	2519.8 (723.4– 3979.7)	333.6 (- 508.0– 946.3)	0.002
Δ HF (n.u.)	- 26.3 (- 42.4– -6.1)	- 26.3 (- 49.1– -13.4)	0.78
Δ LF (n.u.)	26.3 (6.1– 42.4)	24.7 (13.4– 49.1)	0.80
Δ LF/HF	8.7 (3.7– 10.2)	5.4 (1.0– 24.1)	0.73

Data presented as median (interquartile range). RMSSD, square root of the mean of the sum of successive differences in R-R interval; SDNN, standard deviation of normal to normal R-R interval; pNN50%, proportion of successive R-R interval which vary by >50 ms; TP, total power (0.0-0.4 Hz); HF, high-frequency power (0.15-0.4 Hz); LF, low-frequency power (0.04-0.15 Hz)

6.5.2. Longitudinal study of patients with hypertension before (first visit) and after (follow up visit) home-based device-guided slow deep breathing

6.5.2.1. Compliance with the intervention

During the last 30 days of device use patients performed 22±6 successful sessions (interquartile range 20-26), spent 49±15 min per week (interquartile range 42-57) in 'therapeutic zone' (breathing rate reduced to ≤10 breath/min). Average synchronization of the breath was 78±18% (interquartile range 69-85%). There was inverse relationship between the change in office diastolic BP and number of successful device-guided sessions over the last 30 days of intervention (r=-0.54, *P*=0.02) (Figure 6.1).

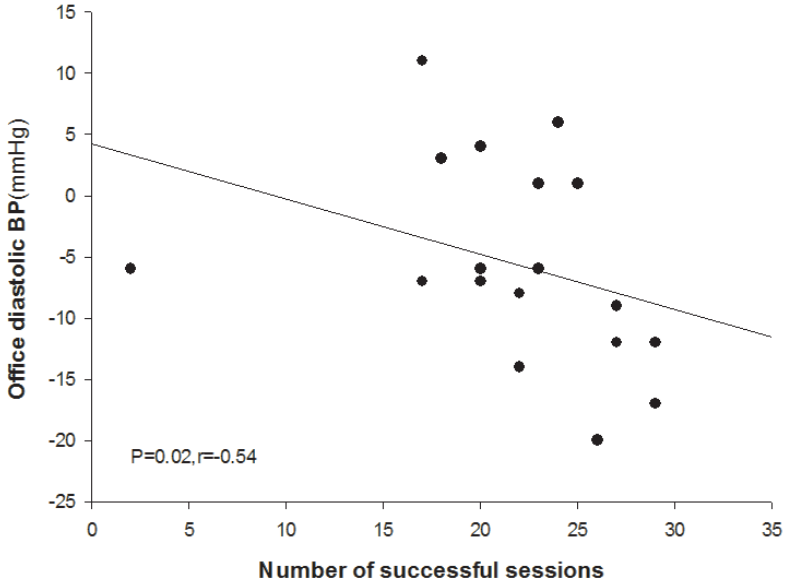

Figure 6.1. The association between adherence to the home-based device-guided slow deep breathing training and long-term changes in diastolic blood pressure.

BP, blood pressure

6.5.2.2. Effect of home-based device-guided slow deep breathing on steady state cardiorespiratory parameters at baseline

After 8 weeks of home-based slow deep breathing training, office systolic BP was significantly reduced from 147±18.1mmHg at the first visit to 133±17.0 mmHg at the follow up visit (*P*=0.001). Office diastolic BP was also significantly reduced over the same time period (84±9.5 vs. 79±12.3 mmHg, *P*=0.008). There was no change in weight (81±13.7 vs. 81±14.2 kg, *P*=0.52) or BMI (28±2.8 vs. 28±3.08 kg/ m^2, *P*=0.55) between the two visits. No significant changes were observed in either the clinical biochemistry or the echocardiography measurements (Tables 6.8 and 6.9). There was no change in respiratory parameters over the 8 weeks of training (Table 6.10). Muscle SNA burst frequency and burst incidence were significantly reduced at the follow up (33.8±8.2 to 28.3±7.1 bursts/min, *P*=0.03 and 59.0±19.5 to 48.6±14.4 bursts/100hb^{-1}, *P*=0.05, respectively; Figure 6.2). There were no changes in cardiac and sympathetic baroreflex control. RMSSD, SDNN, pNN50%, HF were modestly but significantly reduced following home-based slow deep breathing training, whereas TP, LF and LF/HF were unchanged (Table 6.11).

Table 6.8. Baseline blood biochemistry in patients with hypertension before (first visit) and after (follow up visit) home-based device-guided slow deep breathing

	First visit	Follow up visit	*P* value
Total cholesterol (mmol/l)	5.3±1.1	5.2±1.2	0.45
HDL (mmol/l)	1.3±0.45	1.4±0.50	0.19
Triglycerides (mmol/l)	1.5±0.9	1.3±0.6	0.33
Glucose (mmol/l)	4.9±0.4	4.7±0.9	0.65
Creatinine (μmol/l)	86.0±23.2	77.0±14.8	0.49
Estimated glomerular filtration rate (mL/min/1.73 m^2)	75.4±13.7	82.2±7.2	0.60
Lymphocytes ($x10^3$ per μl)	1.8±0.6	1.3±0.3	0.37
Monocytes ($x10^3$ per μl)	0.58 (0.38-0.64)	0.49 (0.37-0.57)	0.31

Data presented as mean±SD or median (interquartile range). HDL, high-density lipoprotein.

Table 6.9. Baseline echocardiography parameters and arterial stiffness in patients with hypertension before (first visit) and after (follow up visit) home-based device-guided slow deep breathing.

	First visit	Follow up visit	*P* value
Left ventricular mass index (g/m^2)	82.3±20.1	84.4±19.7	0.23
Left ventricular ejection fraction (%)	61.8±2.9	60.6±3.5	0.07
Systolic annular septal velocity (cm/s)	6.9±1.6	6.6±1.3	0.32
Systolic annular lateral velocity (cm/s)	7.4±2.4	7.3±2.3	0.97
Stroke volume (ml)	62.2±12.5	61.7±11.8	0.77
Cardiac output (L/min)	3.9±1.0	4.0±1.0	0.35
Mitral valve inflow (E/A) ratio	1.1±0.4	1.1±0.3	0.61
E/e' septal ratio	10.3±2.9	10.7±2.9	0.46
Left atrial volume index (ml/m^2)	16.8±3.5	18.2±3.4	0.06
Aortic Augmentation Index HR@75	23.7±11.5	24.0±8.5	0.9

Data presented as mean±SD. HR, heart rate.

Table 6.10. Baseline cardiorespiratory parameters in patients with hypertension before (first visit) and after (follow up visit) home-based device-guided slow deep breathing.

	First visit	Follow up visit	*P* value
Respiratory frequency (b/min)	12.2±3.6	11.3±3.4	0.22
Tidal volume (L)	0.47 (0.3-0.9)	0.56 (0.4-0.8)	0.74
Minute ventilation (L/min)	6.0±1.9	6.2±1.8	0.67
$P_{ET}CO_2$ (mmHg)	39.7±2.3	40.5±2.2	0.23
Heart rate (beats/min)	61±9.4	61±7.1	0.83
R-R interval (s)	1.01±0.15	1.00±0.12	0.84
Burst incidence (bursts/100hb^{-1})	59.0±19.5	48.6±14.4	0.05
Burst frequency (bursts/min)	33.8±8.2	28.3±7.1	0.03
Burst incidence gain (bursts/100hb/mmHg)	-2.9 (-5.1 – -1.3)	-2.4 (-7.0 – -1.1)	0.58
Total muscle SNA gain (AU/beats/mmHg)	-11.8 (-25.0 – -0.4)	-7.1 (-16.0– -1.7)	0.87
Systolic BP-RRI gain (ms/mmHg)	9.4 (6.9-14.8)	9.5 (5.8-14.2)	0.71

Data presented as mean±SD or median (interquartile range). BP, blood pressure; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; SNA, sympathetic nerve activity.

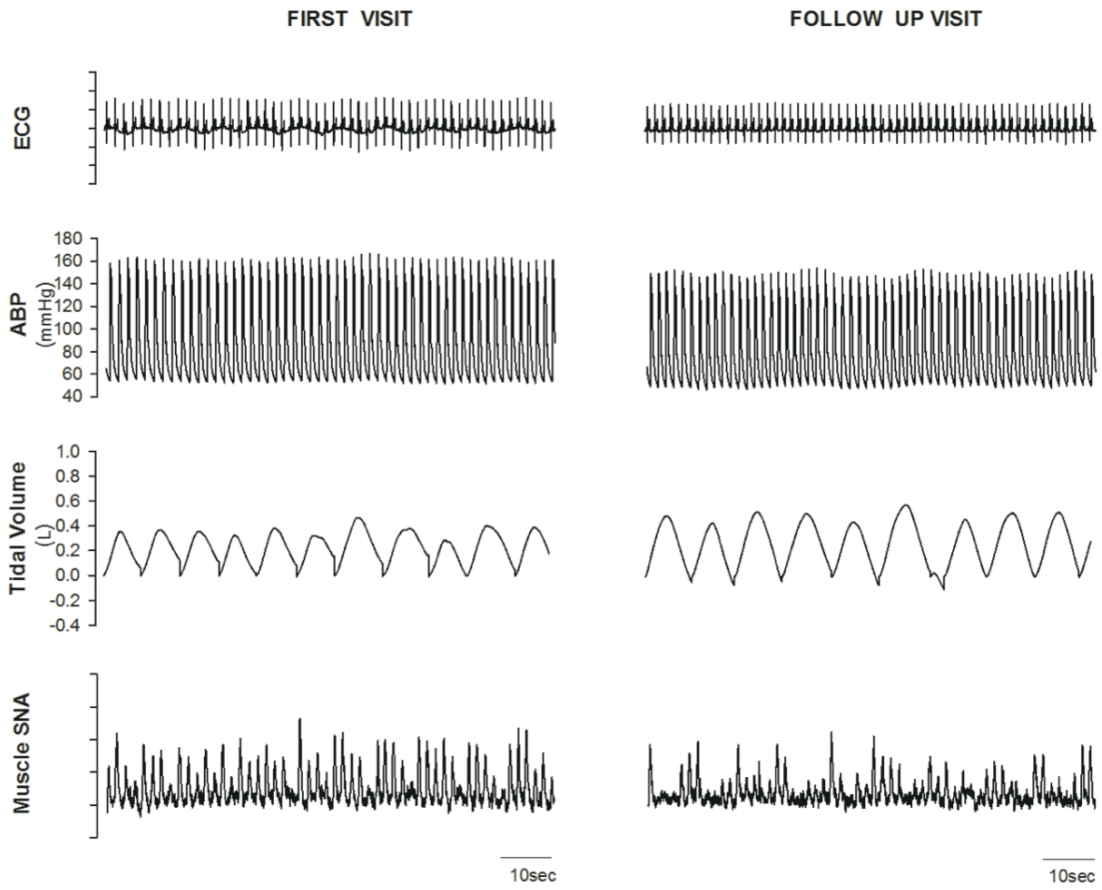

Figure 6.2. Original record showing cardiorespiratory responses to long-term slow deep breathing training (see more details in text).

ABP, arterial blood pressure; ECG, electrocardiogram; SNA, sympathetic nerve activity.

Table 6.11. Baseline HR variability indices in patients with hypertension before (first visit) and after (follow up visit) home-based device-guided slow deep breathing

	First visit	Follow up visit	*P* value
RMSSD (ms)	30.0 (26.2 –38.2)	26.1 (19.3 – 37.6)	0.04
SDNN (ms)	42.2(35.6-63.0)	42.1 (33.0-50.2)	0.02
pNN50 (%)	7.4(1.6–18.4)	5.8 (0.8-11.7)	0.01
HF (ms^2)	330.8 (130.1-612.7)	251.1 (128.5-430.4)	0.04
LF (ms^2)	335.0 (230.1–1058.6)	385.6 (200.9-1165.8)	0.91
TP (ms^2)	1692.4 (1039.1-2743.9)	1571.8 (787.9-2608.0)	0.31
HF (n.u.)	45.2 (24.3-65.2)	33.5 (20.8-58.1)	0.18
LF (n.u.)	54.8 (34.8-75.7)	66.5 (41.9-79.2)	0.18
LF/HF	1.2 (0.5-3.2)	2.0 (0.7-3.8)	0.85

Data presented as median (interquartile range). RMSSD, square root of the mean of the sum of successive differences in R-R interval; SDNN, standard deviation of normal to normal R-R interval; pNN50%, proportion of successive R-R interval which vary by >50 ms; TP, total power (0.0-0.4 Hz); HF, high-frequency power (0.15-0.4 Hz); LF, low-frequency power (0.04-0.15 Hz).

6.5.2.3. Effect of training on acute response to device-guided slow deep breathing

Respiratory frequency, minute ventilation and $P_{ET}CO_2$ responses to acute slow deep breathing were not significantly different between the visits. However, increases in tidal volume with acute slow deep breathing were slightly but significantly greater during the follow up visit, *P*=0.03 (Table 6.12). During the follow up visit, reductions in BP in response to the acute slow deep breathing test were significantly less pronounced compared to the first visit, particularly for diastolic BP, *P*=0.01. The magnitude of the device-guided slow deep breathing evoked change in muscle SNA and baroreflex control parameters was not significantly different between the two visits.

Increase in HR variability indices were more pronounced during the second visit, in particularly TP increased significantly more at the follow up visit (1003.0 (91.5–1861.9) vs. 333.6 (-508.0 –946.3), *P*=0.03) as well as LF power *P*=0.04, RMSSD, *P*=0.02, SDNN, *P*=0.002 and pNN50%, *P*=0.01 in a favor to the follow up visit (Table 6.13). However changes in the HF power and LF/HF were not different between the visits.

Table 6.12. Effect of training on acute response to device-guided slow deep breathing on cardiorespiratory parameters in patients with hypertension before (first visit) and after (follow up visit) home-based device-guided slow deep breathing

	First visit	Follow up visit	*P* value
Δ Respiratory frequency (b/min)	- 5.8 (- 8.2– -3.3)	- 4.9 (- 6.3– -4.2)	0.64
Δ Tidal volume (L)	0.4 (0.1– 0.8)	0.5 (0.4– 0.9)	0.03
Δ Minute ventilation (L/min)	0.3 (- 1.4–2.0)	- 0.1 (- 0.6–2.2)	0.42
Δ $P_{ET}CO_2$ (mmHg)	0.4 (- 3.8– 2.2)	- 0.6 (- 4.7– 1.2)	0.47
Δ Supine systolic BP (mmHg)	- 5.0 (- 8.0– -3.0)	- 1.0 (- 5.0– 2.0)	0.03
Δ Supine diastolic BP (mmHg)	-2.0 (-5.0 – -1.0)	0.0 (- 1.0– 1.0)	0.01
Δ Supine mean BP (mmHg)	-3.0 (-5.0 – -2.0)	- 1.0 (- 3.0– 2.0)	0.03
Δ Supine PP (mmHg)	-3.0 (-7.0 – 0.00)	- 1.0 (- 3.0– 3.0)	0.06
Δ Heart rate (beats/min)	2.0 (-0.7– 4.0)	1.1 (- 0.9– 4.4)	0.9
Δ Burst incidence (bursts/100hb^{-1})	- 1.8 (- 9.9– 6.3)	- 3.5 (- 6.2– 5.2)	0.67
Δ Burst frequency (bursts/min)	- 1.4 (- 3.6– 4.1)	- 1.9 (- 2.5– 2.7)	0.89
Δ Burst incidence gain (bursts/100hb/mmHg)	-1.3 (- 0.1– 2.0)	- 0.2 (- 1.0– 0.6)	0.21
Δ Total muscle SNA gain (AU/beats/mmHg)	1.0 (- 10.1– 9.9)	- 3.6 (- 10.5– 4.9)	0.87
Systolic BP-RRI gain (ms/mmHg)	- 1.5 (- 3.0– 0.4)	0.6 (- 2.9–3.4)	0.15

Data presented as median (interquartile range). BP, blood pressure; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; PP, pulse pressure; SNA, sympathetic nerve activity.

Table 6.13. Effect of training on acute response to device-guided slow deep breathing on HR variability indices in patients with hypertension before (first visit) and after (follow up visit) home-based device-guided slow deep breathing

	First visit	Follow up visit	*P* value
Δ RMSSD (ms)	-4.8 (-8.7 – 0.5)	0.9 (-5.6 – 6.7)	0.02
Δ SDNN (ms)	1.5 (-4.0 – 9.1)	9.6 (4.4 – 19.0)	0.002
Δ pNN50 (%)	-2.3 (-5.9 – -0.03)	-0.1 (-5.0 – 3.1)	0.01
Δ HF (ms^2)	-146.9 (-434.6 – -54.2)	-166.2 (-337.2 – -5.9)	0.17
Δ LF (ms^2)	277.5 (57.1–1177.9)	1009.6 (181.8-1992.0)	0.04
Δ TP (ms^2)	333.6 (-508.0 –946.3)	1003.0 (91.5– 1861.9)	0.03
Δ HF (n.u.)	-26.3 (-49.1 – -13.4)	-20.2 (-51.0 – -9.3)	0.40
Δ LF (n.u.)	24.8 (13.4 – 49.1)	20.2 (9.3 – 51.0)	0.50
Δ LF/HF	5.4 (1.0 – 24.1)	5.2 (3.9 – 20.3)	0.25

Data presented as median (interquartile range). RMSSD, square root of the mean of the sum of successive differences in R-R interval; SDNN, standard deviation of normal to normal R-R interval; pNN50%, proportion of successive R-R interval which vary by >50 ms; TP, total power (0.0-0.4 Hz); HF, high-frequency power (0.15-0.4 Hz); LF, low-frequency power (0.04-0.15 Hz).

There was a strong significant correlation between reduction in the sympathetic nerve burst frequency during the acute slow deep breathing at first visit and reduction in office diastolic BP after the home-based device-guided slow deep breathing training ($r=0.75$, $p=0.008$). Similar association was found between acute reduction in the nerve burst incidence at the first visit and decrease in office diastolic BP after the slow deep breathing training ($r=0.62$, $p=0.04$). However acute response to slow deep breathing was not predictive of changes in office systolic BP during the follow up (Table 6.14). Also there was no significant correlation between the acute response to slow deep breathing at first visit and changes in the nerve parameters after the home-based device-guided training (follow up visit) in hypertensive subjects (Table 6.15).

Table 6.14. Correlation between acute response to slow deep breathing (first visit) and changes in office BP after the home-based device-guided slow deep breathing in hypertensive patients

Parameters	Δ Office systolic BP (mmHg)		Δ Office diastolic BP (mmHg)	
	r	*P* value	r	*P* value
Δ Supine systolic BP (mmHg)	-0.59	0.81	0.01	0.95
Δ Supine diastolic BP (mmHg)	-0.04	0.86	-0.21	0.39
Δ Burst incidence (bursts/100hb^{-1})	0.01	0.97	0.62	0.04
Δ Burst frequency (bursts/min)	0.25	0.46	0.75	0.008

BP, blood pressure

Table 6.15. Correlation between acute response to slow deep breathing (first visit) and changes in nerve parameters after the home-based device-guided slow deep breathing in hypertensive patients

Parameters	Δ Steady burst incidence (bursts/100hb^{-1})		Δ Steady burst frequency (bursts/min)	
	r	*P* value	r	*P* value
Δ Supine systolic BP (mmHg)	-0.34	0.41	-0.20	0.64
Δ Supine diastolic BP (mmHg)	-0.16	0.71	-0.05	0.91
Δ Burst incidence (bursts/100hb^{-1})	-0.38	0.35	-0.48	0.23
Δ Burst frequency (bursts/min)	-0.45	0.26	-0.57	0.14

BP, blood pressure

6.6. Discussion

6.6.1. Cross-sectional assessment of acute device-guided slow deep breathing responses in healthy controls and patients with hypertension

Acute device-guided slow deep breathing leads to a significantly greater reduction in systolic, diastolic and mean BP in patients with hypertension compared to normotensive controls. This observation indicates that acute slow deep breathing may specifically target a pathogenic mechanism activated in hypertension. Given that the effects are observable within minutes the underlying mechanism must be capable of responding promptly to the changes in the triggering stimuli. The sympathetic and parasympathetic branches of the autonomic nervous system and peripheral smooth muscle tone are plausible candidates.

Baseline muscle SNA burst frequency was higher in the hypertensive group. These patients also had reduced baseline spontaneous cardiac baroreflex sensitivity and diminished arterial baroreflex control of muscle SNA. However, the BP responses to acute device-guided slow deep breathing in the hypertensive subjects occurred despite similar magnitude of responses in muscle SNA and baroreflex sensitivity in both groups. This suggests that the more prominent BP reduction in the hypertensive subjects was not due to inhibition of muscle SNA per se. In a previous studies, acute slow deep breathing reduced BP in hypertensive patients,[54, 56] increased cardiac baroreflex sensitivity,[54] and reduced muscle SNA.[33, 44] However, the present study highlights that it is important to interpret these changes in the context of responses seen in healthy

control participants to delineate existence of different BP lowering mechanisms in response to slow deep breathing.

Abnormal (reduced) HR variability was seen in hypertensive patients at rest and this group also showed diminished increase in parameters of HR variability in response to the acute slow deep breathing compared to the healthy group. Consequently changes in parasympathetic nervous system do not appear to be primarily responsible for the differences in BP response. In fact the observed changes in parasympathetic parameters in HR variability could represent normal response to BP reduction and diminished response seen in the hypertensive patients highlights dysfunction of this system in hypertension.

It is not thus entirely clear which exact mechanisms are behind the augmented acute respiration induced BP reduction in hypertensive patients.

6.6.2. Longitudinal study of patients with hypertension before (first visit) and after (follow up visit) home-based device-guided slow deep breathing

The device-guided slow deep breathing training significantly reduced both systolic and diastolic BP. The magnitude of the reduction in office BP observed in this study (14/5 mmHg for systolic/ diastolic BP) was comparable to the results of recent meta-analysis, which indicated a reduction in office BP by 13/7 mmHg and net beneficial effect of 4/3 mmHg (systolic/ diastolic BP) after adjustment for a placebo group.[48] Such reductions in office BP result in a clinically relevant reduction in incidence of cardiovascular

events (stroke, myocardial infarction, heart failure, sudden death, peripheral artery disease and end-stage renal disease).[60, 582-585] Previously, Viskoper et al found that BP reduction in hypertensive patients only occurred if mean BP was 98 mmHg or higher, (i.e., in those with likely higher peripheral resistance).[42] The present study was performed in similar patients.

This study shows for the first time that long-term device-guided slow deep breathing evokes sympathetic inhibition in middle age patients with essential hypertension. These changes were not associated with any significant modulation of arterial baroreflex control of muscle SNA. The sympathetic inhibition was accompanied by the long-term improvement in HR variability in response to the acute test. The strong correlation between acute reduction in the sympathetic nerve burst frequency and magnitude of the diastolic BP after the home-based device-guided slow deep breathing training indicates a possibility of utilization of the acute test for selection of patients who are more likely to respond to the treatment.

Device-guided slow deep breathing training has been suggested to play a role in modulation of autonomic cardiovascular control, likely affecting pulmonary stretch receptors, cardiorespiratory reflexes, arterial baroreflexes, chemoreflex and central respiratory motor output.[26-28, 31, 549, 568-572] Considering the absence of a long-term effect of slow deep breathing on parameters of cardiac baroreflex sensitivity, arterial baroreflex control of muscle SNA, and $P_{ET}CO_2$ the sympathoinhibition could be due to enhancement of the central inhibitory rhythms beyond baroreflex and chemoreflex mechanism.[586] Another possible mechanism is enhancement of the direct sympatho-

inhibitory effects of lung inflation reflex due to activation of slowly adapting pulmonary stretch receptors.[576, 587] Furthermore, slower spontaneous respiratory rate is closely associated with lower level of sympathetic activation. For instance, in study by Narkiewicz at al., respiratory rate was the only independent predictor of muscle SNA in young healthy men.[130] This study results are consistent with above findings as a long-term reduction in muscle SNA in this group of hypertensive was accompanied by numerically lower respiratory rate. Previously only one study evaluated the training effects of the device on muscle SNA and BP and it did not find any effect on either parameter.[44]

Intriguingly, during the follow up visit in the hypertensive group, there was a pronounced improvement in HR variability indices of parasympathetic activity during the acute slow deep breathing. This indicates that the long-term device-guided slow deep breathing training increased the capacity for acute improvement of HR variability, in a response to the acute slow deep breathing. This improvement in HR variability may be explained by an enhanced increase in tidal volume, but not respiratory frequency or minute ventilation. Such improvements in HR variability responsiveness were not paralleled by an increase in steady state HR variability at the follow up. In fact, contrary to the study hypotheses HR variability indices decreased significantly. This is difficult to explain and these observations may indicate involvement of factors responsive to respiration and not accountable for in this study.

Previously, the only study that assessed the long-term effect of the RESPeRATE device on HR variability, was performed in high risk hypertensive patients, with concomitant

diabetes and it showed reduction in BP and increase in LF power, with no effect on HF and total power by slow deep breathing but no direct measurements on sympathetic nervous system activity were collected.[59]

6.6.3. Target organ damage

Despite reductions in BP and muscle SNA no significant changes in cardiac structure and function, arterial stiffness or renal function were detected after the 8-week use of the device. Although previous data showed significant association between high sympathetic activity and development of LV hypertrophy in hypertension these changes in cardiac structure are reflective of chronic sympathetic hyperactivity.[14-16] They take years to occur and resolution of LV hypertrophy would require long-term sustained BP reduction. Despite significant decrease in BP seen during the 8-week training in this study with resulting BP reaching recommended target levels the duration of the intervention was probably not sufficient to observe detectable improvement in target organ damage. Of note, the patients demonstrated good adherence to the required number of sessions, per week and their synchronization was above the previously suggested threshold of 65% for achieving significant systolic BP reduction. [497]Although no significant improvement in the target organ damage was seen the changes were clearly in the direction towards such improvement as suggested by pathophysiological studies. Indeed, reduced HR variability was associated with LV hypertrophy in hypertensive patients[17] and high muscle SNA was associated with LV diastolic dysfunction and increased arterial stiffness independently of BP levels.[18, 19, 238, 580, 581]As thus longer duration of slow deep breathing training may be needed to elicit befits

towards the cardiac structure, but also improvement in arterial stiffness and renal function.

6.7. Conclusion

In conclusion, long-term device-guided slow deep breathing training leads to sympathetic inhibition in middle age patients with essential hypertension. The sympathetic inhibition was associated with longer-term improvement in office BP and responsiveness of HR variability. The slow deep breathing training may provide therapeutic benefits in essential hypertension via inhibition of excessive sympathetic outflow.

Contribution

For this experimental chapter, I contributed to the study design. I undertook identification of the study participants, their screening, recruitment, consent, patient follow-up, examination, data acquisition (except for the microneurography, where positioning of the electrodes into the peroneal nerve was performed by Dr J. Fisher). I performed data analysis, data interpretation and wrote the thesis chapter.

CHAPTER VII. SUMMARY AND OVERALL CONCLUSIONS

7.1. Thesis summary

In **Chapter 1.** (Introduction) a broad introduction to the field with which this thesis is concerned was provided along with a rationale for subsequent experimental chapters. An overview of our present understanding of autonomic nervous system neuro-anatomy and regulation was provided (**Chapter 2.** Literature review). Following this the literature review provides a discussion of the pathways linking dysfunction of sympathetic nervous system and pathogenesis of hypertension, along with an overview of therapeutic approaches targeting the sympathetic nervous system in order to improve BP control. The literature review highlighted the gaps in our present knowledge that this thesis sought to address.

Chapter 3 provides a description of the study methods. With the participants in supine position, standard microneurography techniques were used to record efferent postganglionic multiunit muscle SNA. HR was measured using ECG and beat-to-beat arterial BP non-invasively measured from the middle finger using photo-plethysmography. Respiration related changes in thoracic circumference were measured using strain gauge pneumobelt. Participants were fitted with an oro-nasal mask and two-way valve connected to a heated pneumotachometer to record respiratory parameters. Transthoracic echocardiography was used to assess cardiac structure and function to establish correspondence to inclusion criteria and effects of the slow deep breathing intervention. Arterial stiffness was assessed based on aortic augmentation index.

The raw muscle SNA, ECG, BP and respiratory signals underwent analogue-to-digital conversion at 10 kHz and were stored for offline analysis. Tidal volume and minute ventilation were calculated using the Spirometry module from Labchart (ADInstruments, Dunedin, New Zealand). $P_{ET}CO_2$ level was derived from the relative percentage of expired CO_2 waveform as the maximum value. Analyses of steady-state muscle SNA and its interaction with respiration were conducted using a custom written interactive scoring program (Spike 2, Cambridge Electronic Design, Cambridge, UK). The sequence technique was used to determine spontaneous cardiac baroreflex sensitivity. Calculations of arterial baroreflex control of muscle SNA were obtained from the relationships between diastolic BP vs. burst incidence and total muscle SNA by weighted linear regression analysis.

The aim of the first experimental chapter **(Chapter 4)** was to determine the influence of age on respiratory related bursting of muscle SNA and on the association between the rhythmic fluctuations in muscle SNA and THW that occur with respiration in humans. In this part of the study 10 young and 10 older healthy males participated in the study and were investigated during 10 min of uncontrolled spontaneous breathing at a normal resting rate and depth.

In contrast to the initial hypothesis, it was observed that the strength of the respiratory modulation of muscle SNA parameters (e.g., burst incidence, frequency, amplitude and total activity) were preserved in healthy older individuals. A significant association between the rMSNA and THW amplitude was identified and this was similar in healthy

young and older groups. Collectively, these findings suggest that a potential attenuation of inspiratory-linked inhibition of muscle SNA does not appear to explain the elevated resting muscle SNA in older individuals, and that central respiratory-sympathetic coupling is a component of the THW in both young and older humans

The aim of the second experimental chapter **(Chapter 5)** was to investigate whether slow deep breathing reduces BP and muscle SNA in young healthy individuals, and to investigate the underlying autonomic neural control mechanisms. To achieve this, microneurographic recordings of muscle SNA were obtained in young healthy men during spontaneous breathing and acute device-guided slow deep breathing. Ten young healthy, non-smoking males underwent the experimental protocol, which consisted of 10 min of uncontrolled spontaneous breathing followed by 10 min of slow deep breathing. The latter was guided by the RESPeRATE device (InterCure [UK] Limited, London, UK), which generates melodic tones to assist the individual in slowing their respiratory rate below 10 breaths per minute.

The acute device-guided slow deep breathing led to a reduction of muscle SNA with no significant change in BP, arterial baroreflex control of muscle SNA or cardiovagal baroreflex regulation. These observations indicate that device-guided reduction in central sympathetic outflow are not accompanied by an increase in baroreflex sensitivity (cardiac or muscle SNA), but may be attributable to enhanced sympatho-inhibitory effects of lung inflation reflex and/or changes in central respiratory-sympathetic coupling.

The aim of the last experimental chapter **(Chapter 6)** was to comprehensively evaluate the acute and long-term training effects of device-guided slow deep breathing on autonomic regulation in hypertension reflected by muscle SNA, arterial baroreflex control of muscle SNA and parameters of HR variability effect on hypertension target organ damage (e.g. heart, vessels, kidney). To achieve this cardiorespiratory parameters, muscle SNA, HR variability and arterial baroreflex function were measured at rest and in response to acute slow deep breathing (10 min), both before and after home-based long-term device-guided slow deep breathing training (8 weeks). The influence of this training regime on cardiac (echocardiography), vascular (arterial stiffness) and kidney functions was also determined.

In the cross-sectional study twenty patients with essential hypertension were compared with 19 age, sex and BMI-matched normotensive healthy controls. All hypertensive patients were clinically stable and treated. In the longitudinal study, 19 of 20 patients completed the follow up and were included in the analysis.

In the cross-sectional part of the analysis, acute device-guided slow deep breathing led to a significantly greater reduction in systolic, diastolic and mean BP in the hypertensive group. The magnitude of the change in muscle SNA and baroreflex sensitivity were not different between two groups. Increases in HR variability in response to the acute slow deep breathing were more pronounced in the healthy group.

In the longitudinal part of the analysis, after 8 weeks of home-based slow deep breathing training, office systolic BP was significantly reduced from 147±18.1mmHg at

the first visit to 133±17.0 mmHg at the follow up visit (*P*=0.001). Office diastolic BP was also significantly reduced over the same time period (84±9.5 vs. 79±12.3 mmHg, *P*=0.008). Muscle SNA burst frequency and burst incidence were significantly reduced at the follow up. No significant changes were found the clinical biochemistry, echocardiography measurements, respiratory parameters and in cardiac and sympathetic baroreflex control. RMSSD, SDNN, pNN50%, HF were modestly but significantly reduced following home-based slow deep breathing training, whereas TP, LF and LF/HF were unchanged.

During the follow up visit, the changes in tidal volume with acute slow deep breathing were slightly but significantly greater, whereas reductions in BP reduction were less pronounced. No differences in the responses of muscle SNA, baroreflex sensitivity, minute ventilation and $P_{ET}CO_2$ were observed with slow deep breathing. However, increases in indices of HR variability were more at the follow up visit.

The study provides for the first time evidence of sympathetic inhibition by the long-term device-guided slow deep breathing in middle-aged patients with essential hypertension. These changes were not associated with any significant modulation of arterial baroreflex control of muscle SNA. The sympathetic inhibition was accompanied by the long-term reduction in office BP and an improvement in HR variability in response to the acute test.

7.2. Study limitations

Studies of respiratory modulation of SNA commonly assess respiration using a strain-gauge pneumobelt and this practice was followed in the present work. The choice was made to avoid participants having to breathe through a mouthpiece and thus minimise alteration of breathing pattern and tension of the patient. However, the approach is limited by the fact that the time-delay between the occurrence of respiratory related events within the central nervous system and changes in thoracic circumference is not accounted for. I have assumed that the time-delay is the same in the different study groups. It has been previously shown that that there was not significant difference in muscle SNA during uncontrolled spontaneous breathing and controlled breathing at 12 breaths per minute.

As discussed earlier the processes implicated in regulation of the sympathetic system and BP are extremely complex and they interact with virtually all other systems of the body including very dynamic ones, such endocrine and immune systems. It is thus almost impossible to account for the multitude of factors modulating activity of autonomic system.

The study does not provide insight into details of molecular mechanisms linking breathing modulation and pathways of sympathetic activation, in peripheral nerves but also in the brain, kidneys and blood vessels as well as mechanisms tested. Conduction of such studies in this human study was beyond the aims and available budget and the presented results should ideally be complimented by further mechanistic investigations.

These experiments need to be carefully designed to overcome limitations of available technologies. Some experiments, particularly those focusing on brain processes, may be unethical to be performed in humans but might be conducted on non-human models. Advances in non-invasive imaging technologies need to be fully utilized.

The study measured muscle SNA directed to one region, namely the skeletal muscle vasculature. Although this is a standard approach in the field, having data from different peripheral nerves/target organs would be desirable. Also giving limitations of any used methods of assessment of autonomic nerve function or indirect nature of the measurements (e.g., based on HR variability) it would be ideal to use several different approaches to quantify each tested process to minimise risk of bias.

Although the study populations is of typical size for this type of study a larger study cohort would permit greater confidence in study results and conclusions.

7.3. Overall conclusion

The study confirms links between activity of the sympathetic nervous system and BP and provides further insights into the contribution of ageing and respiration into the complex interplay between these systems. It shows that despite the presence of age-related elevation in muscle SNA the strength of the respiratory modulation of muscle SNA is similar in young and older adults. This indicates that aging-related increase in muscle SNA has no association with a diminished respiratory-sympathetic coupling.

Although slow deep breathing does not affect BP or arterial baroreflex control of muscle SNA in young healthy men it does reduce muscle SNA burst incidence, frequency and total activity. These effects do not appear to be dependent on changes in baroreflex sensitivity, but may reflect an increase in lung inflation afferent input and/or a reduction in central respiratory-sympathetic coupling.

Long-term device-guided slow deep breathing training in middle age patients with essential hypertension leads to significant sympathetic inhibition that was paralleled a significant reduction in both systolic and diastolic BP and improvement in responsiveness of HR variability. These findings indicate the slow deep breathing training may provide therapeutic benefits in essential hypertension via inhibition of excessive sympathetic outflow.

Undoubtedly, complexity of regulatory mechanisms implicated in the processes studies leaves a possibility for alternative contributing factors, and certainly further research is essential to understand intimate details of the phenomenon tested in my study.

7.4. Future research and implication for practice

Given the multitude of interactions between the sympathetic nervous system, hypertension, respiration and aging a more detailed analysis molecular and cellular changes in each of the comportments of the sympathetic system is essential. This information would allow more holistic understanding of the processes involved and help finding ways to manipulate them in the desired direction.

Neuroimaging is a rapidly developing field. Whilst brain and spine magnetic resonance imaging and computer tomography have become standard clinical tools opportunities of functional brain imaging, although limited at present, could allow some insight into the interaction of the SNA and brain function. For example, Fatouleh at al have recently demonstrated linkage between peripheral sympathetic nerve burst size and central activation before and after 6 month treatment with continuous positive airway pressure in patients with obstructive sleep apnoea.[588] However the currently available methods have limited capacity in assessment of brain processes related to SNA at molecular level (e.g., tracing neuromediator activity in specific parts of the brain). This may become possible in the future. For example, development of magnetic resonance tracking agents tagged with monoclonal antibodies can provide insight in processes mediated by proteins and containing them cells/organs. The role of non-invasive imaging in assessment of central and peripheral nerve activity (i.e., to trace changes in interactions of molecules and cells involved in activity of the sympathetic system) function is limited at present this may become an option in future.

The present study assessed 8-week effects of slow deep breathing and both statistical power and the study duration were designed to establish physiological impact and significance of the tested processes in accordance with the study hypotheses. However the study was not designed to determine effects of the findings on long-term clinical outcomes. Appropriately designed randomized trials are needed to determine clinical benefits of this treatment strategy in terms of quality of life, target organ damage (e.g., LV hypertrophy, renal function) and outcomes (e.g., risk of stroke or death). Although

essential hypertension itself is a primary focus of such interventions the approach could also be tested in other disorders where hypertension poses significant risk (e.g., ischemic heart disease and heart failure). In order to determine contribution changes in the sympathetic system to these outcomes it is essential to include minimally obtrusive markers of sympathetic activity as part of the future trials (e.g., plasma catecholamine levels). Identification of specific molecular targets able to modulate the SNA in desirable direction would help to develop new antihypertensive medicines.

This work suggests that testing of acute response of the muscle SNA to slow deep breathing can predict the magnitude of reduction of diastolic BP after 8-week slow deep breathing training. In order to optimize utilization of the technique further research into the predictors of responsiveness to the intervention would be desirable. This will help identify patients who are more likely to gain most benefits from it. Testing the predictive value of acute muscle SNA response for establishment of long term effects of slow deep breathing intervention would be an option, but it can also be complemented by other methods of quantification of acute response of the sympathetic system (e.g., measurement of catecholamine levels) and functional neuroimaging. Additionally it would be reasonable to compare the effects of different timings of the intervention (e.g., 10 min vs. 20 min. vs. 30 min) aiming to both optimise the therapeutic effects and minimise inconvenience to the patients.

A number of pharmacological agents are routinely used in management of hypertension; some are known to interact with the sympathetic system. It would be important to know the effects of these agents on respiratory sympathetic coupling with and without

application of slow deep breathing techniques. Together all this information would facilitate better management of hypertension in the future.

As respiratory disorders are common and they often involve some disturbances of the immune system it would be of interested to test changes in SNA and their interaction with respiration in patients with hypertension and concomitant lung problems, such as asthma and chronic obstructive pulmonary disease.

APPENDICES

Appendix 1. List of the study publications

Manuscript:

Shantsila A, McIntyre DB, Lip GY, Fadel PJ, Paton JF, Pickering AE, Fisher JP. Influence of age on respiratory modulation of muscle sympathetic nerve activity, blood pressure and baroreflex function in humans. Exp Physiol. 2015 Sept; 100: 1039-51.

Abstracts presentation:

Shantsila A, Adlan AM, Lip GYH, Pickering AE, Paton JFR, Fisher JP Does home-based, slow deep breathing training reduce central sympathetic outflow and enhance baroreflex sensivitiy in primary hypertension? *BCS Conference 2015, Manchester, June 8-10.* The top scoring clinical abstract award in the Stable IHD/Prevention/Hypertension/Lipids category

Shantsila A, Adlan AM, Lip GYH, Pickering AE, Paton JFR, Fisher JP. Effect of device-guided slow deep breathing on central sympathetic outflow and arterial baroreflex sensitivity in young healthy individuals. *Experimental Biology 2014*, *San Diego, April 26-30.*

Shantsila A, Adlan AM, Lip GYH, Pickering AE, Paton JFR, Fisher JP. Device-guided slow deep breathing in essential hypertension: is cardiac or sympathetic baroreflex sensitivity altered? *Experimental Biology 2014*, *San Diego, April 26-30.*

Shantsila A, McIntyre DB, Lip GYH, Paton JFR, Fadel PJ, Pickering A E, Fisher JP. 'Influence of age on respiratory modulation of muscle sympathetic nerve activity and blood pressure in humans' *Experimental Biology 2013, Boston, April 20-24*:

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