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Hanna Mussalo

Cardiovascular Autonomic Regulation in Patients with Sustained Hypertension of Different Etiology and Severity

Doctoral dissertation

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ABSTRACT

The control of cardiovascular system is the result of complex interactions between reflex mechanisms and humoral factors. A large amount of both experimental and clinical data about blood pressure regulation have been collected, but the current knowledge of the cardiovascular autonomic nervous and humoral control mechanisms in hypertensive patients representing different severity and different etiology of the disease is still limited.

The majority of the hypertensive patients have essential hypertension, and only a minority of the patients have potentially curable forms of hypertension, such as renovascular hypertension. In the early phases of essential hypertension particularly the sympathetic nervous system seems to have an important role. In renovascular hypertension it is difficult to define exactly if changes in cardiovascular autonomic regulation are primarily due to factors that are behind the hypertensive condition or only the consequence of blood pressure elevation. When hypertension becomes established, blood pressure regulation changes in any case. In the long-term regulation of the blood pressure, the autonomic nervous system is though to be less important, and that other humoral mechanisms, such as, vasoactive peptides might have more influence. Cardiovascular autonomic nervous function tests and measurements of vasoactive peptides may give us interesting information of blood pressure regulation in established hypertension. In addition, to treat hypertensive patients better, greater basic knowledge based on limited and well-defined hypertensive groups are needed.

The basic hypothesis of this study was to evaluate whether patients with different etiology and severity of hypertension also have differences in cardiovascular and humoral control mechanisms. To evaluate this issue 30 patients with mild essential hypertension (MEHT), 40 patients with severe essential hypertension (SEHT) and 14 patients with renovascular hypertension (RVHT) were studied, and each group were compared with age and sex-matched control subjects and with each other. Heart rate variability (HRV), short-term blood pressure variability (BPV) and baroreflex sensitivity (BRS) and atrial (NT-proANP) and brain-type natriuretic peptide (BNP) were measured. The principal findings were: HRV was reduced in patients with SEHT but not in patients with MEHT compared to healthy control subjects. RVHT was characterised by reduced short-term BPV. Despite similar blood pressure level, patients with SEHT showed increased short-term BPV. In the MEHT group the results were somewhat inconsistent. BRS was impaired in patients with RVHT and SEHT but not in patients with MEHT. Plasma concentrations of NT-proANP and BNP were increased in RVHT but not in SEHT, despite similar blood pressure levels.

The results show that both the etiology and the severity of hypertension influence cardiovascular regulation. In conclusion, this study suggests that different control mechanisms are operating in clinically distinctly different hypertension groups.

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To Akseli, Venla and Pekko

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Kuopio, August 2003

Hanna Mussalo

ABBREVIATIONS

ANP	Atrial natriuretic peptide
BNP	Brain natriuretic peptide
BPV	Blood pressure variability
BRS	Baroreflex sensitivity
ECG	Eletrocardiography
E/A-ratio	Ratio between peak velocity in early diastole (E) and late diastole (A) of mitral inflow in Doppler echocardiography
HF	High frequency (0.15 - 0.4 Hz)
HRV	Heart rate variability
LF	Low frequency (0.07 - 0.15 Hz)
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
LVMi	Left ventricular mass index (g/m^2)
MEHT	Mild essential hypertension
MSNA	Muscle sympathetic nerve activity
NT-proANP	N-terminal fragment of proANP
RMSSD	The square root of the mean sum of the squares of differences between adjacent RR intervals
RR-interval	Time between two successive R-waves in ECG
RVHT	Renovascular hypertension
SDNN	Standard deviation of RR intervals over the selected time interval
SEHT	Severe essential hypertension

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, which will be referred in the text as Studies I - IV.

I Mussalo H, Vanninen E, Ikäheimo R, Laitinen T, Laakso M, Länsimies E, Hartikainen J. Heart rate variability and its determinants in patients with severe or mild essential hypertension. Clinical Physiology 2001;5:594-604.

II Mussalo H, Vanninen E, Ikäheimo R, Laitinen T, Hartikainen J. Short-term blood pressure variability in renovascular hypertension and in severe and mild essential hypertension. Clinical Science 2003. DOI 10.1042/CS20020268. In Press.

III Mussalo H, Vanninen E, Ikäheimo R, Laitinen T, Laakso M, Länsimies E, Hartikainen J. Baroreflex sensitivity in essential and secondary hypertension. Clinical Autonomic Research 2002;12:465-471.

IV Mussalo H, Vanninen E, Ikäheimo R, Hartikainen J. NT-proANP and BNP in renovascular and in severe and mild essential hypertension. Kidney&Blood Pressure Research 2003;26:34-41.

CONTENTS

1 INTRODUCTION

The management of hypertension constitutes one of the largest health care expenses and it has also an enormous economical impact on individuals. The reason for identifying and treating hypertension is to reduce the risk of cardiovascular diseases and associated morbidity and mortality (Burt et al. 1995). It is well known that the treatment of hypertension is still inadequate, although awareness, detection and treatment of hypertension has improved during the recent decades (Burt et al. 1995).

A great majority of hypertensive patients have essential hypertension, i.e. hypertension of unknown cause. It has been estimated that less than 5 % of the patients have secondary hypertension and about 1 % of all hypertensive patients have renovascular hypertension (Derkx and Schalekamp 1994). The prevalence of renovascular hypertension increases when selected patient groups are considered and among patients with malignant hypertension it can be as high as 30-40 % (Semple and Dominiczak 1994).

Essential hypertension can be described as a complex syndrome which is accompanied by multiple underlying pathophysiological abnormalities (Figure 1, Kaplan 1998 (pp. 41-101), Carretero and Oparil 2000). In peripheral arteries smooth muscle hypertrophy develops in response to permanently elevated blood pressure and in response to neurogenic and hormonal factors. As result, arterial intima media becomes thicker. Smaller lumen to wall ratio and reduction in vascular compliance together with endothelial dysfunction are mechanisms responsible for the increase in vascular resistance (Rosei et al. 1995, Franke and Tegeler 1997).

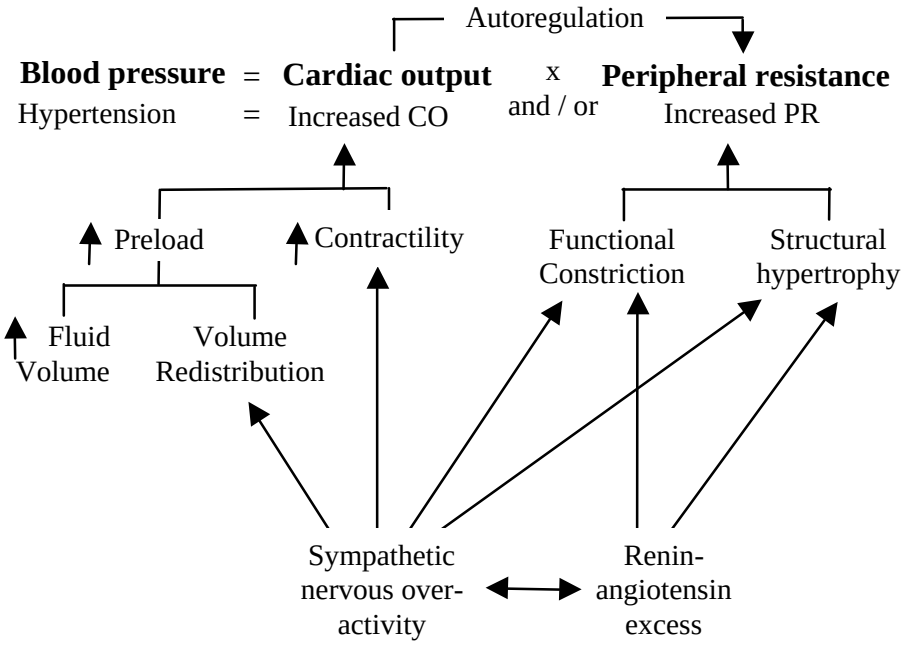


Figure 1. Hemodynamic changes in essential hypertension (modified from Kaplan 1998)

In renovascular hypertension the primary cause of hypertension is better known, renal artery stenosis and increased renin secretion (Figure 2, Kaplan 1998 (pp. 301-323)). Later on, plasma renin concentration falls towards normal levels and in prolonged phases also renovascular hypertension becomes a multifactorial condition (Kaplan 1998 (pp. 301-323)).

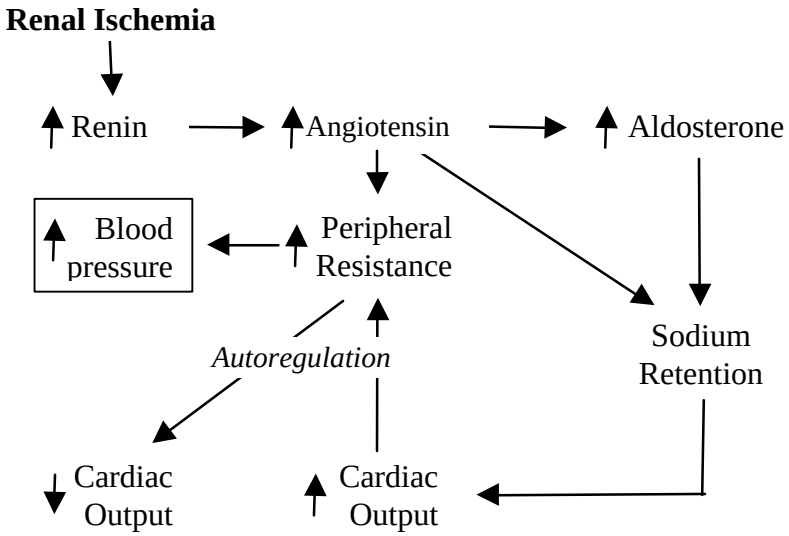


Figure 2. Hemodynamic changes in renovascular hypertension (modified from Kaplan 1998)

Cardiovascular autonomic nervous control and neurohumoral factors are known to play

important roles in blood pressure regulation. There is strong evidence that the progression of hypertension is associated with excessive sympathetic activation, which leads to structural changes in the most critical organs of blood pressure regulation (Julius 1996). Renal function and elevated concentrations of vasoactive hormones are also important in blood pressure regulation (Kaplan 1998).

Untreated essential hypertension is known to be associated with decreased heart rate variability (HRV) (Mancia et al. 1983, Guzzetti et al. 1988, Liao et al. 1996, Aono et al. 1996), increased blood pressure variability (BPV) (Siché et al. 1995), decreased baroreflex sensitivity (BRS) (Gribbin et al. 1971, Takeshita et al. 1975) and increased levels of plasma natriuretic peptides (Sugawara et al. 1985, Saganella et al. 1986, MacDonald et al. 1986). In renovascular hypertension BRS is also known to be impaired (Mancia et al. 1982, Gao et al. 2002) and natriuretic peptide levels to be increased (Larochelle et al. 1987, Schreij et al. 1996).

However, our knowledge of these factors in hypertensive patients with different severity and etiology of medically treated hypertension is limited. Autonomic nervous system has interactions with other mechanisms, that also regulate blood pressure. Natriuretic peptides are part of an important neurohumoral control system, the significance of which in established hypertension is not well understood (fluid balance Bucley et al. 1994, genetics Nakayama et al. 2000, Kato et al. 2000). The present series of studies were designed to investigate cardiovascular autonomic nervous function and neurohumoral control mechanisms in patients with long-lasting hypertension of different etiology and severity.

2 REVIEW OF LITERATURE

2.1 Hypertension

2.1.1 Pathophysiology of essential hypertension

There are a number of pathophysiological changes that are known to be involved in the development of hypertension and it is evident that these factors interact with each other (Figure 1 and 2). The principal aim is to keep the vascular control system in balance.

Generally, blood pressure can be described in simple terms as a product of cardiac output and peripheral resistance. The main factors that influence cardiac output are increased preload and contractility of the heart. Functional and structural changes of the vessels are responsible for increased peripheral resistance. These physical forces seem simple, but their origin is in fact complex, and there is a risk to oversimplify the blood pressure regulation system.

Increasing fluid volume (= preload), as well as, increasing contractility of the heart increases cardiac output which can elevate blood pressure. In chronic hypertension the typical hemodynamic finding is an elevated peripheral resistance with normal cardiac output (Julius 1991). Small resistance arterioles are of special interest because most of the hemodynamic abnormalities occur there, and structural changes in resistance arterioles are responsible for the increased peripheral resistance in hypertension. Peripheral resistance is usually determinated by measuring blood pressure and cardiac output. In hypertension vessel compliance is decreased. Only 20 % is due to structural changes of the aorta and large arteries, and in middle size arteries compliance may increase. The greatest loss of compliance occurs in the resistance vessels. Pulse pressure acts as a stimulus for alteration of vascular changes (Jokiniitty et al. 2001, O'Rourke et al. 2002). Probably there is a link between compliance and humoral factors.

In addition, other factors, such as the autonomic nervous system (discussed later), high salt intake (Intersalt study 1988), insulin resistance and obesity (Landsberg et al. 1986, Leiter et al. 1999), the renin-angiotensin system (Page et al.1975, Laragh 1991a and 1991b, Katz et al. 1992), stress (Herd et al. 1969, Leary et al. 2002), endothelial dysfunction (Cleland et al. 2000, Schiffrin et al. 2000), the central nervous system (Smith et al. 2002) and genetic factors (Lifton et al. 2001, Timberlake et al. 2001), may have an important role in established hypertension. These factors may also interact with each other and many are additive.

2.1.2 Diagnosis of essential hypertension

Blood pressure shows great variability in healthy subjects. Blood pressure changes in different part of vessels according to cardiac cycle and it also changes over the 24-hour period. Blood pressure is the pressure of the blood in a large artery, most often brachial artery, because at this point also indirect blood pressure measurements are usually performed. Blood pressure declines progressively until it reaches capillaries with a very low pressure (13 mmHg).

Hypertension is defined as systolic blood pressure of 140 mmHg or greater, diastolic blood pressure of 90 mmHg or greater or the use of antihypertensive medication. The positive relationship between high systolic and diastolic blood pressure and cardiovascular risk has long been recognised (Janeway 1904), and it is strong, independent, predictive, continuous and consistent in patients with or without cardiovascular disease (Stamler 1991, Falck et al. 1995).

Detection and confirmation of hypertension begins with proper blood pressure measurements. Blood pressure should be measured in a standardized way using an equipment that fulfills certification criteria (O´Brien et al. 1990, Beever et al. 2001, O´Brien et al. 2001a and 2001b). Measurement of blood pressure outside the clinician's office provides valuable information for the evaluation of hypertensive patients (Appel et al. 1993, Asmar et al. 2000, Mancia et al. 2001, O´Brien et al. 2001a and 2001b). In addition, a variety of commercial monitors for ambulatory blood pressure recording are available (O'Brien et al. 1990, White et al. 1993). In ambulatory blood pressure measurements the normal values (135/85 mmHg) are lower than office blood pressure values (Appel et al. 1993). Ambulatory blood pressure correlates more closely with target organ damage, particulary left ventricular hypetrophy, than office blood pressure (Parati et al.1987, Appel et al. 1993, Palatini et al. 1999, Parati et al. 2000).

Evaluation of hypertensive patients (medical history, physical examination, laboratory tests and other diagnostic tests) has following objectives: to identify the cause of hypertension, assess the presence or absence of target organ damage and cardiovascular disease and identify cardiovascular risk factors. To find an identifiable cause of hypertension additional diagnostic tests are used. These are particularly important in patients whose age, history, physical examination, severity of hypertension or laboratory test findings suggest such a cause. It is also important in patients whose blood pressure responds poorly to medical therapy and when blood pressure in previously well-controlled hypertension begins to increase, when the onset

of hypertension is sudden or when hypertension is severe.

2.1.3 Patophysiology of renovascular hypertension

In contrast to essential hypertension, renovascular hypertension is a disease with well known mechanisms (Martinez-Maldonado 1991, Semple and Dominiczak 1994). Renovascular hypertension can be defined as hypertension which is cured or improved by correction of renal artery stenosis. Renal artery stenosis is most often due to atherosclerosis (60 - 70 %) and the rest is mainly fibromuscular dysplasia (20 - 30 %), especially in younger female patients (Pickering 1989, Semple and Dominiczak 1994, Weaver et al. 1996). The etiology of fibromuscular dysplasia remains a mystery. It may be low-grade arteritis that may affect all vascular layers and other vessels (carotid and cerebral arteries) also, not only the renal artery. However, renal artery stenosis is not always accompanied by hypertension. About 10 % of patients with renovascular hypertension have other reasons such as vasculitis, trauma or tumor that compress renal artery. Renovascular hypertension is relatively uncommon, but it is the most frequent curable type of hypertension. The prevalence of renovascular hypertension increases in selected groups of hypertensive patients.

2.1.4 Diagnosis of renovascular hypertension

The most important clinical clues suggesting renovascular hypertension are: onset of hypertension before the age of 30, recent onset of significant hypertension after the age of 55, abdominal bruit, sudden worsening of previously controlled hypertension, recurrent pulmonary oedema, renal impairment of unknown cause, elevation of serum creatinine by ACE inhibitors or angiotensin II receptor blockers, known asymmetry of renal size or coexisting diffuse atherosclerotic vascular disease (Mann and Pickering 1992).

In patients with suggested indications of renovascular disease, the captopril-enhanced radionuclide renal scan (Fommei et al. 1987, Johansson et al. 2000, Fine et al. 2001), duplex Doppler flow studies (Johansson et al. 2000, de Haan et al. 2002), renin measurements of the renal veins (Sellars et al. 1985, Roubidoux et al. 1991, Semple and Dominiczak 1994) and magnetic resonance angiography and magnetic resonance flow quantification in renal arteries (de Haan et al. 2003) has been used as screening tests (Qanadli et al. 2001). Spiral computed tomography is also used, but it requires intravenous contrast (Farrés et al. 1996, Kaatee et al. 1997, Gayard et al. 2000). Definitive diagnosis of renovascular hypertension requires renal

intra-arterial digital subtraction angiography, a gold standard for the diagnosis of renal artery stenosis (Vidt 1997).

There are three possibilities for the therapy of RVHT: medical, surgical revascularization or percutaneus transluminal renal angioplasty, possibly associated with renal artery stenting (Baumgartner et al. 2000, Cherr et al. 2002). There are no large-scale controlled studies of the relative value of these methods. Medical therapy is often effective in unilateral disease and many patients remain stable for prolonged periods, if blood pressure is well controlled (Zierler et al. 1996). Younger patients with fibromuscular dysplasia have excellent results when treated with surgery or angioplasty (Davidson et al. 1996). Renal artery stents have improved the success of angioplasty (van de Ven et al. 1999, Leertouwer et al. 2000, Baumgartner et al. 2000). Patients with normal renal function and atherosclerotic stenosis are managed similarly to those with fibromuscular dysplasia (Sos 1991). Renal parenchymal damage is a major determinant of renal dysfunction and the outcome of renovascular disease (Wright et al. 2002).

2.2 Cardiovascular autonomic regulation

In early studies, to assess cardiovascular autonomic nervous function in hypertension, plasma and urine norepinephrine and heart rate were used to measure the overall sympathetic activity of the body (Julius 1971, Goldstein 1983, Esler 1989, Anderson 1989). However, plasma levels of norepineprine represent combined effects of norepinephrine release, uptake and catabolism, not only cardiovascular sympathetic activity. It has been criticised that norepinephrine (Esler et al. 1990, Grassi et al. 1997) and heart rate (Grassi et al. 1998) are not real measures of adrenergic tone and that these do not separate well enough normotensive subjects from hypertensive patients.

Other methods that have been used to assess cardiovascular autonomic regulation are measurement of HRV (Akselrod et al. 1981, Kleiger et al. 1987, Guzzetti et al. 1988), BPV (Parati et al. 1995a and 1998) and BRS (Sayers 1973, Malliani et al. 1991, Parati et al. 1995b). In addition, microneurography (Wallin and Sundlöf 1979, Anderson et al. 1989, Jennings 1998), norepinephrine spillover (Esler et al. 1981, Johansson et al. 1999) and radionuclide studies with ^{123}I- MIBG (^{123}I- metaiodobenzylguanidine) (Wieland et al. 1981, Sisson et al. 1987) and other tracers, such as ^{11}C -meta-hydroxyephedrine, has also been used (Schwaiger et al. 1990, Rosenspire et al. 1990).

2.2.1 Heart rate variability

HRV is an indirect marker of cardiovascular autonomic nervous function reflecting to the autonomic control of the sinus node (Malik and Camm 1995 (pp. 3-19)). HRV can be measured using conventional linear time and frequency domain methods for assessing autonomic nervous control both in scientific and clinical work, and can also be used to define patients' prognosis (Task Force 1996). Aging, respiratory rate, smoking, physical fitness and drugs may influence HRV (Task Force 1996).

HRV in time and frequency domain can be analysed from successive RR-intervals from the short-term or long-term recordings (Akselrod et al. 1981). Short-term recordings are often 3-5 min ECG-data segments, obtained either under ambulatory or stationary laboratory conditions. Ambulatory long-term recordings can be several hours or days long allowing patients to continue their normal or almost normal daily activities. For reliable HRV analysis, accurate detection of R waves and stationarity of ECG signal are crucial. ECG data should be free of any artefact and ectopic beats. This means, in fact, that RR-interval data should not change with time, and that statistical parameters, such as mean values and frequency contents of the signal should not change. It is quite obvious that cardiovascular signals in general seldom, if ever, fulfill all those criteria. In contrast to controlled laboratory conditions, the lack of stationarity is a problem in long-term ambulatory recordings. Longer ECG -recordings permit measurements of very low frequency (VLF, between 0.003 - 0.04 Hz) band of HRV. On the other hand, they do not allow the control of common factors known to affect HRV such as posture, physical activity and breathing frequency without additional measuring equipment. Nevertheless, with ambulatory recordings it is possible to measure HRV in real life conditions.

2.2.1.1 Time-domain parameters of heart rate variability

The two most common time domain parameters of HRV are the mean of RR-intervals and the standard deviation of normal to normal RR-intervals (SDNN) resulting from rate of sinus node depolarisation (Kleiger et al. 1987, Malik and Camm 1995 (pp. 33-45), Task Force 1996). These are usually obtained from 24-hour recordings and they reflect to overall variability of that period. SDANN, the standard deviation of the average normal to normal RR-interval reflects long-term HRV and can be calculated over short recordings, typically five

minutes. It also results from the rate of sinus node depolarisation. RMSSD, the square root of the mean squared differences of normal to normal RR-intervals, is perhaps the most common measure derived from RR-intervals. It also is an estimate of short-term HRV and an index to quantify the parasympathetic tone. Other indexes, such as HRV triangular index, have been used (Davies et al. 2002). This analysis is usually derived from 24-hour ECG-recording and it is less sensitive to artefact.

2.2.1.2 Frequency domain parameters of heart rate variability

Power spectral analysis quantifies the oscillatory components of the heart rate and quantifies the different frequency components in their relative power (Malik and Camm 1995 (pp. 63-74), Task Force 1996). Total power is represented by the total area under the power spectral curve. The power of low and high frequency components are represented by the area under the portion of the curve related to each component. The total power (a frequency domain measure) is mathematically identical to the variance (a time domain measure). However, power spectrum is not constant. It may vary quite much as a function of different conditions (Parati et al. 1995a), especially when oscillations are around 0.05 Hz. This explains why the lowest frequency components of the power spectrum are so often left out because it is considered to be noise.

Spectral analysis permits the quantification of the absolute power. The power spectrum can be expressed also as normalised power, also called as a relative or fractal power centered to the frequency of interest (i.e. LF or HF) divided by total power (Öri et al. 1992, Task Force 1996). There are four principal power spectral components. These are: a high frequency band (HF) due to respiratory-driven oscillation around 0.2 - 0.4 Hz and a low frequency band (LF) - Mayer waves - around 0.1 Hz, very low frequency band (VLF, 0.003 - 0.04 Hz) and ultra low frequency bands (ULF, below 0.003 Hz) believed to be due to thermoregulation and renin-angiotensin system and dependent primarily on the presence of parasympathetic outflow (Sayer et al. 1973, Akselrod et al. 1981, Taylor et al. 1998).

Good-quality ECG recording is very important, and perhaps even more important for frequency domain analysis than in time-domain measurements of HRV. Spectral analysis uses either a Fast Fourier transform algorithm model (FFT, non-parametric) or an autoregressive model (AR, parametric) (Parati et al. 1995a, Task Force 1996) to transform RR-interval to power spectral components. FFT is a frequency-domain representation of a signal as a

complex-valued function characterised by a modulus function and a phase function. AR model is time-series modelling based on the assumption that each value of the series depends on a weight sum of the previous values of the same series plus noise. In the AR model, the model in order is crucial and too low model order results in a low resolution power spectrum and too high model order can generate false peaks in the spectrum (Pincus et al. 1994). However, FFT and AR methods provide comparable results (Task Force 1996).

2.2.1.3 Other methods to analyse heart rate variability

Assumption that RR-interval time series are stationary and that variation is harmonic or sinusoidal is seldom perfectly true. Awareness of this had lead to the development of several other methods to assess HRV based on non-linear mathematical models and chaos theory (Goldberger et al. 1987, Poon et al. 1997, Mäkikallio et al. 1998 and 2001 Kuusela et al. 2001). These methods are thought to describe better the complex and dynamic elements of heart rate regulation, but the magnitude of HRV cannot be calculated. Several non-linear methods, such as geometric methods (Poincare plots), power law behavior of 24 -hour FFT spectrum and other methods quantifying the fractal properties of given time series (Mäkikallio et al. 1998, Huikuri et al. 1999) have been used. Although the physiological backgrounds of the non-linear HRV is poorly understood, the methods may still provide additional information beyond the conventional methods of HRV in patients risk stratification (Mäkikallio et al. 1999).

2.2.1.4 Physiological meaning and clinical use of heart rate variability

HRV is the result of continuous interplay between spontaneous activity of sinus node, sympathetic and vagal neural activity and the humoral factors (Malliani et al. 1991). During systole blood pressure rises and stretches the baroreceptores increasing their discharge rate. In the medulla, nucleus tractus solitarius responds to this and stimulates preganglion neurones of vagal nerve and increases cardiac vagal activity (McAllen and Spyer 1978). In the sinus node the spontaneous depolarisation rate decreases and the RR-interval becomes longer (Eckberg et al. 1976). Parasympathetic nervous system causes high frequency variation to the heart rate and also low frequency variation via the vagal nerve. Sympathetic nervous system causes variation at low frequency (Task Force 1996). High-frequency variation is regulated partly via receptors in lungs and partly via the central nervous system (Brown et al. 1993, Penttilä et al.

2001). Baroreceptors, mechanoreceptors and chemoreceptors in the blood vessels and heart have a major role in the regulation of low frequency variation of the hear rate.

Time domain measures correlate with vagal activity (Task Force 1996). Mean of RR-intervals and SDNN measure both sympathetic and parasympathetic control. RMSSD measures mostly parasympathetic activity due to respiration. In frequency domain measures, fluctuations in the power spectrum reflect the cardiovascular sympathetic and vagal modulation at the heart level. HF power of HRV is a marker of vagal influence (Task Force 1996). LF power of HRV had been suggested to reflect both sympathetic and vagal control of the heart. The balance between sympathetic and vagal components have been expressed by dividing HF power by LF power (HF/LF –ratio) (Pagani et al. 1985 and 1986, Eckberg et al. 1997). However, that can be criticised because spectral power do not reflect absolute levels of autonomic traffic. It has been proposed that there is marked vagal component around 0.1 Hz and changes of sympathetic and vagal nerve activities do not occur reciprocally (Eckberg 2000). The precise origin of VLF and ULF powers of HRV are not clear. VLF and ULF oscillations cannot be assessed reliably from short ECG recordings. Because HRV represents combined effects of vagal, sympathetic and humoral inhibitory and excitatory effects, change in HRV is not specific to certain component of autonomic nervous system.

HRV in pathological conditions has a particular interest. Reduced HRV predicts poor prognosis in post-myocardial infarction patients (Kleiger et al. 1987, Bigger et al. 1992) and patients with heart failure (Saul et al. 1988, La Rovere et al 2003), neurally mediated syncope (Lepicovska et al. 1992) and diabetic autonomic neuropathy (Malpas and Maling 1990). It may be speculated that reduced HRV may predict poor prognosis also in hypertensive patients.

2.2.2 Short-term blood pressure variability

Blood pressure is characterised by continuous fluctuations known since famous studies of Hales (1773) and Ludwig (1852). BPV has been studied ever since to our days (Turjanmaa et al. 1987 and 1990, Takalo et al. 1994, Mancia et al. 1997 (pp 117-169)). A major step for BPV measurements was the possibility in 1960s to record intra-arterial blood pressure over 24 hours with so called "Oxford system" (Bevan et al. 1969, Mancia et al. 1983). In addition to invasive methods, beat-to-beat blood pressure can now be estimated non-invasively using finger photoplethysmograph (Penáz 1973, Parati et al. 1989, Imholz et al. 1993, Langewouters

et al. 1998). Systolic or mean BPV has been studied most often whereas diastolic BPV is often neglected.

2.2.2.1 Measurement of short-term blood pressure variability with frequency domain method

Like HRV measurements, short-term BPV can be quantitatively analysed from continuous blood pressure recordings by power spectral analysis (Imholz et al. 1998). As in HRV analysis, high signal quality is very important for reliable analysis of short-term BPV. Similarly to HRV, power spectrum analysis can be divided in different frequency bands. However, there are no consensus recommendations of the frequency bands that should be used. Lower ranges for LF power varies from 0.05 to 0.075 Hz while the upper limit in 0.129 to 0.15 Hz (Takalo et al. 1994, Parati et al. 1995, Fluckiger et al. 1999).

2.2.2.2 Physiological meaning and clinical use of short-term blood pressure variability

The factors affecting BPV and the mechanisms determining the degree of short-term BPV, is an issue that is under debate (Mancia et al. 1997). Short-term BPV is under the influence of autonomic nervous system but during daytime, variability reflect more to the physical and mental activity (Conway 1986, Bernardi et al. 1997). The best known rhythms are associated with respiratory frequency around 0.25 - 0.3 Hz and other components around 0.04 - 0.10 Hz. These can be observed parallel to HRV.

The HF component of BPV is associated with respiratory rate, and it can be detected in both systolic blood pressure and HRV. It is considered to reflect to mechanical effects of stroke volume which are due to changes in venous return and changes in respiration (Task Force 1996).

The LF component of BPV, "Mayer waves", have been described almost 130 years ago but the underlying mechanisms are not yet resolved. There is preliminary evidence that changes in BPV are associated with changes in sympathetic activity, and that central mechanisms can buffer the degree of BPV at LF band (Parati et al. 1998). In addition, LF component of blood pressure is believed to rise from changes in vascular tone and peripheral resistance (Radaelli et al. 1994, Bernardi et al. 1997).

The VLF component of BPV is poorly understood and thus remain speculative. The idea that the VLF band reflects sympathetic activity has been challenged. It has been argued that it

influenced by the renin-angiotensin system and that it primarily depends on the presence of parasympathetic outflow (Taylor et al. 1998).

Increased long-term BPV has been associated with increased end-organ damage and therefore BPV may thus have prognostic value (Parati et al. 1997). In humans, the prognostic role of short-term BPV is open. However, present availability of non-invasive devices (Portapres®), which are able to measure blood pressure beat-to-beat, should make studies on BPV easier to perform (Castiglioni et al. 1999). With these methods is could also be possible to study the clinical relevance of short-term BPV in larger patient groups.

Behavioural factors, such as emotions, exercise and sleep, which increase or decrease BPV, have important role when determining BPV. In addition, neurohumoral and endothelial factors and BRS are also important. It is believed that impaired BRS and also reduced HRV, typical in aging and also seen in hypertension, are responsible for elevated blood pressure and increased BPV (Mancia et al. 1980, Parati et al. 1995). In elderly people, there is a strong correlation between 24-h BPV and age without sex-related differences (Cicconetti et al. 2000).

2.2.3 Baroreflex sensitivity

BRS gives information on the vagal control of the blood pressure regulation (Eckberg 1992, Piepoli et al. 1997). Baroreceptors are sensory nerve endings that are located in the wall of aortic arch and sinus caroticus. Vascular stretch due to increased blood pressure activates the baroreceptors. Baroreceptors have a central role in short-term blood pressure control, and they respond to the continuous changes in blood pressure produced by various stimuli occurring in daily life. The baroreflex mediates short-term changes in autonomic outflow, influences heart rate, myocardial contractility and peripheral resistance in order to maintain blood pressure in normal range. Mechanisms determining the magnitude of BRS are increased blood pressure and stiffness of arterial wall at the site of the baroreceptors (aortic arch and sinus caroticus), viscoelastic properties of the vessel walls, cellular and molecular mechanisms and pathological states (endothelial dysfunction, oxidative stress, platelet activation) and baroreceptor deformation.

2.2.3.1 Measurement of baroreflex sensitivity with phenylephrine method

The baroreflex is an important cardiovascular reflex. BRS can be measured with phenylephrine technique, which is considered to be safe, minimally invasive and also

clinically useful. With this method, intravenous phenylephrine injections (doses of 50 to 150 µg) induce an increase in blood pressure (20 to 30 mmHg) that is followed by lengthening of RR-intervals (Smyth et al.1969, Bristow et al. 1969, Eckberg et al. 1971, Hartikainen et al. 1994). There is a linear correlation between length of RR intervals and changes in systolic blood pressure. The slope of regression line is used to quantification of BRS. In order to reduce the measurement variability, the measurements are often repeated several times, usually at five minutes intervals, and the corresponding regression slopes are then averaged.

2.2.3.2 Other methods to measure and analyse baroreflex sensitivity

The fact that the baroreflex is possible to quantify has increased its clinical research and led to the development of creative and innovative ways to measure BRS. In addition to phenylephrine, other vasoactive agents such as nitroprusside, nitroglycerine (vasodilator) and angiotensin (vasoconstrictor) has been used to assess BRS (Pickering et al. 1972, Chen et al. 1982).

The Valsalva test, which means prolonged expiration against a closed glottis so that there is increasing of intrathoracic and intra-abdominal pressures, has also been used to assess BRS (Smith et al. 1987). The phase 4 of the Valsalva manoeuvre (elevation of blood pressure and slowing down of heart rate) is used for BRS calculations.

In neck chamber technique (Eckberg et al. 1980), the advantage is the activation and deactivation of the carotid baroreceptors changing pressures in the neck area. Thus, the stimulus is confined to the baroreceptor areas and it allows to study the baroreflex responses of both heart and circulation. The technique is, however, complicated and time-consuming and uncomfortable for patients.

There are also ways to measure BRS non-invasively from recordings of finger blood pressure and heart rate without the need to inject vasoactive drugs or to set up complex experimental conditions (Pellizzer et al. 1996). Spontaneous BRS can be measured from normal fluctuations of blood pressure and RR interval or peripheral sympathetic nerve activity (Robbe et al. 1987, Airaksinen et al. 1997, Persson et al. 2001). The analysing methods are based on time domain and frequency domain methods. It has been observed that these techniques provide comparable information about BRS with other techniques, particularly when BRS estimates are averaged over time window of a few minutes (Robbe et al. 1987, Persson et al. 2001).

2.2.3.3 Physiological meaning and clinical use of baroreflex sensitivity

Baroreflex contributes to the short-term blood pressure regulation and cardiovascular variability. In 1969 Bristow et al. showed that in high blood pressure BRS is diminished (Bristow et al. 1969). Already in early hypertension BRS may be reduced (Moreira et al. 1992). Depressed BRS is believed to be the result of increased BPV. The fluctuation of systolic blood pressure may contribute to increased end-organ damage (Frattola et al. 1993). During sustained increase of blood pressure baroreflex adapts over time as elevated blood pressure is maintained (McCubbin et al. 1956). Decreased vascular compliance contributes to decreased BRS in chronic hyperetension but there are also similar finding in atherosclerosis and aging. In chronic hypertension BRS is suppressed and pressure threshold is increased i.e. reset. Regardless, the basic issue, whether a low BRS is a cause or a consequence of high blood pressure, is still unsolved.

In addition, the measurements of BRS has gained particular interest since it was known that decreased BRS is an independent marker of cardiovascular risk, increased mortality and sudden cardiac death in patients with myocardial infarction (Kleiger et al. 1987, Farrell et al. 1991, Hartikainen et al. 1994, LaRovere et al. 1998).

2.2.4 Other methods to assess cardiovascular autonomic regulation

More recently, our understanding of the character of the autonomic nervous dysfunction has been expanded with the aid of microneurography and norepinephrine spillover analysis. Sympathetic nerve activity can be measured directly in peripheral nerves using microneurography technique (Hagbarth and Vallbo 1968, Wallin and Sundlöf 1979). MSNA provides reliable estimate of sympathetic nerve traffic to the heart, kidneys and muscles (Kingwell et al. 1994). However, this method is time-consuming and there are often difficulties to find adequate recording sites for best detection of sympathetic nerve activity. One limitation is also the background activity. MSNA and power spectral analysis of HRV correlate well (van de Borne 1997).

Measurement of the norepinephrine spillover gives a better and more specific picture of cardiac sympathetic activity (Esler et al. 1979 and 1989). Norepinephrine spillover is measured by radiotracer method and calculated according the formula were the infusion rate of the labeled norepinephrine is divided by the specific activity measured in the arterial

plasma (Esler et al. 1979). The limitation of this methods is that it is invasive and requires arterial and central venous cannulation. Therefore it is not suitable to use on a large scale. However, total body norepinephrine spillover is also used.

^{123}I-MIBG is a radionuclide tracer that is a norepinephrine analogue. It shares the same neuronal transport and storage mechanisms as norepinephrine (Wieland et al. 1981, Sisson et al. 1987). With this tracer it is possible to study adrenergic neuronal function *in vivo* in human body. ^{123}I-MIBG distribution in the heart, detected with gamma-cameras, reflects to the amount of regional sympathetic nerve endings in the heart (Sisson et al. 1987). Also other tracers, like ^{11}C -meta-hydroxyepinephride, are available for assessment of sympathetic nerve endings (Schwaiger et al. 1990).

2.2.5 Reproducibility of the methods

Reproducibility and comparability of the methods depend on the measurement technique, study population, time interval between measurements and the length of the recordings. The reproducibility of frequency domain measures of HRV have found to be at least moderate, and reproducibility is better when paced breathing is used during the measurements (Ahmed et al. 1994, Freed et al. 1994, Sinnreich et al. 1998). There is also an acceptable correlation (correlation coefficient range from 0.6 to 0.9) between long-term and short-term recordings of HRV (Bigger et al. 1993) and between parameters of the time and frequency domain measurements. Reproducibility of the BPV is not well known. In a very small group of hypertensive and normotensive subjects it was found to be only moderate, measured at a one week interval (Girard et al. 1994). Reproducibility of BRS varies from good to poor (coefficient of variation between 7.4 % and 28 %) depending on the method that is used for measurement (phenylephrine, Hartikainen et al. 1995; neck suction, Bene et al. 1999).

2.3.Cardiovascular autonomic regulation in essential hypertension

2.3.1 Role of cardiovascular autonomic regulation in the development of essential hypertension

There are two basic hypothesis of the role of autonomic nervous function in the genesis of hypertension: increased sympathetic and decreased parasympathetic activity and reduced baroreflex sensitivity.

In borderline and early hypertension, especially in young patients, the sympathetic drive to the heart and blood vessels is increased (Julius et al. 1971, Esler et al. 1977, Goldstein 1983, Anderson 1989, Julius et al. 1991, Esler et al. 1996, Esler 1989, Esler 1990, Julius 1996, Esler 2000) while the parasympathetic drive is decreased (Julius et al. 1971, Guzzetti et al. 1988). These observations are based mainly on pharmacological studies blocking autonomic nervous system and measuring heart rate and epinephrine. It has been criticised that these measures are not real measures of adrenergic tone (Grassi et al. 1998). It has also been criticised that plasma epinephrine measurements cannot separate normotensive subjects from hypertensive subjects (Esler et al. 1990, Grassi et al. 1997).

In the later phases of hypertension, the hyperkinetic circulation is less evident since ß-adrenergic responsiveness and cardiac compliance tend to decrease (Julius et al. 1991, Esler 1989 and 2000). When blood vessels have became hyper-reactive, the same degree of vasoconstriction per blood pressure elevation can be achieved with less amount of sympathetic tone. In that phase the sympathetic activity is less evident as brain resets itself to maintain the same blood pressure elevation with a smaller amount of sympathetic discharge (Julius et al. 1971, Guzzetti et al. 1988). Decreased sympathetic activity may be useful and protect hypertensive patients from cardiac arrhythmia and sudden death (Cerati and Schwarts 1991, Barron and Lesh 1996) whereas increased parasympathetic activity is associated with high blood pressure and poor prognosis (Floras 1993, Julius 1998). In chronic essential hypertension sensitivity of ß-receptors, atrial compliance and cardiac stroke volume decrease, while cardiac output remains normal. In resistance vessels, wall-to-lumen ratio changes and peripheral resistance increases, leading to structural remodelling of the vessels.

2.3.1.1 Plasma catecholamine concentrations and adrenergic blocking agents

In essential hypertension measurements of catecholamine levels have shown that catecholamines are elevated in young hypertensive patients (Goldstein 1983). With adrenergic blocking agents it has been shown that heart rate and stroke volume are increased in young, mildly hypertensive patients and, in addition, that they return to normal after autonomic blockade (Julius 1971, Esler et al. 1989). Egan et al. studied the mechanism of increased alfa adrenergic vasoconstriction by combining the measurements of plasma catecholamine and adrenergic blocking agents and found that in hypertensive patients there were abnormalities in adrenergic function, which were explained to be due to increased sympathetic drive (Egan et al. 1987).

2.3.1.2 Muscle sympathetic nerve activity

Wallin and Sundlöf (Wallin and Sundlöf 1979) showed that sympathetic nerve traffic to heart, blood vessels and kidney are similar at rest. In essential hypertension MSNA has shown conflicting results. In early studies of Wallin and Sundlöf no increase in MSNA was found in hypertensive patients when compared to normotensive subjects (Wallin and Sundlöf 1979). In addition, some other studies did not detect elevated MSNA in essential hypertension at rest (Somers et al. 1988, Rea and Hamdan 1990), whereas some other studies have found increased levels of MSNA (Anderson 1989, Miyajima et al. 1991). Matsukawa et al. (Matsukawa et al. 1993) found that patients with accelerated hypertension have more increased MSNA bursts than patients with mild hypertension. Increased MSNA is closely related to renin-angiotensin system because treatment with ACE-inhibitors decreases MSNA and lowers blood pressure (Johansson et al. 2000). The discrepancy between these studies can possibly be explained by differences in sodium intake, obesity and BRS.

2.3.1.3 Heart rate variability

In untreated essential hypertension, several studies have found decreased HRV (Mancia et al. 1983, Guzzetti et al. 1988, Furlan et al. 1990). In hypertensive patients who have been already under antihypertensive medication, factors affecting autonomic nervous system are largely unknown (La Rovere et al. 1988, Kupari et al. 1993, Huikuri et al. 1996, Pikkujämsä et al. 1998). It is known that when essential hypertension becomes sustained, previously elevated plasma norepinephrine levels falls because of negative feedback (Julius 1990). Under standardised laboratory conditions HF power of HRV is lower in subjects with essential hypertension than in normotensive subjects (Guzzetti et al. 1988, Langerwitz et al. 1994, Siché et al. 1995, Liao et al. 1996). In patients with borderline hypertension, HF power of HRV has found to be reduced compared with controls (Langerwitz et al. 1995) or unchanged (Guzzetti et al. 1988). LF power of HRV in essential hypertension has been found to be both higher (Guzzetti et al. 1988), equal (Aono et al. 1996) or lower (Siché et al. 1995) than normotensive control subjects. LF power of BPV was observed to be higher in hypertensive than in normotensive subjects (Siché et al. 1995, Aono et al. 1996). Under ambulatory conditions, no differences were observed among borderline, hypertensive and normotensive subjects in the HF power of HRV or BPV (Parati et al. 1990). Reductions of human

cardiovascular hemodynamic and neural fluctuations have important prognostic implications, but only one study deals with predictive value of power spectral analysis in developing hypertension (Liao et al. 1996).

In conclusion, these studies indicate, that increased sympathetic activity exists in young hypertensive patients, in mild hypertension and early phases of hypertension and contributes to the hemodynamic profile, development of vascular structure, appears to involve the kidneys and could therefore contribute to long-term elevation of blood pressure.

2.3.1.4 Baroreflex sensitivity

A diminished baroreflex and high blood pressure are closely related (Bristow et al. 1969, Takeshita et al. 1975, Eckberg et al. 1979, Moreira et al. 1992, Watkins et al. 1996). That has led to the hypothesis that if the baroreflex is not functioning properly, hypertension may result (Takeshita et al. 1975, Yamada et al. 1988, Matsukawa et al. 1991, Parmer et al. 1992). In patients with untreated essential hypertension several studies have found decreased BRS (Cribbin et al. 1971, Takeshita et al. 1975, Eckberg et al. 1977). However, the basic issue is still not resolved. It is not known whether low baroreflex is the cause or the consequence of elevated blood pressure. Permanently elevated blood pressure leads to increased arterial stiffness and low BRS and, on the other hand, a low BRS opposes insufficiently high blood pressure. A prospective follow-up study with a group of young subjects with low BRS and normal blood pressure could possible resolve this question. However, that kind of study has not yet been done.

2.3.1.5 Renin in essential hypertension

A small group of patients with essential hypertension has high plasma renin activity and a larger group may have elevated renin activity due to antihypertensive drugs (diuretics and vasodilators). However, the majority of patients with essential hypertension do not have suppressed renin-angiotensin levels (Folkow et al. 1982). This has been explained in two ways. The first explanation is nephron heterogeneity (Sealey et al. 1988), and the second is increased sympathetic drive (Julius 1988b). Low renin activity means better prognosis of hypertension because high renin activity indicates more severe intrarenal vascular damage. Thus, it is not surprising that there are more complications among patients with high renin activity.

2.3.3 Mechanisms of increased sympathetic activity and decreased parasympathetic activity in essential hypertension

2.3.3.1 Increased sympathetic activity

One explanation is that blood pressure becomes elevated because of too strong individual reactivity to stressful stimuli from the environment. At the beginning blood pressure is elevated for short moments and later on becomes permanently elevated. This theory has been proven in animals (Herd et al. 1969, Folkow et al. 1973). In humans it is still under debate (Cobb and Rose 1973, Timio et al. 1988, Schnall et al. 1990, Schwartz et al. 1996). However, it is true that sympathetic nervous system may be directly activated by stress. This over-activity can in turn interact with high sodium intake (Intersalt study 1988). In addition, excess of renin-angiotensin activity could interact with sympathetic nervous system and mediate its effects. Also the associations between hypertension and insulin resistance are well known, but the meaning is uncertain (Julius 1998). It is also known that insulin stimulates sympathetic nervous system which can be detected with increased plasma epinephrine levels and MSNA brushes (Rowe et al. 1981, Scherrer et al. 1997). Increased sympathetic activation can also be of central origin (Jannetta et al. 1985, Schobel et al. 2002). Lesions in the medulla (nucleus tractus solitarius) produce labile hypertension in rats (Jannetta et al. 1985). Recently in humans, Schobel et al. found that essential hypertension is accompanied by a significantly augmented central sympathetic outflow in patients who had compression in the rostral ventrolateral medulla (magnetic resonance tomography evaluation, Schobel et al. 2002). Hypertension can be linked to genetically mediated hyper-reactive response to stress or an overly strong stimulus from the environment or both (Timio et al 1988, Schnall et al. 1990). The hypothalamus may have important role in increased sympathetic activity (Folkow et al. 1982, Zanchetti 1977). It has also been explained that this increased activation is due to early weakening of BRS.

2.3.3.2 Decreased parasympathetic activity

Hypertension is characterized with a higher threshold for activation of baroreflex and diminished BRS. This can be explained by as a result of chronic increases in blood pressure and has partly been explained by genetic factors (in human, Parmer et al. 1992). Normally the

baroreflex reduces heart rate and lowers blood pressure by vagal stimulation and sympathetic inhibition. In rabbits, the baroreflex resets rapidly when (renal) hypertension becomes sustained (Xie et al. 1991). Reduced BRS is the principal determinant of increased blood pressure variability (Floras et al. 1988) and reduced BRS is assumed to lead to increased sympathetic activity (Mancia et al. 1997).

2.3.4 Role of cardiovascular autonomic regulation in established, long-lasting essential hypertension

Increased sympathetic activation leads to structural changes in the heart and vessels that can lead to left ventricular hypertrophy and to progression of atherosclerosis. In addition, maximal oxygen consumption of the heart increases and peripheral resistance increases. In addition to tachycardia and vasoconstriction, cardiovascular autonomic nervous control may contribute to blood pressure levels and regulation in long-term by other mechanisms: by effects on the kidney, blood vessel growth, blood vessel permeability and resetting of baroreflex sensitivity. Increase in efferent sympathetic traffic promotes sodium retention and renin release and increases blood pressure (DiBona 1992). This favors the maintenance of hypertension by interfering with the ability of renal compensatory (homeostatic) mechanisms. Increased sympathetic activity appears to occur particularly during the early stages of hypertension influencing to the structure of vessels and promoting the growth of vascular muscle. Thus, it also influences the long-term regulation of blood pressure. In addition, genetically determined sympathetic influence may cause that long-term alteration in membrane properties contribute to hypertension. Sustained increases in blood pressure may contribute to baroreflex resetting in hypertension. Baroreflex resetting is an important mechanisms that allow sympathetic activation and blood pressure to increase.

Sympathetic activity is a key factor in the genesis of essential hypertension but it also has an important role in established hypertension while it promotes cardiovascular complications. The knowledge of sympathetic nervous system in different types of hypertension and end organ damage is limited and therefore further studies are needed.

2.4 Cardiovascular autonomic regulation in renovascular hypertension

2.4.1 Mechanisms of renovascular hypertension

In 1934 Goldblatt succeeded in making a dog model of renovascular hypertension by constricting the renal artery with clips. He also found that venous plasma from ipsilateral kidney contain a vasopressor substance. However, already in 1898 a "pressure substance", later called renin, had been discovered by Tigerstedt at Karoniska Institute who extracted it from the rabbit kidney. Later, it was found that this enzyme releases angiotensin (Page et al. 1975). Although increased secretion of renin from the kidney was detected it took a long time to find out that increased renin activity was responsible for renovascular hypertension (Barger et al.1979) and this finding led highly productive renin-angiotensin research. Renin is at same time a growth factor and a pressure hormone. There is multiple forms of active renin (Katz et al. 1992). Expression and regulation of human renin gene and the structure of the enzyme have been evaluated and the molecular biology of the renin-angiotensin system has been found to be increasingly more complicated (different forms of renal renin, control of renal renin secretion, prorenin, extrarenal renin) (Grendling et al. 1993).

Increased renin, released from the stenotic renal artery, is also responsible for renovascular hypertension in humans (Pickering 1989). In acute phase, the elevated blood pressure is related to the increased amount of angiotensin II in circulation and the stenotic kidney secretes renin excessively leading to elevated fluid volume and increased peripheral resistance (Tarazi and Dustan 1973). In the chronic hypertensive phase, plasma renin activity falls towards normal. Blood pressure falls when the stenosis is relieved and it is followed by a fall in fluid volume and peripheral resistance. The sooner the stenosis is relieved, the greater the chance of relieving hypertension. In prolonged renovascular hypertension, hypertension is fixed and can only poorly be affected by removal of the stenosis (Martinez-Maldonado 1991). In this phase renovascular hypertension is considered to be multifactorial, just like essential hypertension.

Plasma renin activity varies within both normal and hypertensive population. While renin is involved in cardiovascular homeostasis, it may also have an important role in the pathogenesis of hypertension (Laragh 1991a).

2.4.2 Role of cardiovascular autonomic regulation in renovascular hypertension

Permanent elevation of blood pressure in renovascular hypertension causes disturbances in cardiovascular control mechanisms as well as in essential hypertension. However, these

neurohumoral disturbances are different than in essential hypertension (Mancia et al. 1982, Johansson et al.1999, Petersson et al. 2002, Gao et al. 2002). The data of sympathetic nervous system in secondary hypertension is more scanty and less univocal when compared to essential hypertension. It is uncertain whether increased sympathetic nerve activity is specific to renovascular hypertension per se or whether it is a partial cause of elevated blood pressure. One important point is that increased sympathetic activity may contribute to the progression and poor prognosis of renovascular hypertension (Johansson et al. 1999, Petersson et al. 2002). It has been showed that patients with renovascular hypertension have altered sympathetic function, increased sympathetic drive and impaired catecholamine extraction which can also explain poor prognosis and high cardiovascular mortality of these patients (Oparil et al. 1986, Miyajima et al. 1991, Johansson et al. 1999, Petersson et al. 2002). In addition, plasma and urine catecholamines are increased in patients with renovascular hypertension (Januszewicz et al. 1978, Izumi et al. 1980, Gordon et al. 1982). Increased MSNA has also been found in renovascular hypertension compared with essential hypertension and also compared with primary aldosteronism, and in addition, MSNA was decreased after successful dilatation of renal arterial stenosis (Miyajima et al. 1991). This finding is accompanied with decreased plasma renin activity and angiotensin II concentration, suggesting that renin-angiotensin-system might have something to do with sympathetic nerve activity possibly via central nervous system. All these findings support the idea that sympathetic nervous system is important also in the pathophysiology of renovascular hypertension.

2.5 Natriuretic peptides in hypertension

Natriuretic peptide system consists of three peptides, atrial natriuretic peptide (ANP), brain-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). These peptides contribute to blood pressure regulation and fluid homeostasis by forming a humoral link between the heart and kidney (Lang et al. 1985, Ruskoaho 1992, Levin et al. 1998). ANP is mainly synthesised in the atria where it is stored as the 126-amino acid peptide proANP (Thibault et al. 1987). BNP is mainly synthesised by both atria and ventricles and it is also produced in central nervous system (Levin et al. 1998). CNP is synthesized in central nervous system, kidney and endothelium, and acts as an endothelium-derived relaxing peptide (Levin et al. 1998).

The major determinant of ANP secretion is atrial wall stretch and for BNP both atrial and ventricular stretch. In addition several hormones (angiotensiini II, vasopressin, catecholamines) stimulate the ANP secretion (Ruskoaho 1992). Cardiovascular effects of ANP and BNP are very similar. They balance the effect of renin-angiotensin system, contribute against salt and water retention, have inhibitory effects against vasoconstrictor peptides, promote vascular relaxation and inhibit sympathetic outflow (Levin et al. 1998). Therefore, natriuretic peptide system is implicated in the pathophysiology of hypertension, congestive heart failure atherosclerosis and renal diseases.

Clinical application of natriuretic peptide system is actively on going (Wallén et al. 1997). Determination of plasma ANP and BNP levels have found to be useful for the evaluation of heart failure (Mukoyama et al. 1991, Brunner-LaRocca et al. 2001, Yoshimura et al. 2001), cardiac hypertrophy (Hasegawa et al. 1993) and after myocardial infarction (Motwani et al. 1993). In patients with chronic heart failure or myocardial infarction ANP has prognostic information (Gottlieb et al. 1989, Hall et al. 1994). In the general elderly population, elevated BNP predicts mortality even in subjects without any known cardiovascular disorders (Wallen et al. 1997).

ANP and BNP are primarily produced by the heart and they have beneficial hemodymanic and neurohumoral effects and inhibition of the renin-angiotensin system and sympathetic nervous system (Clarkson et al. 1996). Activation natriuretic peptides is thought to be a protective response to cardiac damage, dysfunction and overload (McDonagh et al. 1998). Patients with established hypertension, especially those who have LVH, have increased ANP and BNP (Schreij et al. 1996, Nishikimi et al. 1996). It is not known what the role of natriuretic peptides is in the early phases of hypertension. It is also not known whether plasma ANP and BNP concentrations are related to the severity or to the etiology of established hypertension.

NT-proANP is released in equal amounts as ANP into circulation. The normal distribution of both ANP and BNP are skewed. ANP has a short, 2 - 5 minutes, half-life in the circulation, whereas half-life of NT-proANP is eight times longer. Therefore it can be used as a measure of ANP in clinical practice (Ruskoaho 1992).

2.5.1 Natriuretic peptides in essential hypertension

It has been reported that plasma concentrations of both ANP (Sugawara et al. 1985, Saganella

et al. 1986, MacDonald et al. 1986, Buckley et al. 1993) and BNP (Kohno et al. 1992 Buckley et al. 1993, Cheung et al. 1994, Takeda et al. 1995) become elevated in essential hypertension, and that there is considerable overlapping in ANP and BNP concentrations between hypertensive and normotensive subjects.

In hypertensive patients the increase in plasma ANP can be explained to be due to feedback mechanism in order to decrease elevated blood pressure and increase sodium excretion. In hypertension, it is common that left ventricular compliance decreases which leads to increased atrial work, the major stimulus for ANP release. Therefore hypertensive patients with LVH are considered to have higher plasma ANP concentrations than those without LVH, but this is not an uniform finding (Saganella et al. 1986, Yamaji et al. 1986). In addition, age, renal function, body fluid volume, sodium intake and blood pressure levels contribute to ANP release (Yamaji et al. 1986, Montorsi et al. 1987).

2.5.2 Natriuretic peptides in renovascular hypertension

Studies on natriuretic peptides in renovascular hypertension are scanty. There is one study of Schreij and co-workers who found increased levels of ANP in hypertensive patients with renal artery stenosis compared to patients with essential hypertension (Schreij et al. 1996).

3 AIMS OF THE STUDY

The purpose of the present study was to examine the cardiovascular control in patients with sustained hypertension of different etiology and severity. The specific questions were:

1. Are there differences in HRV in patients with differing in severity of long-lasting essential hypertension, and what are the clinical determinants of HRV?
2. Are there differences in short-term BPV in patients with differing in severity of long-lasting essential hypertension and in patients with renovascular hypertension?
3. Are there differences in BRS in patients with different severity of essential hypertension and in patients with renovascular hypertension, and what are the clinical determinants of BRS?
4. Are there differences in ANP (measured as NT-proANP) and BNP in patients with differing in severity of essential hypertension and in patients with renovascular hypertension, and what are the clinical determinants of ANP and BNP?

4 SUBJECTS AND METHODS

4.1 Study design

This study is a cross-sectional clinical study on cardiovascular autonomic nervous system and natriuretic peptides in patients with renovascular hypertension (RVHT), severe essential hypertension (SEHT) or mild essential hypertension (MEHT). It was performed in co-operation with the Department of Clinical Physiology and Nuclear Medicine, Department of Internal Medicine and Department of Clinical Radiology at the Kuopio University Hospital.

Patients in the RVHT and SEHT groups were selected out from 54 consecutive patients who were referred for evaluation of possible renovascular hypertension to the Kuopio University Hospital. The following inclusion criteria were used (Renal Working Group Guidelines 1987, Vidt 1991, Mann and Pickering 1992). Moderate suspicion for renovascular hypertension were: office diastolic blood pressure ≥115 mmHg, refractoriness to standard treatment, sudden onset of hypertension with age under 30 or over 55 years. Criteria for a high suspicion for renovascular hypertension were: malignant hypertension, elevation of serum creatinine during angiotensin-converting enzyme inhibitor (ACE) therapy and known asymmetry in renal size. The exclusion criteria were known non-vascular nephropathy, type 1 diabetes and aortic stenosis. Patient with MEHT were separately selected from the recruitment phase of an ongoing clinical trial (LIFE study, Dahlöf et al. 1997). They were patients who were actually excluded from the LIFE study because their blood pressure was too low or their ECG did not fulfil the criteria for LVH. The inclusion criteria for the MEHT group were mild hypertension and absence of any other chronic disease.

Patients were evaluated with detailed medical history, physical examination and routine laboratory tests (Figure 3). A history of vascular and other diseases, medication, duration of hypertension and smoking-habits was obtained from all patients. Office and ambulatory blood pressure measurements were performed one week before the other tests under normal antihypertensive medication. Thereafter medication was totally withdrawn, or if it was not possible, markedly reduced. Diuretics were suspended for at least three days and ACE-inhibitors at least one week prior to the tests. β-Blocker medication was reduced within four days to at least one half and thereafter terminated two days before the measurements. If this was not possible, because of very high blood pressure, patients used one quarter of normal β-blocker dose.

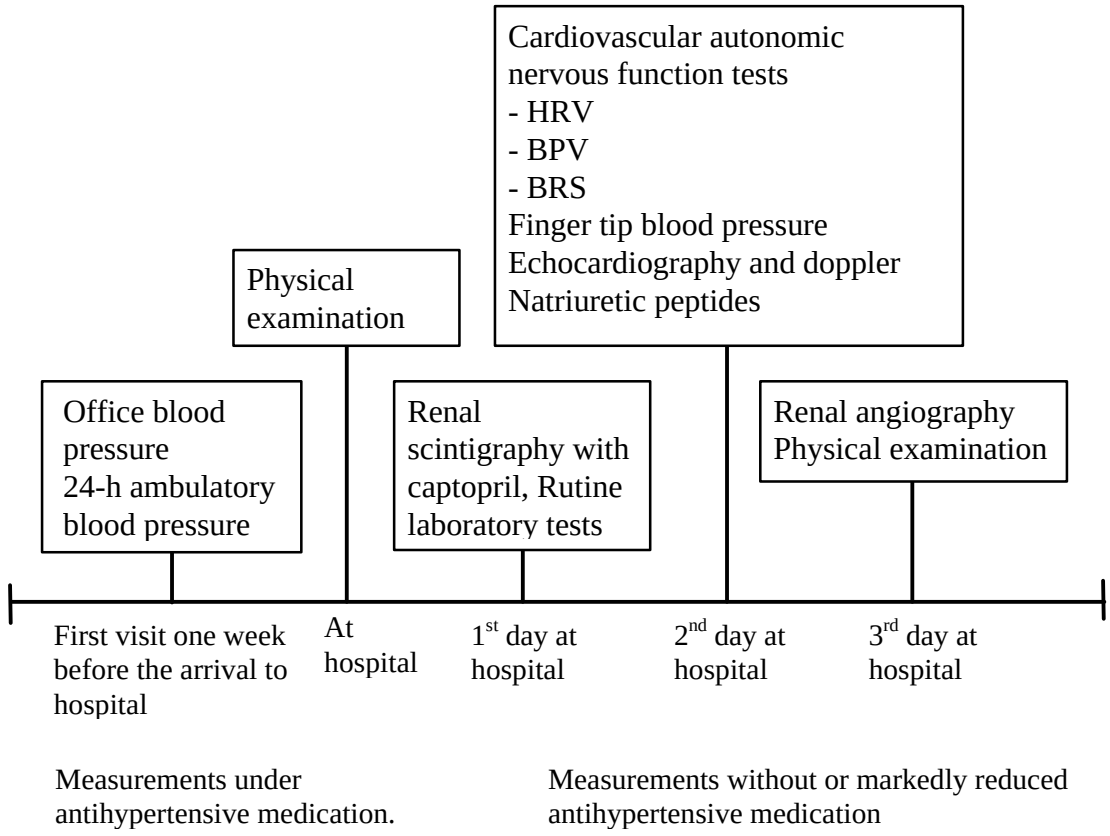


Figure 3. Schedule of the clinical visit

In the RVHT and SEHT groups cardiovascular autonomic nervous tests, echocardiography, natriuretic peptide measurements, captopril renography and renal digital subtraction angiography, measurement of glomerular filtration rate and routine laboratory tests were then performed during the three days patients were in the hospital. Patients in the MEHT group were outpatients. They were studied otherwise in the same way, but without captopril renography and renal digital subtraction angiography (Figure 3).

4.2 Study subjects

4.2.1 Hypertensive patients

Table 1 shows the clinical characteristics of the hypertensive patients and control subjects. Fourteen patients (5 men, 9 women, mean age 60 ± 4.1 years, range 28 - 84 years, average office blood pressure 190 ± 7.5 / 113 ± 2.9 mmHg) with significant renal arterial diameter stenosis in renal subtraction angiography (> 50 %) were considered to have RVHT (Bookstein

et al. 1972). The etiology of RVHT was atherosclerosis (n = 12) or fibromuscular dysplasia (n = 2). The remaining 40 patients (22 men, 18 women, mean age 46 ± 2.1 years, range 18 - 76 years, average office blood pressure 191 ± 5.2 / 119 ± 2.0 mmHg) were considered to have SEHT. The 30 patients in the MEHT group were separately selected (15 men and 15 women, mean age of 59 ± 1.2 years, range 43 - 69 years, average office blood pressure 142 ± 3.3 / 88 ± 1.7 mmHg).

Patients in the RVHT and SEHT groups used several antihypertensive drugs (Table 2). In the RVHT group 36 % and in the SEHT group 57 % of the patients used three or more antihypertensive drugs. Even so, over 4/5 of the patients had poor control of blood pressure. In the MEHT group the situation was better, but still somewhat surprisingly only 40 % of the patients reached the target i.e. they had blood pressure clearly under 140/90 mmHg with treatment. Patients in the MEHT group had been treated as normal outpatients by local health care centers.

4.2.2 Healthy control subjects

Healthy age- and sex-matched control subjects were selected for each hypertensive patient from an other study population performed during the same period with similar HRV, BPV and BRS measurements methods (Laitinen et al.1998). All control subjects were previously carefully evaluated and found to be in good health and free of clinically apparent systemic diseases. None of them smoked or was taking any antihypertensive or cardiovascular medication.

Table 1. Clinical characteristics of the study subjects

	RVHT n = 14	CTR n = 14	SEHT n = 40	CTR n = 40	MEHT n = 30	CTR n = 30
Gender (male/female)	5/9	5/9	22/18	22/18	15/15	15/15
Age (years)	59.9 ± 4.1	60.2 ± 4.1	45.8 ± 2.1	46.3 ± 2.3	58.5 ± 1.2	55.9 ± 1.5
Height (cm)	166 ± 2	164 ± 2	171 ± 1.6	171 ± 1.7	167 ± 1.4	169 ± 1.7
Weight (kg)	71 ± 3	64 ± 3	81 ± 2.5‡	70 ± 2.0	77 ± 1.7†	68 ± 2.1
Body mass index (kg/m^2)	23.8 ± 1.5	23.1 ± 0.7	27.5 ± 0.7‡	23.9 ± 0.3	27.7 ± 0.6‡	24.0 ± 0
Office blood pressure						
Systolic (mmHg)	167 ± 7.9	151 ± 5.9	156 ± 3.9†	139 ± 3.2	141 ± 3.3	137 ± 3.7
Diastolic (mmHg)	97 ± 5.0	86 ± 2.8	98 ± 2.1‡	84 ± 1.8	89 ± 1.7*	83 ± 1.6
Ambulatory blood pressure						
Systolic (mmHg)	151 ± 22		142 ± 15		132 ± 12	
Diastolic (mmHg)	92 ± 16		89 ± 11		84 ± 7	

*P< 0.05, †P< 0.01, ‡ P< 0.001 for hypertensive vs. controls. Values are mean ± SEM or numbers of cases. In hypertensive patients, blood pressure is measured under antihypertensive treatment.

Table 2. Antihypertensive medications

	RVHT n = 14	SEHT n = 40	MEHT n = 30
Number of antihypertensive medication used before the study (%)			
0	0	10	7
1	14	6	83
2	50	27	10
=3	36	57	0
Antihypertensive drugs (%)			
ß-blockers	57	78	17
ACE-inhibitors	84	65	67
Ca-blockers	50	65	13
Diuretics	43	30	7

4.3 Methods

4.3.1 Office blood pressure

In hypertensive patients, the office blood pressure was measured from non-dominant arm, in a sitting position, just before ambulatory blood pressure measurements were started, with a standard sphygmomanometer. The bladder width was 40 % of the arm's circumference and the bladder length was 80 % of the length of the upper arm or as near as possible. Three measurements were performed after a 10-minute rest and a mean of three consecutive blood pressure readings was used for the analyses.

4.3.2 Ambulatory blood pressure

The 24-hour ambulatory blood pressure was monitored from non-dominant arm by using a previously validated non-invasive recorder (Space Labs 90207, Redmond, Washington, USA), which is a device that uses an oscillometic method. The recording day was a typical weekday, and subjects were encouraged to pursue their typical activities and to relax their arm at their side when the cuff inflated. Blood pressure measurements were performed automatically at 15 min intervals during the daytime period and 30 min intervals during the night-time period. The accuracy of the recorder was checked by performing three simultaneous readings with a standard mercury manometer both at the beginning and at the end of the monitoring session (O'Brien et al. 1990, Padfield et al. 1991). The difference between measurements was allowed to be ± 5mmHg. Patients kept detailed diaries of their daily activities and medications. All recordings obtained in this study had a success rate of at least 80 % for all readings.

4.3.3 Echocardiography

Pulsed waved M-mode echocardiography and Doppler were recorded with a 2.25 MHz transducer (Aloka 870, Aloka Ltd., Tokyo, Japan). Parasternal view was used to find an adequate site for M-mode recordings. All tracings were printed on paper with 100 mm/s and were analysed with custom-built software (Vanninen et al. 1992). Measurements from three cardiac cycles were digitised and averaged (MM1201, Summagraphics Co., Fairfield, Conneticut; resolution 0.1 mm). M-mode were measured according to the recommendations of The American Society of Echocardiography (Shan et al. 1978). Left ventricular mass (LVM) was calculated according to the formula: LVM = 1.04[(LV end-diastolic diameter^3 +

septum^3 + posterior wall^3)- LV end-diastolic diameter^3] - 13.6 g (Devereux et al. 1977) and LVM index was calculated by dividing the LVM by body surface area (g/m^2) (Shan et al. 1978). Doppler signals were recorded from apical four-chamber view placing sample volume parallel to maximal mitral inflow. Doppler waveforms were digitised, and the ratio of peak early to peak late flow velocities (E/A-ratio) was calculated to assess the left ventricular diastolic function (Vanninen et al. 1992).

4.3.4 Cardiovascular autonomic nervous function tests

HRV, short-term BPV and BRS were measured before noon, between 0800h and 1200h (Laitinen et al.) in a quiet, dim room. Before the tests begun, there was a 15 - 30 min resting period.

4.3.4.1 Heart rate variability and short-term blood pressure variability

During the HRV and short-term BPV measurements subjects lay in the supine position and breathed with 0.2 Hz frequency according to a paced signal for ten minutes with their normal tidal volume. Continuous ECG signal and finger tip blood pressure were recorded. The continuous non-invasive blood pressure recording was performed using the plethysmographic finger-cuff method (Finapres, Ohmeda, Inc., Englewood, Co., USA). The cuff was placed around the middle finger of the right hand and the right arm was kept at the level of the heart. Self-adjustment of Finapres was performed immediately before the recordings and then turned off. ECG- and blood pressure signals were simultaneously analogue-to-digital converted with a temporal resolution of 200 Hz/channel with an amplitude resolution of 12 bits and transferred into a computer and analysed with a menu-driven software package (CAFTS, Medikro Oy, Kuopio, Finland, Tahvanainen et al. 1992). All recordings were interactively reviewed.

HRV was obtained from stationary regions of sinus rhythm containing at least 250 beats. Only signals free from ectopic beats and artefacts were accepted. SDNN and RMSSD were used as time domain measures of RR-interval variability. The autoregressive model (model order 14) was used to calculate the frequency domain estimate of HRV. Total power (0 - 0.5 Hz) and powers of high-frequency (HF, 0.15 - 0.4 Hz) and low-frequency bands (LF, 0.07 - 0.15 Hz), were calculated. Powers were determined in absolute (ms^2) and in normalised units (n.u.). Normalised units were calculated by dividing the absolute power of a given component

by the LF power plus HF power and multiplying by 100. LF/HF-ratio was calculated.

Mean values of systolic and diastolic blood pressure and a power spectral analysis of short-term systolic and diastolic BPV were obtained from the same steady-state recordings as HRV. The powers of each frequency bands were calculated as an integral under the respective power spectral density and expressed in absolute units ($mmHg^2$) and in normalised units (n.u.) which were calculated in a similar way as described above and divided in similar frequency bands.

4.3.4.2 Baroreflex sensitivity

BRS was evaluated in according with a modification of the method first described by Smyth (Smyth et al. 1969). A bolus of 150 μg phenylephrine was given as an intravenous injection into the left antecubital vein. Continuous ECG and non-invasive arterial pressure were recorded from the right middle finger (Finapres, Ohmeda, Inc., Englewood, Co, USA). All data acquisition and analysis were performed with a menu driven software package (CAFTS, Medikro Oy, Kuopio, Finland, Tahvanainen et al. 1992). During the period of increasing blood pressure, beat-to-beat values of RR-interval were plotted against the SAP values [RRI(i+1) vs. SAP (i)]. The slope of the regression line and corresponding correlation coefficient were calculated. Three acceptible tests with correlation coefficients of at least 0.8 or statistically significant correlation ($p < 0.05$) were required. The mean value of the accepted slopes was considered to be the index of baroreflex sensitivity (La Rovere et al. 1988, Hartikainen et al. 1995).

4.3.5 Natriuretic peptides

The blood samples for measurements of NT-proANP and BNP were directly drawn into chilled tubes containing 1.5 mg K_2-EDTA/ml blood, after 30 minutes rest at supine position. Plasma was immediately separated by centrifugation and then frozen and stored at -70°C. Samples were analysed later by the Department of Physiology at the University of Oulu. NT-proANP was assayed directly from 25 μl of unextracted plasma with the protocol that has been previously described by Vuolteenaho and co-workers (Vuolteenaho et al. 1985). BNP were then extracted from plasma using SepPak C_{18} cartridges. BNP assay was performed with the same protocol as ANP. The sensitivities of the NT-proANP and BNP assays were under 30 pmol/l and under 0.5 pmol/l plasma, respectively. The within- and between-assay coefficients of variation in each assay were < 10 % and < 15 %, respectively. The following

plasma levels were detected in healthy adults (20 - 55 years): NT-proANP 110 - 600 pmol/l (mean ± 2SD, n = 411), BNP 2.0 - 10.5 pmol/l (mean ± 2SD, n = 40).

4.3.6 Statistical methods

Data were analysed by SPSS statistical programs (versions 7.0 - 9.0 SPSS Inc., Chicago, IL, USA). In Studies I - III the data were based on matched pairs (patient groups versus control groups), a paired t-test was used for group comparisons. The normal distribution of the variables were tested with the Kolmogorov-Smirnov test. If the variable did not show normal distribution, a natural logarithmic transformation was performed. Analysis of variance (ANOVA) with appropriate covariates was used to compare the different hypertension groups. Univariate linear correlations were calculated using Pearson's correlation analysis. In addition, significant determinants of HRV and BRS were assessed using multiple-regression analysis in Studies I and III. Receiver operating characteristics (ROC-curve) was used in Study IV. A value of $p < 0.05$ was considered statistically significant. The results are mainly expressed as mean ± SEM.

4.3.7 Approval of the Ethics Committee

The study protocol was approved by the Ethics Committee of the Kuopio University Hospital. Written informed consent was obtained from all subjects before they entered the study.

5 RESULTS

5.1 Clinical characteristics of hypertensive subjects

Patients in the RVHT and MEHT group were older (p = 0.002 for both) than patients in the SEHT group (Table 1). SEHT and MEHT patients were overweight, but only slightly, while patients in the RVHT group were on average normal weight. Seventeen % of hypertensive patients were smokers, 29 % in the RVHT, 15 % in the SEHT and 13 % in the MEHT group (differences between groups ns.). All control subjects were in normal weight and they were also non-smokers.

In the RVHT group seven (50 %) patients had high and seven (50 %) patients had moderate clinical suspicion of renovascular hypertension. In the SEHT group only one (2.5 %) patient had had originally high suspicion of renovascular hypertension and the rest (97.5 %) had primarily a moderate suspicion of renovascular hypertension. All patients in the MEHT group had low suspicion (mild hypertension and absence of clinical clues) of renovascular hypertension.

Patients in the RVHT group had shortest duration of hypertension (5 ± 1.8 years) when compared to the SEHT and MEHT groups (11 ± 1.3 and 12 ± 1.6 years; p = 0.02 and p = 0.007, respectively). The average duration of hypertension did not differ between the SEHT and MEHT groups.

A positive family history of hypertension (firstdegree relatives) was found in 64 % of patients in the RVHT group, 78 % in the SEHT group and 70 % in the MEHT group. In the RVHT groups ten patients had other disease such as mild stable coronary artery disease (n = 3), previous non-disabling stroke (n = 1) or other diseases (n = 6). In the SEHT group ten patients had co-existing disease such as mild stable coronary artery disease (n = 4), previous non-disabling stroke (n = 1) or other diseases (n = 5). In the MEHT group two patients had stable mild coronary artery disease and four other diseases.

Blood pressure was poorly controlled in the RVHT and SEHT groups. Although there was a tendency for higher office blood pressure in the RVHT group than in the control group, the difference was not statistically significant (Table 1, p = 0.057). Comparison between hypertensive groups (age, gender and BMI as covariates) showed that patients in the RVHT and SEHT groups had higher systolic office blood pressure than patients in the MEHT group (p = 0.002 for both). RVHT and SEHT groups did not differ from each other in this respect. In patients with RVHT, 24-hour systolic blood pressure was higher than in patients with SEHT

and MEHT (p = 0.015 and p = 0.007, respectively).

Baseline renin levels were higher in the RVHT group than in the SEHT group (p = 0.007). The difference between baseline levels and levels after the captopril challenge were greatest in the RVHT group, 1.6 µg/l/h vs. 50.7 µg/l/h (p = 0.003). Renin measurements were not performed in patients with MEHT because all patients in that group had low clinical suspicion of renovascular hypertension. In the RVHT group 17 %, in the SEHT group 25 % and in the MEHT group 20 % had serum creatinine > 120 mmol/l (differences between groups ns.).

Table 3 shows the result of the echocardiography and doppler. Left ventricular hypertrophy (> 125 g/m^2), defined by echocardiography, was present in 55 % in the RVHT group and in 66 % in the SEHT and MEHT groups (differences between groups ns.).

Table 3. Echocardiography

	RVHT n = 14	SEHT n = 40	MEHT n = 30
Left atrium (mm)	38.5 ± 2.0	36.3 ± 0.9	38.2 ± 0.7
Intervetricular septun (mm)	17.8 ± 1.1*†	12.5 ± 0.4	12.1 ± 0.5
Left ventricular posterior wall (mm)	14.3 ± 0.8*†	11.2 ± 0.3	10.9 ± 0.4
Fractional shortening (%)	35 ± 3.7	37 ± 1.0	40 ± 1.6
E/A-ratio	1.1	1.2	1.1
Left ventricular mass index (g/m^2)	170 ± 16	156 ± 10	151 ± 10

Values are mean ± SEM or percentages. *P< 0.05 RVHT vs SEHT and, †P< 0.01 RVHT vs MEHT.

5.2 Heart rate variability (Study I)

5.2.1 Comparison of heart rate variability between the hypertensive groups and the control groups

The RVHT group remained relatively small, and it was therefore inadequate for statistical analysis for time and frequency domain analysis of HRV. In time domain analysis, the SEHT group had a lower SDNN (p < 0.001) and RMSSD (p = 0.017) than the control group (28 ± 3 vs 45 ± 4 ms and 21 ± 3 vs. 40 ± 4 ms, respectively) (Table 4). In frequency domain analysis, the SEHT group had lower total, HF and LF powers of HRV than the control group but in

normalised units the significant difference was found only in HF power. In the MEHT group there was a trend to reduced total power when compared to the control subjects (p = 0.053) (Table 4).

5.2.2 Comparison of heart rate variability between the hypertensive groups

Comparison of the SEHT and MEHT groups (age, sex and body mass index as covariates) showed that SDNN, total and LF power were lower (p = 0.01, p = 0.009, p = 0.002, respectively) and HF power (p = 0.006) was higher in the SEHT than in the MEHT group. RMSSD and normalised unit of LF and HF did not differ between the SEHT and MEHT groups (Table 4).

Table 4. Heart rate variability in the hypertensive and healthy control groups (Study I)

	SEHT n = 34	CTR n = 34	p	MEHT n = 29	CTR n = 29	p
Time-domain measurement of heart rate variability						
RR-interval (ms)	859 ± 29	997 ± 19	< 0.001	937 ± 21	1005 ± 28	0.018
LnSDNN (ms)	3.19 ± 0.1	3.71 ± 0.1	< 0.001	3.24 ± 0.1	3.49 ± 0.1	ns
LnRMSSD (ms)	2.96 ± 0.2	3.53 ± 0.1	0.017	2.85 ± 0.1	3.07 ± 0.1	ns
Frequency-domain measurement of heart rate variability						
LnTotal power (ms^2)	6.17 ± 0.2	7.24 ± 0.2	< 0.001	6.27 ± 0.2	6.84 ± 0.2	ns
LnLF power (ms^2)	4.09 ± 0.2	5.11 ± 0.2	< 0.001	4.21 ± 0.2	4.64 ± 0.3	ns
LnHF power (ms^2)	4.88 ± 0.3	6.27 ± 0.2	0.006	4.92 ± 0.2	5.27 ± 0.3	ns
LF power (n.u)	33 ± 3	27 ± 2	ns	35 ± 3	35 ± 3	ns
HF power (n.u.)	67 ± 3	73 ± 3	< 0.001	65 ± 3	65 ± 5	ns
LF/HF-ratio (%)	64 ± 6	43 ± 1	ns	67 ± 1	81 ± 2	ns

Values are mean ± SEM or percentages.

5.1.3 Determinants of heart rate variability in the hypertensive groups

To study determinants of HRV in patients with essential hypertension, a stepwise regression model included age, gender, RR-interval, use of ß-blockers and separately systolic or diastolic finger blood pressure and HRV measures, as a dependent variable, was used. Several separate analyses revealed that age and RR-interval are the two most important determinants of HRV

in patients with SEHT (r^2 values were between 0.558 and 0.692). In MEHT RR-interval was the most significant determinant of HRV.

5.3 Short-term blood pressure variability (Study II)

5.3.1 Comparison of short-term blood pressure variability between the hypertensive and the control groups

In the RVHT group there was a tendency of total power diastolic BPV to be lower than in the control group ($p = 0.094$, Table 5). LF power of systolic and diastolic BPV were lower in the RVHT group when compared to the control group ($p = 0.004$ and $p = 0.003$, respectively), but HF power of systolic and diastolic BPV of the RVHT group and controls did not differ. In the SEHT group total power of systolic BPV tended to be higher, and diastolic BPV was higher when compared to the control group ($p = 0.077$ and $p = 0.044$, respectively). In absolute values, LF and HF powers of both systolic and diastolic BPV were higher than in the controls, but the difference was not statistically significant. In the MEHT group, total power of systolic and diastolic BPV did not differ from control group. On the other hand, LF power of both systolic and diastolic BPV ($p = 0.028$ and $p = 0.003$, respectively) and HF power of diastolic BPV were lower in the MEHT group ($p = 0.020$) when compared to the control group.

Table 5. Short-term variability of blood pressure in the hypertensive and healthy control groups (Study II)

	RVHT n = 10	CTR n = 10	p	SEHT n = 34	CTR n = 34	p	MEHT n = 29	CTR n = 29	p
Variability of systolic blood pressure ($mmHg^2$)									
Total Power	18.7 ± 4.0	25.7 ± 3.8	ns	36.7 ± 5.1	25.2 ± 3.6	ns	30.8 ± 5.4	25.8 ± 2.5	ns
LF	1.4 ± 0.5	3.4 ± 0.7	0.004	3.8 ± 0.6	3.0 ± 0.5	ns	3.0 ± 0.5	3.9 ± 0.7	0.028
HF	6.8 ± 2.8	6.3 ± 2.0	ns	5.1 ± 0.9	3.7 ± 0.4	ns	4.0 ± 0.6	3.7 ± 0.4	ns
Variability of diastolic blood pressure ($mmHg^2$)									
Total Power	5.0 ± 0.3	6.2 ± 0.2	ns	8.5 ± 0.8	6.5 ± 0.9	0.044	5.9 ± 0.2	6.3 ± 0.1	ns
LF	0.4 ± 0.3	1.1 ± 0.2	0.003	1.4 ± 0.2	1.1 ± 0.2	ns	0.9 ± 0.2	1.3 ± 0.1	0.003
HF	0.7 ± 0.5	2.1 ± 0.4	ns	1.0 ± 0.2	0.7 ± 0.1	ns	0.4 ± 0.2	0.6 ± 0.1	0.020

Values are mean ± SEM.

5.3.2 Comparison of short-term blood pressure variability between the hypertensive groups

Systolic BPV did not differ between hypertensive groups (Table 5). Total power and LF power of diastolic BPV ($p = 0.043$ and $p = 0.039$, respectively) were lower in the RVHT group compared to the SEHT group, but no significant differences were found between the RVHT group and the MEHT group. Total power of diastolic BPV ($p = 0.030$) in the SEHT group was higher and HF power of systolic BPV ($p = 0.058$) also tended to be higher than in the MEHT group.

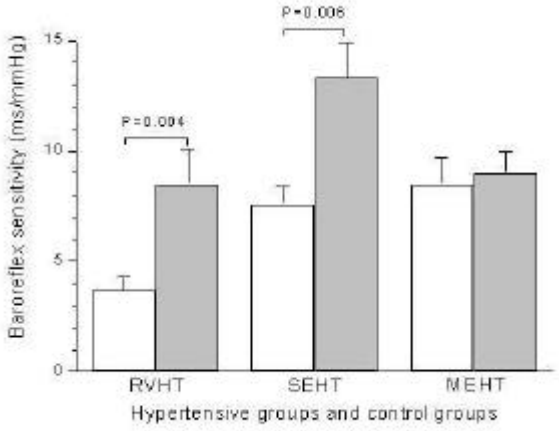
5.4 Baroreflex sensitivity (Study III)

5.4.1 Comparison of baroreflex sensitivity between the hypertensive and the control groups

BRS was significantly reduced in the RVHT group (3.7 ± 0.6 vs. 8.5 ± 1.6 ms/mmHg, $p = 0.004$) and in the SEHT group (7.6 ± 0.8 vs. 13.3 ± 1.6 ms/mmHg, $p = 0.006$) when compared to the corresponding control groups (Figures 4 and 5). No difference was detected in BRS between the MEHT group and the corresponding control group (8.5 ± 1.2 vs. 9.0 ± 1.0 ms/mmHg). Compared to the healthy controls, the average reduction of BRS was 57 % in the

RVHT group, 43 % in the SEHT group and only 6 % in the MEHT group.

Figure 4. Baroreflex sensitivity in patients (open bars) with renovascular hypertension, severe



essential hypertension and mild essential hypertension and healthy control subjects (shaded bars).

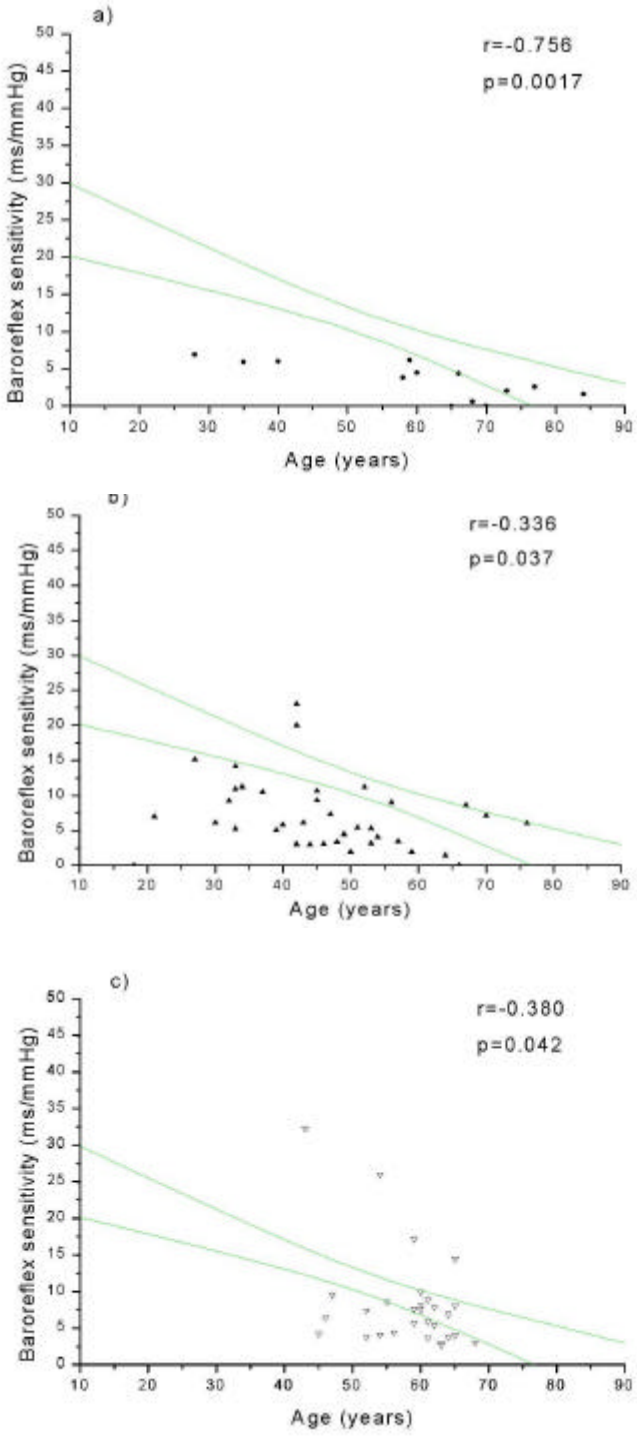


Figure 5. The relationship between BRS and age for patients with RVHT (a), SEHT (b) and MEHT (c) and controls. The lined area indicates the 95 % confidence interval for the regression line of the controls.

5.4.2 Comparison of baroreflex sensitivity between the hypertensive groups

Comparison between all hypertensive groups (age, gender and BMI as covariates) showed that BRS was significantly lower in the RVHT group (ANOVA, $p = 0.008$) as well as in the SEHT group than in the MEHT group ($p = 0.016$). BRS in the RVHT and SEHT groups did not differ significantly from each other. The difference between the RVHT and MEHT groups as well as between the SEHT and MEHT groups remained statistically significant even after the adjustment for the use of β-blockers during the measurements ($p = 0.021$ and $p = 0.029$, respectively).

The result remained the same when RVHT and SEHT subgroups were studied, matched either according to age ($n = 9$, ± 4 years) or 24–hour mean blood pressure ($n = 14$, ± 10 mmHg). On the other hand, BRS was significantly lower in the RVHT ($p = 0.008$) and SEHT ($p = 0.016$) groups than in the MEHT group.

5.4.3 Determinants of baroreflex sensitivity in the hypertensive groups

In the RVHT group there was an inverse correlation between BRS and age, and positive correlation between BRS and SDNN, RMSSD, LF power and HF powers. In the SEHT group, BRS was associated inversely with age, systolic office blood pressure, and positively with SDNN, total power and LF power. In the MEHT group BRS did not correlate significantly with any of the parameters.

After the hypertensive groups were combined, the association of age, gender, RR-interval, HRV parameters (SDNN, RMSSD, total power, LF power, HF power and LF/HF-ratio), systolic and diastolic office blood pressure (included separately in two models) and hypertensive group on BRS was studied using stepwise multiple regression analysis. Total power and LF power and systolic office blood pressure turned out to be significant determinants of BRS (ß = -0.445, $p = 0.048$; ß = 0.862, $p < 0.001$; ß = -0.328, $p = 0.001$, respectively). LF power of HRV, hypertensive group, age and gender were the significant determinants when diastolic office blood pressure was used in the model (ß = 0.404, $p < 0.001$; ß = 0.274, $p = 0.004$; ß = -0.274, $p = 0.008$; ß = -0.200, $p = 0.030$, respectively).

5.5 Natriuretic peptides (Study IV)

5.5.1 Comparison of natriuretic peptides between the hypertensive groups

The concentrations of NT-proANP were higher in patients with RVHT than in patients with SEHT or MEHT (593 ± 80 vs. 320 ± 33 pmol/l, $p < 0.001$ and 593 ± 80 vs. 356 ± 30 pmol/l, $p = 0.004$, respectively; Figure 6). The mean NT-proANP concentration between SEHT and MEHT groups did not differ from each other and were in fact within normal limits. When taking age, body mass index and systolic blood pressure into consideration NT-proANP was still higher in the RVHT group than in the MEHT group ($p = 0.049$). Fifty percent of patients in the RVHT group had elevated (> 600 pmol/l) NT-proANP levels, where as this was true only in 5 % in patients of SEHT group and 11 % of the patients in the MEHT group.

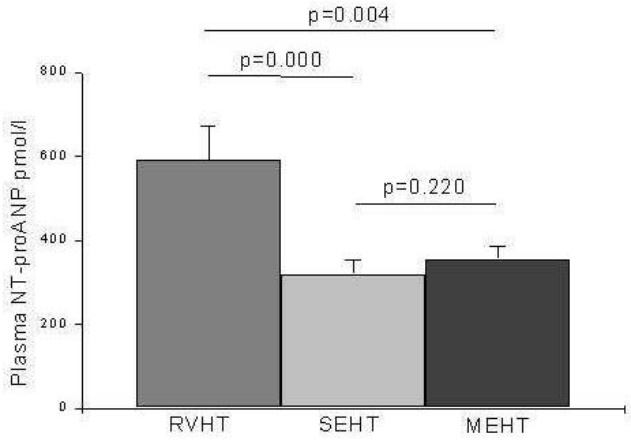


Figure 6. Plasma NT-proANP concentrations in the hypertensive groups. Values are mean ± SEM.

The plasma concentrations of BNP were also higher in the RVHT (25.0 ± 9.3 pmol/l) when compared to the SEHT (4.7 ± 0.6 pmol/l, $p < 0.001$) and MEHT (7.0 ± 1.0 pmol/l, $p = 0.006$) groups (Figure 7). The BNP concentration did not differ between SEHT and MEHT groups and were within normal limits in both groups. After adjusting for age, body mass index and systolic blood pressure, BNP was still significantly higher in the RVHT group than in the SEHT and MEHT groups ($p = 0.008$ and $p = 0.012$, respectively). In the RVHT group 50 %, in the SEHT group 7 % and in the MEHT group 24 % showed elevated plasma BNP levels (> 10.5 pmol/l).

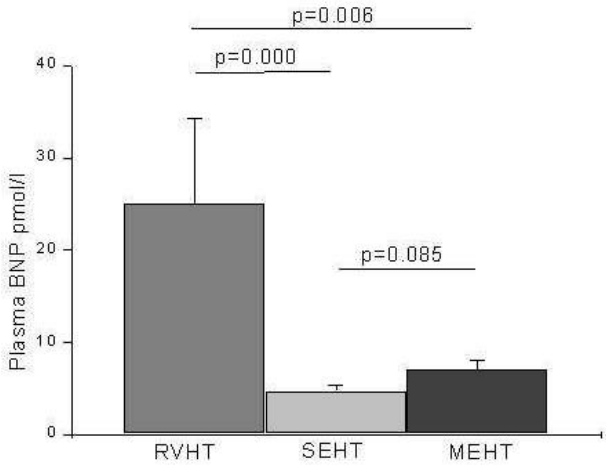


Figure 7. Plasma BNP concentrations in the hypertensive groups. Values are mean ± SEM.

5.5.2 Determinants of NT-proANP and BNP in the hypertensive groups

When all hypertensive groups were pooled together, age, serum creatinine and E/A-ratio were significant independent determinants of NT-proANP concentration, whereas age and systolic office blood pressure were independent determinants of BNP concentration. When the hypertensive groups were evaluated separately, in the RVHT group serum creatinine correlated independently with NT-proANP and BNP. In the SEHT group NT-proANP and BNP correlated with age. No significant independent determinants of natriuretic peptides were found in the MEHT group.

5.5.3 NT-proANP and BNP in the detection of renovascular hypertension

All hypertensive patients were evaluated by ROC-curve analysis in order to study the possible diagnostic accuracy of NT-proANP and BNP measurements to identify patients with RVHT and define the optimal cut-off levels of these peptides in the diagnosis of RVHT. The area under the ROC curve was 0.793 for NT-proANP. The discriminative cut-off value that best separated RVHT patients from patients with essential hypertension was 530 pmol/l for NT-proANP giving a sensitivity of 67 % at the specificity level of 86 %. The area under the ROC curve for BNP was 0.782. For BNP the best separation of RVHT from essential hypertension was 9.8 pmol/l, resulting in a sensitivity of 58 % at the specificity level of 90 %. When a high clinical suspicion of renovascular hypertension was added to the model, the specificity improved up to 97.5 %, but this had no effect on the sensitivity.

6 DISCUSSION

6.1 Patients and study design

The present study was based on the evaluation of three clinically distinctly different hypertensive patient groups in a cross-sectional setting. It allowed that patient groups were carefully selected and examined in detail. In addition, assessment of cardiovascular autonomic nervous function, baroreflex sensitivity and measurements of natriuretic peptides occurred under well-controlled, constant laboratory conditions, which provides physiologically and clinically valid information (Task Force 1996) and also allows a comprehensive and reliable data analysis.

The study design was based on highly selected homogenous patient groups. Therefore a relatively small number of patients were studied on each hypertensive group, particularly the RVHT group. Fifty-four hypertensive patients who fulfilled the clinical criteria of suspected secondary hypertension were studied. Among these carefully pre-selected hypertensive patients, 26 % had RVHT, indicating strict and accurate pre-selection criteria. SEHT and MEHT patients were selected absolutely separately and these groups represented clinically totally different entities of patients with essential hypertension.

6.2 General methodological considerations

Most of the data were collected in laboratory settings, which can be considered an artificial environment that may be somewhat stressful. It therefore is also a limitation of the study. It can be argued that only momentary quantification of HRV, BPV and BRS was obtained, without information on daily life. It is common to measure HRV and sometimes also BPV and BRS from ambulatory ECG- and AMBP-recordings lasting from 18 to 24 hours. Although such recordings may give prognostically important data in patients with heart disease (Kleiger et al. 1987, Woo et al. 1992, Mäntysaari et al. 1995, La Rovere et al. 1998), it is difficult to estimate the contribution of sympathetic and parasympathetic activity from non-stationary long-term recordings during which the physical and mental activity of the patients cannot be standardized. Some of the discrepancies between the observations of this study and previous studies may be due to differences in the study population, the length of recordings and the physiological conditions during recordings. In present study, duration of the ECG- and blood pressure recordings for HRV and BPV were relatively short, but the measurements were

carried out under physiologically strictly controlled conditions at rest using a standardized protocol and paced breathing (according to auditory signals). In addition, hypertensive patients were compared to healthy controls, separately matched by age and sex for each patient. It can also be argued that BRS, which was obtained with the phenylephrine method, gives only momentary quantification of BRS. Other techniques (sequence techniques, alfa-coefficient) that can analyze spontaneous blood pressure and heart rate fluctuation allow BRS measurement in real life conditions and there is no need for external stimulus (Robbe et al. 1987, Airaksinen et al. 1997, Persson et al. 2001). These techniques may give deeper and richer information about BRS and autonomic nervous dysfunction in daily-life and may improve the evaluation of hypertensive patients.

6.3 The use of antihypertensive medication during the study

One confounding fact of the study design was that some patients were using antihypertensive medications, which increase statistical noise. Patient selection is always difficult when there are several medications and all medication cannot be dropped out. When the study was planned it was considered neither clinically nor ethically possibly to discontinue all antihypertensive medication in patients who had very high blood pressure. However, even those patients were taking markedly reduced medication, i.e. ¼ of their normal ß-blocker dose. Some of the patients possibly had a wash-out from drug therapy that was too short. In any case, the majority of patients were free of all antihypertensive medication.

It is not very clear what is the effect of continuous use of β-blockers on autonomic nervous and natriuretic peptides in established hypertension. The use of β-blockers attenuates sympathetic activity. β-Blockers have been shown to increase HF component (Cook et al. 1991, Niemelä et al. 1994, Vesalainen et al. 1998, Vaile et al.1999) of HRV while LF component has remained unchanged or increased (Cook et al. 1991, Niemelä et al. 1994, Vaile et al. 1999). Lipophilic and hydrophilic β-blockers seems to increase cardiac vagal activity equally (Vaile et al.1999), but the dose-response of β-blockers on autonomic control is unknown. β-blockers could possible reduce BPV, but there are no studies dealing with that question. The effect of β-blockers on BRS in hypertensive patients have also yielded conflicting results (Ylitalo et al. 1999, Vesalainen et al. 1998). There are reports of increased BRS during β-blocker treatment in patients with mild hypertension (Vesalainen et al. 1998)

and in older patients with more severe hypertension (Ylitalo et al. 1999). The time when antihypertensive therapy is started might also be important. The effect of small β-blocker dose on ANP and BNP in hypertensive patients is not well known. According to a retrospective population based study β-blockers might increase ANP and BNP levels, whereas diuretics and ACE-inhibitors have no effect on these peptide concentrations (Lunchner et al. 1998). A small β-blocker dose was used equally in RVHT and SEHT groups (difference between groups ns). The use of β-blockers cannot explain the difference between the groups. In summary, it is unlikely that medication had contributed a significant bias to the study.

6.4 Heart rate variability (Study I)

Study I showed that hypertensive patients differing in severity of essential hypertension have as well differences in HRV. This study demonstrates that impairment of cardiac autonomic control is associated with the severity of essential hypertension. Cardiovascular autonomic control was decreased in the SEHT group, but not in MEHT group, when compared to age and sex-matched control subjects. In the SEHT group the decrease in HF power was slightly more pronounced (27 %) than the decrease in LF power indicating a trend towards sympathetic predominance.

There is no "golden standard" method for assessing autonomic dysfunction. HRV is one method, and when several parameters are used, it is likely that accuracy improves. In patients with borderline and mild essential untreated hypertension, there are several previous studies reporting altered sympatovagal balance characterized by increased sympathetic (Goldstein 1983, Esler et al.1989, Anderson 1989, Mancia et al. 1993, Julius, 1996) and reduced parasympathetic tone (Julius et al. 1971). However, the reported results in sustained essential hypertension have been inconsistent, showing changes in LF and HF in opposite directions (Furlan et al. 1990, Guzzetti et al. 1991, Rizzoni et al. 1991, Petretta et al. 1995, Liao et al. 1996, Huikuri et al. 1996, Picirillo et al. 1996, Kohara et al. 1996, Lazzeri et al. 1998, Singh et al. 1998). In most studies HRV measures have been lower in subjects with hypertension compared to normotensive controls (Guzzetti et al. 1988). Compared with Study I, the hypertensive patient populations have been more heterogeneous in these studies and patients with mild or more severe essential hypertension were not selected or examined separately.

In population based studies (Liao et al. 1996, Singh et al. 1998) on essential hypertension total HRV was reduced while the LF/HF -ratio remained unchanged. Liao and co-workers

(Liao et al. 1996) demonstrated that total HRV was lower in medically treated hypertensive patients compared to normotensive controls. In contrast to Study I, the data were obtained from free breathing. Moreover, the study population consisted of a heterogeneous group of treated and untreated patients, and patients with different severities of essential hypertension were not analysed separately. In the study of Singh et al., the average duration of hypertension was unknown (Singh et al. 1998). In both studies blood pressure levels were only mildly elevated, and were therefore closer to the MEHT group than the SEHT or RVHT groups (Liao et al. 1996, Singh et al. 1998). Huikuri et al. (Huikuri et al. 1996) showed that LF component of HRV and LF/HF-ratio were lower in middle-aged men with long-lasting (over 5 years) treated hypertension when compared with age-matched controls. In more severe, established essential hypertension in which left ventricular hypertrophy was also present, Petretta et al. (Petretta et al. 1995) demonstrated lower HRV measured from 24-hour recordings. They also found a negative correlation between LVM and HRV. In younger hypertensive patients with LVH Piccirillo et al. (Piccirillo et al. 1996) reported higher LF component and LF/HF-ratio measured from short-term recordings and found an inverse correlation between HRV and LVMi. In contrast, no correlations were found in Study I between HRV and LVMi in mild and severe essential hypertension.

Age and RR-interval were the most important determinants in the SEHT group, accounting for 13-58 % of the total variance of HRV. Similar finding has been found also in healthy subjects (Tsuij et al. 1996). In the MEHT group age was not a significant determinant, probably due to the narrow age range of this group.

Impairment of HRV is associated with the severity of essential hypertension and there may be clinically relevant subgroups that are characterised by differences in cardiac autonomic control. Difference in cardiac autonomic control between the SEHT and MEHT groups may reflect the heterogeneity of the clinical disease itself. RVHT group was too small for adequate statistical comparisons with the other groups.

6.5 Short-term blood pressure variability (Study II)

Hypertensive patients presenting with a different etiology and severity of hypertension also had differences in short-term BPV. Patients with RVHT had reduced BPV while, in spite of the same blood pressure level, patients with SEHT had increased BPV compared to control subjects. This may suggest that the etiology of hypertension has an important role in short-

term blood pressure regulation. Although the clinical significance of short-term BPV is not fully understood, blood pressure fluctuations are thought to reflect differences in cardiovascular modulation which can be due to central regulation (Parati et al. 1995).

Spectral analysis of short-term BPV has been previously used maily in animal studies (Hedman et al. 1992), in patients with orthostatic hypotension (Lipsitz et al. 1989) and in studies with healthy subjects (Veerman et al. 1994, Laitinen et al. 1998). There are only few studies in hypertensive patients (Parati et al. 1990, Lossius et al. 1993, Siché et al. 1995). There are no previous studies on short-term BPV with a similar study design like Study II, which included three clinically distinct hypertensive patient groups.

In the SEHT group we found no significant differences in LF components compared to control subjects at all, and surprisingly, LF power of both systolic and diastolic BPV were reduced in the MEHT group and also in the RVHT group when compared with control subjects. Our patients were highly selected. Previously generally unselected essential hypertension was studied, which could be one explanation for the differences in results.

LF power of short-term BPV has been observed to be higher in subjects with essential hypertension without LVH (Sichè et al. 1995) and in elderly hypertensive patients (Aono et al. 1996) compared with normotensive subjects. In Study II LVH was equally common in every hypertensive group, and only $^1/_3$ of patients were without LVH. Siché and co-workers (Siché et al. 1995) found LF power of systolic and diastolic BPV to be increased in hypertensive patients without LVH when compared with normotensive controls or hypertensive patients with LVH. In a subgroup of patients with essential hypertension without LVH, in the SEHT groups (n = 10) only HF power of systolic and diastolic BPV were increased when compared to controls ($p = 0.013$ and $p = 0.049$, respectively), while no difference in BPV was noted in the MEHT group (n = 10). There was no significant difference in LF power of BPV in either subgroup. In SEHT patients with LVH, it was not possible to find any difference in BPV when compared with controls. In the MEHT patients with LVH, LF powers of systolic and diastolic BPV were found to be reduced when compared with controls. These findings are similar to those of Siché et al. (Siché et al. 1995).

Under ambulatory conditions, Parati et al. (Parati et al. 1990) studied intra-arterial BPV. They observed no differences in BPV between borderline hypertensive and normotensive subjects. In this study the populations and measurement conditions were markedly different than in Study II, which makes direct comparisons difficult. In addition, differences in duration and severity of hypertension may also explain the results.

In healthy subjects, it has been shown that age, gender, body mass index, baroreflex sensitivity and blood pressure are independent determinants of BPV (Laitinen et al. 1998, Veerman et al. 1994). In addition, it has also been shown that total power and VLF and LF components of BPV represent sympathetic modulation of cardiovascular regulation (Laitinen et al. 1998). The LF component of BPV is believed to rise from changes in vascular tone and peripheral resistance (Parati et al. 1996). The HF component of BPV is considered to reflect more the mechanical effects of stroke volume, which in turn are due to changes in venous return and changes in respiration (Radaelli et al. 1994, Bernardi et al. 1997). These factors may differ between the hypertensive groups. In addition, BPV may reflect to differences in central regulation and mental and physical activities between hypertensive groups (Parati et al. 1997).

6.6 Baroreflex sensitivity (Study III)

In Study III BRS was markedly decreased in patients with RVHT and SEHT. These two patient groups had the same severity of hypertension but the etiology was different. BRS was similarly decreased in the RVHT and SEHT group and when compared with control subjects. Surprisingly, no significant difference in BRS between patients with mild, medically treated hypertension and healthy control subjects were found. The data support the idea that the decrease of BRS reflects the severity of the disease rather than the initial cause of the elevated blood pressure.

Earlier studies have reported that systolic and diastolic blood pressure correlate inversely with BRS in healthy normotensive subjects (Bristow et al. 1969, Gribbin et al. 1971, Laitinen et al. 1998, Dawson et al. 1999). Decreased BRS has been found in borderline hypertension (Takeshita et al. 1975, Eckberg et al. 1979, Watkins et al. 1996), and it has been suggested that impaired BRS plays a role in the development of hypertension (Takeshita et al. 1975, Yamada et al. 1988, Matsukawa et al. 1991, Parmer et al. 1992). BRS is decreased also in established hypertension when compared to normotensive control subjects (Parmer et al. 1992, Ylitalo et al. 1997, Pikkujämsä et al. 1998). Parmer et al. (Parmer et al. 1992) suggested that decreased BRS in essential hypertension may be partly genetically determined and may contribute to the pathogenesis of hypertension. Ylitalo et al. (Ylitalo et al. 1997) reported depressed BRS in drug-treated hypertensive patients and speculated that reduced BRS may be a primary feature related to hypertension itself rather than a consequence of hypertension. In

Study III, in contrast to the previous studies, no difference in BRS between patients with mild, drug-treated essential hypertension and healthy control subjects was found. This suggests that mild hypertension is not associated with depressed BRS, i.e. that decreased BRS is the consequence, not the cause, of elevated blood pressure. This is in line with experimental studies in which an increase in blood pressure is followed by resetting of baroreceptors towards higher pressure level and reduction of BRS (McCubbin et al. 1956, Moreira et al. 1992).

BRS was equally diminished in RVHT and SEHT groups. These groups had similar blood pressure levels, but the etiology of hypertension was different. This is in line with Mancia et al. (Mancia et al. 1982), who found that BRS was markedly decreased both in patients with renovascular and essential hypertension. These findings also support the concept that the decrease in BRS is predominantly secondary to elevated blood pressure.

In hypertensive patients, the most important determinants of BRS were systolic blood pressure and LF power of HRV, explaining about 45 % of the variability of BRS. The LF component of HRV represents predominantly cardiac sympathetic regulation and gives evidence that high blood pressure is related to increased sympathetic activity. LF power might partly depend on a resonance in the baroreflex loop. Strong dependency has been found between age, gender and BRS in healthy subjects (Laitinen et al. 1998, Dawson et al. 1999), and the same was found in hypertensive patients. The strong age-dependency is the main reason why the healthy controls of the SEHT groups had higher BRS than the other control groups. Systolic blood pressure correlated inversely with BRS in the SEHT group. The correlation seemed moderate also in the RVHT group, but probably because of small number of patients it did not reach statistical significance. These findings nontheless demonstrate that BRS impairment correlates with the severity of blood pressure.

Decreased BRS has been associated with increased risk of ventricular arrhythmia and sudden cardiac death in patients after myocardial infarction (Kleiger et al. 1987, Farrell et al. 1991, Hartikainen et al. 1994, La Rovere et al. 1998) and in patients with heart failure (Osterziel et al. 1995). Particularly, BRS ≤ 3 mm/mmHg seems to predict increased mortality (La Rovere et al. 1998). In this study, a markedly decreased BRS (≤ 3 mm/mmHg) was found in 46 % of patients with RVHT, in 13 % of patients with SEHT and in 13 % patients with MEHT. It is an interesting question whether low BRS predicts mortality also in hypertensive patients. To address this question, large follow-up studies are needed.

BRS in the MEHT group was not reduced compared with age- and sex-matched controls

subjects. In the MEHT group blood pressure was in general in good control. It is possible that vascular properties such as vascular compliance and viscoelastic characteristics were better preserved. Differences in lifestyle, for example in exercise habits, cannot be totally ruled out. Increased BRS after exercise has been observed in hypertensive subjects (Pagani et al. 1988, Somers et al. 1991). Even low intensity exercise training (50 % of maximal oxygen uptake) reduces heart rate and blood pressure and sympathetic activity to the heart and increases BRS.

6.7 Natriuretic peptides (Study IV)

In the Study IV, despite comparable office blood pressure, plasma NT-proANP and BNP concentrations were higher in patients with RVHT when compared to patients with SEHT. Interestingly, no difference was found in natriuretic peptide concentrations between the SEHT and MEHT groups, even though the SEHT group had significantly higher blood pressure.

In previous studies, considerable overlapping between hypertensive and normotensive subjects in plasma ANP (Saganella et al. 1985, Sugawara et al. 1985, MacDonald et al. 1986) and BNP concentrations (Buckley et al. 1993, Cheung et al. 1994, Tadeka et al. 1995) has been found. Similar finding, were also observed in Study IV. Still, it was somewhat surprising that mean NT-proANP and BNP concentrations did not differ between MEHT and SEHT groups despite the different blood pressure levels. Recently, a similar observation has also been made in mice (Holtwick et al. 2002). On the other hand, in patients with RVHT both NT-proANP and BNP concentrations were elevated. Thus, it seems that blood pressure level per se does not play a big role in release of natiruretic peptides. It can be suggested that secondary hypertension is associated with the elevation of NT-proANP and BNP.

In Study IV NT-proANP concentration was above the normal upper limit only in 5 % of patients in the SEHT group and 11 % of patients in the MEHT group, but in 67 % of patients with RVHT. Our finding that patients with RVHT have higher NT-proANP concentrations than patients with SEHT or MEHT is in line with Schreij et al., who also found increased concentrations of ANP in hypertensive patients with renal artery stenosis when compared to patients with essential hypertension (Schreij et al. 1996). In that study, however, there were differences in blood pressure between these two groups as well (Schreij et al.1996).

BNP concentration was above the normal upper limit in half of the patients in the RVHT group, in 7 % of patients in the SEHT group and 24 % of patients in MEHT group. There was even a tendency suggesting increased BNP concentration in the MEHT group ($p = 0.085$),

even though SEHT group had higher office blood pressure. However, despite comparable office blood pressure, higher mean BNP concentrations were found in patients with RVHT than in patients with SEHT.

Patients with RVHT differed from patients with essential hypertension in thickness of interventricular septum (IVS) and left ventricular posterior wall (LVPW) and in the E/A-ratio on echocardiography, which may explain the difference between the groups. In the RVHT group, the IVS, and LVPW were significantly higher, and there was also a tendency for greater left ventricular mass ($p = 0.076$) than in the SEHT group. Previously, in essential hypertension a positive correlation between ANP and BNP concentrations and LVH, LVMi, IVS and LVPW has been reported (Schreij et al. 1996, Nishikimi et al. 1996, Bettencourt et al. 1999), while in renovascular hypertension ANP concentration has been found to be similar in patients with or without LVH (Schreij et al. 1996). As the RVHT group was rather small, it is difficult to judge whether selection bias, either in Study IV or in Schreij's study (Schreij et al.1996), could explain this discrepancy concerning relation of peptide concentrations and LVH in renovascular hypertension.

The increased ANP levels is thought to be due to compensatory feedback mechanism in order to normalize elevated blood pressure and increase sodium excretion. In hypertension, left ventricular compliance decreases and leads to increased atrial work. This atrial stretch is a major stimulus for ANP release, and therefore hypertensive patients with LVH are considered to have higher ANP levels than those without LVH (Nishikimi et al. 1996). In Study IV, the echocardiography findings were almost identical between SEHT and MEHT groups, which may explain why NT-proANP and BNP concentrations were also similar.

NT-proANP and BNP are strongly age-dependent, and renal function and diet also contribute to ANP release (Buckley et al. 1993, Montorsi et al 1987, Yamaji et al. 1986, Rascher 1985). In Study IV both NT-proANP and BNP were age and renal function dependent. Differences in renin-angiotensin system, endothelin secretion and sympathetic nervous system are other pathophysiological mechanisms that may explain differences in our hypertensive groups (Brunner- La Rocca et al. 2001).

The diagnosis of RVHT is considered clinically challenging. Early treatment of stenosis may avoid life-long antihypertensive drug treatment and progressive renal failure. It is very important to detect those patients who are suspected to have RVHT. None of the non-invasive test have proved to be good enough for proper RVHT diagnosis, and angiography is still the gold standard for diagnosing RVHT.

In Study IV it was found that elevated plasma NT-proANP and BNP concentrations predict RVHT almost as well as a moderate-to-high clinical suspicion of renovascular hypertension (50 % vs. 58 %). The combination of these two improved specificity but did not improve sensitivity. Three quaters of patients with elevated NT-proANP concentrations and $^{2}/_{3}$ with elevated BNP concentrations were found to have RVHT. Thus, measurement of NT-proANP or BNP may have some value when evaluating patients with clinical suspicion of RVHT. The cut-off values that give best sensitivity and specificity are near normal upper limit of healthy adults but this should to be prospectively validated in a larger group of hypertensive patients.

7 SUMMARY AND CONCLUSIONS

The present study contributes to our knowledge about cardiovascular autonomic function and vasoactive peptides in different types of chronic hypertension. It shows that there are differences in HRV, short-term BPV, BRS, NT-proANP and BNP in clinically different types of established hypertension. This study also assessed the value of natriuretic peptide measurement in detecting RVHT. This study provides new information about differences in blood pressure regulation in patients with different types and severities of sustained hypertension.

First, the impairment of HRV in established essential hypertension is related to the severity of the disease. Blood pressure, age and heart rate were the most important determinants of HRV in hypertension. Second, RVHT is characterised by reduced BPV. Despite a similar blood pressure level, patients with SEHT showed increased BPV. Interestingly, in the MEHT group systolic BPV was increased, wheras diastolic BPV was reduced. When the hypertensive groups were compared with each other, in the SEHT group diastolic BPV was higher than in the RVHT and MEHT groups. The mechanisms and clinical significance of short-term BPV needs to be further studied and it remains to be shown whether BPV has clinical significance in hypertensive patients. Third, impairment of BRS reflects the severity of long-lasting hypertension rather than the initial pathophysiology leading to hypertension. Fourth, ANP and BNP are increased in RVHT, which may suggest that the etiology of hypertension has a significant influence on plasma NT-proANP and BNP levels. Measurement of plasma natriuretic peptides may be of value evaluating patients with clinical suspicion of RVHT. Finally, these data suggest there are important differences in control mechanisms in these clinically distinctly different hypertension groups.

The mechanisms responsible for altered reflexes differ among diseases and are not well understood. More work is needed to clarify the roles of HRV, BPV and BRS and other mechanisms causing autonomic dysfunction in hypertension and other diseases.

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