

**CARDIOVASCULAR AUTONOMIC  
AND HORMONAL DYSREGULATION  
IN ISCHEMIC STROKE WITH  
AN EMPHASIS ON SURVIVAL**

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AN EMPHASIS ON SURVIVAL**

Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium 8 of Oulu University Hospital, on October 21st, 2005, at 12 noon.

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## ***Abstract***

Ischemic stroke is associated with cardiovascular autonomic nervous system (ANS) disturbances, including reduced heart rate (HR) variability and acute phase neurohumoral activation with elevated stress hormone levels. The impact of HR variability and neurohumoral factors such as natriuretic peptides on the long-term survival of patients with ischemic stroke has not been studied previously. This study was designed to evaluate cardiovascular autonomic regulation in ischemic stroke patients by assessing HR dynamics and various neurohumoral factors. The values of the assessed variables in predicting mortality were evaluated.

HR variability assessments were performed in the acute phase of ischemic stroke and for a general elderly population. Various neurohumoral factors were also assessed in the acute phase of stroke. After follow-up, the survival of the subjects was assessed and the prognostic values of the measured factors were evaluated.

Stroke patients were found to have cardiovascular autonomic and hormonal disturbances manifested as reduced traditional time and frequency domain measures of HR variability, altered long-term HR dynamics and elevated levels of natriuretic peptides in the acute phase. Altered long-term HR dynamics in the acute phase of stroke predicted long-term mortality after stroke and cerebrovascular mortality in the general elderly population. Neuroendocrine activation involving elevated natriuretic peptide values that were associated with high cortisol and catecholamine levels was observed in the acute phase of ischemic stroke. Neurohumoral disturbance was prognostically unfavourable. The most powerful predictors of poststroke mortality were altered long-term HR dynamics and elevated levels of natriuretic peptides and cortisol, which predicted mortality independently of the conventional risk factors in multivariate analysis.

Prognostically unfavourable cardiovascular autonomic dysfunction with disturbances in the long-term behaviour of HR dynamics was found to be related to ischemic stroke. Neurohormonal activation with elevated natriuretic peptide and cortisol levels in the acute phase predicts long-term mortality after ischemic stroke.

*Keywords:* cerebral infarction, heart rate, mortality, natriuretic peptides



*Learn as if you were going to live forever. Live as if you were going to die tomorrow.*

*Mahatma Gandhi*



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## Abbreviations

$\alpha_1$	short-term scaling exponent
$\alpha_2$	intermediate-term scaling exponent
$\beta$	slope of the power-law relationship
ACTH	adrenocorticotrophic hormone
AMI	acute myocardial infarction
ANP	atrial natriuretic peptide
ANS	autonomic nervous system
ApEn	approximate entropy
AV	atrioventricular
BI	Barthel index
BNP	brain (B-type) natriuretic peptide
BP	blood pressure
CAD	coronary artery disease
CAN	central autonomic network
CI	confidence interval
CNS	central nervous system
DFA	detrended fluctuation analysis
DVN	dorsal vagal nucleus
ECG	electrocardiography
GCS	Glasgow Coma Scale
HF	high frequency
HR	heart rate
HPA	hypothalamus-pituitary-adrenal
IML	intermediolateral cell column
LF	low frequency
MRS	Modified Ranking Scale
MRI	magnetic resonance imaging
NA	nucleus ambiguus
NP	natriuretic peptide
NPRA	natriuretic peptide receptor type A

NTS	nucleus tractus solitarius
PAG	periaqueductal gray
PFC	prefrontal cortex
PNS	parasympathetic nervous system
PVN	periventricular nucleus
RAS	renin-angiotensin system
RR	risk ratio
RR interval	R-peak-to-R-peak interval
SA	sinoatrial
SD	standard deviation
SD1	instantaneous beat-to-beat RR interval variability
SD2	long-term continuous RR interval variability
SDNN	standard deviation of all RR intervals
SNS	sympathetic nervous system
SSS	Scandinavian Stroke Scale
ULF	ultra low frequency
VLF	very low frequency

## **List of original publications**

This thesis is based on the following five publications, which are cited in the text using the Roman numerals I-V.

- I Korpelainen JT, Sotaniemi KA, Mäkikallio AM, Huikuri HV, Myllylä VV (1999) Dynamic behavior of heart rate in ischemic stroke. *Stroke* 30:1008-13.
- II Mäkikallio TH, Huikuri HV, Mäkikallio AM, Sourander LB, Mitrani RD, Castellanos A, Myerburg RJ (2001) Prediction of sudden cardiac death by fractal analysis of heart rate variability in elderly subjects. *J Am Coll Cardiol* 37:1395-402.
- III Mäkikallio AM, Mäkikallio TH, Korpelainen JT, Sotaniemi KA, Huikuri HV, Myllylä VV (2004) Heart rate dynamics predict poststroke mortality. *Neurology* 62:1822-6.
- IV Mäkikallio AM, Mäkikallio TH, Korpelainen JT, Vuolteenaho O, Tapanainen J, Ylitalo K, Sotaniemi KA, Huikuri HV, Myllylä VV (2005) Natriuretic peptides and mortality after stroke. *Stroke* 36:1016-1020.
- V Mäkikallio AM, Korpelainen JT, Mäkikallio TH, Vuolteenaho O, Sotaniemi KA, Huikuri HV, Myllylä VV. Neurohormonal activation in ischemic stroke. Effects of acute phase disturbances on long-term mortality. Submitted.



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# 1 Introduction

In Finland, where brain infarction is the third most common cause of death, about 14 000 persons suffer an ischemic stroke each year (Statistics Finland 2004). About 5 to 20% of ischemic stroke patients die either during the acute phase or within the first year (Petty *et al.* 1998, Petty *et al.* 2000, Pajunen *et al.* 2005). The annual direct and indirect costs of stroke are 800 million euros, comprising 6.1% of our health care budget (Fogelholm *et al.* 2001). In addition to the economic burden, the social and psychological effects of stroke are immeasurable, as many of the survivors remain disabled for the rest of their lives. Accurate information about the survival after stroke is important to the patient and the family and helps the stroke team to target the preventive treatment effectively and to balance the potential risks and benefits of the treatment options, and it also aids in making decisions on the allocation of limited resources. In addition to the traditional predictors of mortality after stroke, such as high age, male sex, stroke subtype, comorbidity and neurological symptom severity (Petty *et al.* 1998, Hankey *et al.* 2000, Kernan *et al.* 2000, Yokota *et al.* 2004), more practical and reliable prognostic measures are needed.

The autonomic nervous system (ANS) is responsible for the extrinsic regulation of cardiac muscle, smooth muscle and all glandular secretions. It provides control over the visceral functions critical to homeostasis, mostly independently of volitional activity (Appenzeller 1990, Adams *et al.* 1997, Ravits 1997). The central nervous system (CNS), through its modulation of autonomic activity, plays an important role in maintaining homeostasis in the cardiovascular system and in integrating cardiovascular responses with the constantly changing internal and external circumstances (Benarroh 1993). Central disturbances, such as brain infarction, may lead to profound alterations in cardiac or vascular control manifested as cardiac arrhythmias, myocardial necrosis, hypertension and lability of arterial pressure (Talman 1997). These cardiovascular autonomic disturbances may predispose patients to potentially fatal complications and thus worsen their long-term survival (Drislane & Samuels 1990, Ropper 1997).

Several new methods for the assessment of cardiovascular ANS functions have emerged over the past decade (Huikuri *et al.* 1996, Huikuri *et al.* 1998, Perkiömäki *et al.* 2005). Non-invasive and easily performed assessment of heart rate (HR) variability has been used widely to evaluate the cardioautonomic regulation in patients with various

cardiac diseases. The prognostic value of HR variability measures and especially the non-linear HR variability methods in cardiac patients has been established (Mäkikallio *et al.* 1999, Huikuri *et al.* 2000). HR variability is also known to be reduced in ischemic stroke patients (Korpelainen *et al.* 1996a, Korpelainen *et al.* 1996b, Naver *et al.* 1996, Orlandi *et al.* 2000, Phillips *et al.* 2000, Meglic *et al.* 2001), but the prognostic value of these measures in stroke patients has not been studied previously. Non-linear HR variability measures have not been used before to assess the ANS functions of ischemic stroke patients.

Natriuretic peptides (NP) are cardiac vasoactive peptide hormones that also function as neuromodulators in the ANS (Floras 1990, Brunner-LaRocca 2001, Herring *et al.* 2001, Thomas & Woods 2003). They have become valuable for the rapid diagnosis of heart failure (Maisel *et al.* 2002, Doust *et al.* 2004) and predict mortality after acute coronary syndromes (Morrow *et al.* 2003, Galvani *et al.* 2004). In the acute phase of stroke, the plasma levels of NPs have been reported to be elevated (Estrada *et al.* 1994, Etgen *et al.* 2005), but the prognostic value of NPs in stroke populations and their relations to other neurohumoral factors such as catecholamines and cortisol in the acute phase of stroke are unresolved.

The present study was designed to evaluate the cardiovascular autonomic disturbances in ischemic stroke by novel methods of investigating HR variability and by assessment of NPs. A special emphasis was placed on the prognostic value of nonlinear HR variability methods and neurohumoral disturbances in the acute phase of ischemic stroke.

## **2 Review of the literature**

### **2.1 Ischemic stroke**

#### ***2.1.1 Epidemiology of ischemic stroke***

The WHO has defined stroke as “a clinical syndrome characterised by rapidly developing clinical symptoms and/or signs of focal, and at times global, loss of cerebral function, with symptoms lasting for more than 24 hours or leading to death, with no apparent cause other than that of vascular origin” (Hatano 1976, Warlow *et al.* 1996). ‘Stroke’ as a term is non-specific, encompassing a heterogeneous group of distinct pathophysiologic causes, including thrombosis, embolism and hemorrhage. Approximately 75-80% of stroke cases are ischemic in origin, the remaining 20-25% being haemorrhagic, i.e due to subarachnoidal or intracerebral haemorrhage (Murray & Lopez 1997). Ischemic stroke is classified according to the etiological mechanisms into five diagnostic subgroups: large-artery atherosclerosis, cardioembolism, small-vessel occlusion, stroke of other determined etiology and stroke of undetermined etiology (Adams *et al.* 1993, Goldstein *et al.* 2001).

In the last two decades, studies of both stroke incidence and mortality have revealed significant variations between different populations and nationalities. Population-based stroke registers, including all age groups, show that the age- and sex-standardised annual incidence rates are approximately 300-500/100 000 population in most countries (Sudlow *et al.* 1997, Wolfe *et al.* 2000). The incidence of ischemic stroke in Finland in 2002 was about 400/100 000 in men and 200/100 000 in women (Pajunen *et al.* 2005), meaning that each day about 40 persons sustain brain infarction.

Stroke is a major cause of mortality and morbidity in industrialised countries. Of all first-ever ischemic stroke patients, about 50% either die or end up dependent in activities of daily living a year after their stroke (Bamford *et al.* 1990). In the latest official mortality statistics from the year 2003, stroke ranked the third most common cause of death in Finland, right after cardiac diseases and cancer. Over 4000 persons die from brain infarction in Finland each year (Statistics Finland 2004). Declining trends in stroke

mortality have been observed since the beginning of the 1980s (Lehtonen *et al.* 2004, Sivenius *et al.* 2004) possibly owing to better control of cardiovascular risk factors (Rothwell *et al.* 2004), improved acute treatment of stroke (Lindsberg *et al.* 2003) and decreasing severity of stroke events (Numminen *et al.* 2000). The latest report from the years 1999-2001 showed an annual decline of 5-6% in mortality from ischemic stroke in Finland (Pajunen *et al.* 2005). Similar findings have been reported from the United Kingdom (Rothwell *et al.* 2004), whereas in the United States the annual reduction in stroke mortality has begun to level off (Cooper *et al.* 2000).

### ***2.1.2 Mortality after ischemic stroke***

Altogether 9-21 percent of brain infarctions result in death within the first month of illness (Wolfe *et al.* 2000, Pajunen *et al.* 2005). Case fatality rates of 5 - 20 % in the first year after ischemic stroke, with a 5-11% annual risk of death for each year thereafter, and 5-year mortality rates of approximately 40% to 50% have been reported (Petty *et al.* 1998, Hartmann *et al.* 2001, Kimura *et al.* 2005, Pajunen *et al.* 2005). In the first few days after brain infarction, most patients who die do so as a result of the direct effects of the brain damage (Bamford *et al.* 1990). Later, the patients are at risk of a recurrent event affecting the brain or ischemic events involving the coronary arteries. The risk of a recurrent cerebrovascular event is higher during the first month (and year) after a brain infarction, but thereafter the risk of a cardiac event becomes equal, and as more time elapses, non-cerebral cardiovascular disease becomes the major cause of death amongst patients with ischemic stroke. (Petty *et al.* 1998, Hankey *et al.* 2000, Brønnum-Hansen *et al.* 2001, Hartmann *et al.* 2001, Kimura *et al.* 2005)

Prior research has identified age, male sex, stroke subtype, comorbidity, neurological symptom severity, prestroke functional status and place of residence as important predictors of mortality after stroke (Petty *et al.* 1998, Hankey *et al.* 2000, Kernan *et al.* 2000, Yokota *et al.* 2004). Level of consciousness is an indicator of stroke severity and decreased consciousness is one of the most powerful predictors for poor outcome after stroke (Kwakkel *et al.* 1996, Arboix *et al.* 2000). The etiological subtypes of brain infarction yield substantial differences in long-term survival and recurrence. A recent population-based prospective study on the long-term survival of brain infarction subtypes found the highest 2-year survival in small-vessel occlusion (85%) and the lowest in cardioembolic brain infarction (55%). Survival in large-artery atherosclerosis was 58%, survival in strokes of undetermined etiology 61% and survival for all subtypes 64% (Kolominsky-Rabas *et al.* 2001). Stroke recurrence not only potentially adds to physical impairment and disability but also increases mortality (Sacco *et al.* 1997). The risk of early recurrent stroke is about 12% within the first week and about 15% within the first month after minor ischemic stroke (Coull *et al.* 2004). This substantial early risk is 3 times higher if the primary stroke was caused by large-artery disease and 5 times lower if the cause was small-artery disease (Lovett *et al.* 2004). The prevalence and level of other causative vascular risk factors also influence the risk of recurrence (Dippel *et al.* 2004).

## 2.2 Autonomic nervous system

### 2.2.1 Autonomic nervous system anatomy

The autonomic nervous system (ANS) is an extensive neural network whose main role is to regulate the human internal environment by controlling homeostasis and visceral functions. ANS adjusts the functions of various organs in changing internal and external conditions, maintaining these homeostatic functions essential to life mostly independently of volitional activity, but profoundly influenced by somatosensory inputs and emotions (Appenzeller 1990, Ravits 1997). ANS also plays an important role in pain modulation and perception (Benarroh 2001). The functions of heart muscle, smooth muscle, secretory glands and hormone secretions are regulated by ANS (Appenzeller 1990, Ravits 1997). When the autonomic nerve transmissions are interrupted, the end organs continue to function, but can no longer effectively maintain homeostasis and adapt to the demands of the changing internal conditions and external stress (Adams et al 1997).

ANS has components at every level of the nervous system. The major part of ANS is located outside the cerebrospinal system, close to the visceral structures that it innervates. In distinction to the somatic neuromuscular system, two motor neurons bridge the gap between the central nervous system (CNS) and the effector organ – one (preganglionic) arising from its nucleus in the brainstem or spinal cord and the other (postganglionic) arising from specialised peripheral ganglia. The autonomic nervous system has three divisions: the sympathetic, parasympathetic and enteric nervous systems (Harati & Machkhaus 1997, Iversen 2000). Functionally, the sympathetic (SNS) and parasympathetic nervous systems (PNS) are complementary in maintaining the balance in the tonic activities of many visceral structures and organs. The viscera are mostly innervated by both sympathetic and parasympathetic fibers, with such exceptions as sweat glands and some blood vessels with single innervation only (Appenzeller 1990). The enteric nervous system in the wall of the gastrointestinal tract is responsible for the reflex activity involved in peristalsis and segmentation during the passage of food through the bowel (Jänig & McLachlan 1999).

The preganglionic sympathetic fibers are myelinated and originate from the intermediolateral and intermediomedial cell columns of spinal gray matter between the first thoracic and the third lumbar segments. The preganglionic fibres synapse with the postganglionic neurons in paravertebral and prevertebral ganglia. Postganglionic unmyelinated fibres supply the blood vessels, sweat glands and hair follicles and also form plexuses that supply the heart, bronchi, kidneys, intestines, pancreas, bladder and sex organs (Collins 1999, Jänig & McLachlan 1999, VanZwieten 1999). PNS consists of a cranial division originating from the midbrain, pons and medulla and the sacral part originating from the lateral horn cells of the second, third and fourth sacral segments. The preganglionic parasympathetic fibres traverse the distinct cranial nerves (III, VII, IX, X) and sacral nerves and synapse in ganglia that lie in the proximity of their end organ (Jänig & McLachlan 1999, Iversen *et al.* 2000).

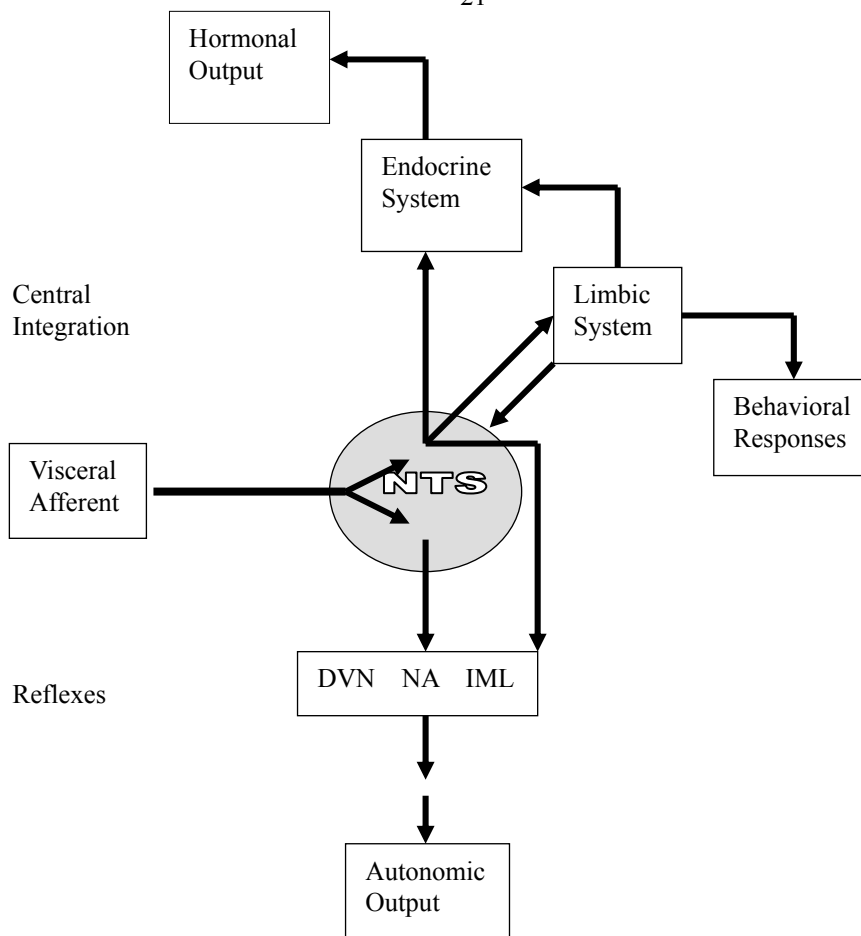
SNS is a diffuse system capable of generating mass responses by epinephrine release from the adrenal medulla. Because of high sympathetic postganglionic/preganglionic

fibre ratio and long postganglionic fibres, widespread responses are easily executed in SNS. In PNS the postganglionic/preganglionic fibre ratio is much lower than in SNS and preganglionic axons synapse with postganglionic neurons in close proximity to the effector organs, thus leading to a more selective way of action (Loewy 1990a). Acetylcholine is the neurotransmitter for preganglionic neurons in both parasympathetic and sympathetic nervous systems. Sympathetic postganglionic neurons are adrenergic, with the exception of sudomotor fibres, which are cholinergic. Postganglionic parasympathetic neurons are all cholinergic (Collins 1999, Jänig & McLachlen 1999). A variety of neuropeptides and putative neurotransmitters coexist with acetylcholine- and norepinephrine-containing neurons in both the pre- and postganglionic terminals, the spinal cord and at various levels of the central autonomic network. They play an important role in modulating the SNS and PNS functions (Joyner & Shepherd 1997, Benarroh 1999, Burnstock & Milner 1999).

### ***2.2.2 Central autonomic network***

The central control of autonomic function consists of various reciprocally interconnected areas in the cortex, basal forebrain, hypothalamus, midbrain, pons and medulla, forming a functional entity called the central autonomic network (CAN). This network controls autonomic functions in a tonic, reflexive and adaptive manner and integrates autonomic with hormonal, behavioural, immunomodulatory and pain-controlling responses to internal or external environmental challenges (Benarroh 1993). CAN receives and integrates visceral, humoral and environmental information and gives efferents to preganglionic autonomic neurons as well as to neuroendocrine, respiratory and sphincter motoneurons (Loewy 1990b).

The nucleus tractus solitarius (NTS) is the major visceral sensory relay cell group in the brain and receives inputs from all major organs of the body. Cardiovascular afferents from arterial, cardiac and pulmonary baroreceptors and carotid and aortic chemoreceptors via glossopharyngeal and vagus nerves project to specific regions of NTS. Ascending fibres are organised in a viscerotopic fashion with two modes of fibre sorting in NTS: one is involved in reflex modification of the end organ, and the other projects to higher CNS regions (Loewy 1990b). Neurons in the dorsolateral subnucleus of NTS phase the cardiac cycle and initiate vasodepressor and bradycardic responses (Andersen & Kunze 1994, Spyer 1995). Figure 1 illustrates how the afferent information is organised and projected further in NTS.



**Fig. 1. Drawing illustrating how afferent information is either processed for reflex responses (down) or projected to higher CNS regions (up). NTS=nucleus tractus solitarius, DVN=dorsal vagal nucleus, NA=nucleus ambiguus, IML= intermediolateral column. (Modified after Loewy 1990b).**

The highest level of integration of autonomic function is executed by the cortical autonomic structures, including the insular, anterior cingulate and medial prefrontal cortices, which integrate the viscerosensory and visceromotor responses (Cechetto 1987, Loewy 1991). Stimulation of the medial prefrontal cortex, which has connections with the amygdala, hippocampus, thalamus, hypothalamus, parabrachial nucleus and NTS, induces bradycardia and hypotension and modulates gastric secretion (Cechetto & Saper 1990). The medial prefrontal cortex may also influence the autonomic processes underlying the appreciation and expression of emotions (Barbas *et al.* 2003). Activation of the insular cortex induces changes in blood pressure and pulse, piloerection and epinephrine secretion and alters gastrointestinal activity (Cechetto & Chen 1990).

Sympathetic innervation is suggested to arise from a more rostral part of the posterior insula than parasympathetic innervation (Oppenheimer & Cechetto 1990). The left insular cortex is suggested to elicit predominantly parasympathetic responses, whereas the right insular cortex predominates in sympathetic responses (Oppenheimer et al 1992a and b).

The extended amygdala, intercalated between the cerebral cortex, hypothalamus and mesencephalic regions, integrates autonomic, neuroendocrine and behavioural responses to emotions (Amaral et. al 1992, LeDoux 1992). The hypothalamus has been claimed to be the most important ANS organ, as it controls every vital function and integrates the neuroendocrine and autonomic systems. Particularly its paraventricular nucleus deserves attention, since it innervates all autonomic centres, integrates responses to stress and regulates cardiovascular function, energy metabolism and immune responses (Swanson 1987, Holstege 1990). At the mesencephalic level, the nucleus parabrachialis and periaqueductal gray (PAG) are integrative relay areas. PAG is also a crucial structure in pain modulation (Benarroh 2001).

Transmission of information within CAN involves several neurotransmitters, including amino acids, acetylcholine, monoamines and neuropeptides. Amino acids mediate rapid communications through ion channel receptors. Acetylcholine, monoamines and neuropeptides mediate slower modulatory influences by acting on specific receptors (Benarroh 1997, Burnstock & Milner 1999, Iversen *et al.* 2000). Angiotensin II, vasopressin, natriuretic peptides, opioids, corticotrophin-releasing hormone and a variety of cytokines affect central cardiovascular control by acting as endogenous neurotransmitters/neuromodulators in the central autonomic pathways and as circulating signals acting directly on the peripheral target organs (Benarroh 1999).

### ***2.2.3 Cardiovascular autonomic control***

#### *2.2.3.1 Anatomical aspects*

The heart possesses an inherent ability for spontaneous, rhythmic initiation of the cardiac excitation impulse, but its function is significantly modulated by innervations from both the sympathetic and parasympathetic divisions of ANS (Benarroh 1997, Crick *et al.* 2000). The parasympathetic innervation of the heart originates in the cardiovagal motoneurons in the nucleus ambiguus and the dorsal vagal nucleus. These neurons are excited by baroreflex and inhibited by hypothalamic and inspiratory influences (Ciriello & Calaresu 1980, Loewy & Spyer 1990, Spyer 1999). The parasympathetic pathway passes through two sets of cardiac nerves arising from each vagus nerve. The cardiac branches of the vagus nerve separate in the thorax and innervate several cardiac ganglion cells (Rossi 1994). Most parasympathetic nerves are distributed near the sinus node and atrioventricular (AV) conduction tissue. The left and right vagi are distributed differentially, with the left vagus nerve inhibiting AV conduction tissue and the right vagus nerve affecting predominantly the sinus node. This anatomico-functional separation in innervation enables the CNS to selectively influence the sinoatrial (SA) and

AV nodes either together or independently (Richter & Spyer 1990, Waller & Schlant 1996).

The sympathetic preganglionic neurons receive central inputs from the paraventricular nucleus, ventrolateral medulla, lateral hypothalamic area, zona inserta, NTS and PAG (Loewy 1990) and synapse in the cervical and thoracic ganglia (Gibbins 1990). Further connections are received from the vasopressin- and oxytocin-secreting cells of the hypothalamus and the noradrenergic cells of the A5 group. Sympathetic preganglionic neurons do not have any direct connection with the cortex. The cortex, however, exerts its influence through its connections with the NTS, limbic system, hypothalamus and parabrachial nuclei (Loewy 1982, Benarroh 1993). Similarly to the parasympathetic system, sympathetic innervation of the heart also functions in a lateralised manner. The right sympathetic pathway predominantly excites the SA node, increasing heart rate, whereas the left sympathetic pathways predominantly innervate the AV node and the ventricles, resulting in increased AV conduction, cardiac contractility and oxygen consumption (Cowley 1992). Stimulation of the left-sided cardiac sympathetic nerves induces arrhythmias more easily than corresponding stimulation on the right side (Talman 1985).

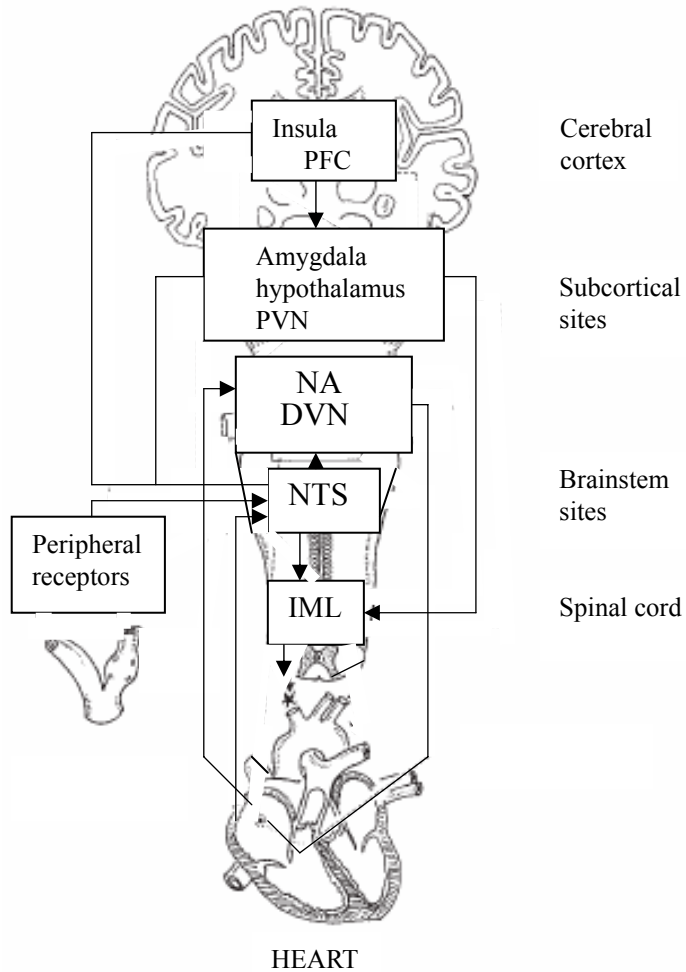
### *2.2.3.2 Physiology of cardiovascular autonomic control*

The cardiovascular system, with its complex interactions between the local and neurohumoral mechanisms, controls cardiac output, systemic vascular resistance and local organ blood flow to regulate mean arterial pressure. Fluctuations in the HR and blood pressure (BP) reflect the dynamic response of the cardiovascular control system to physiological changes (Joyner & Shepherd 1997). The brain receives and integrates all external and internal stimuli to enable proper control of cardiovascular functions through ANS and the endocrine-humoral system (Figure 2). Neural regulation of the circulatory function is operated through the interplay of sympathetic and vagal outflows. The sympathovagal balance is tonically and phasically modulated by the interaction of the CAN and peripheral reflex mechanisms. In most physiologic conditions, activation of either of these outflows is accompanied by inhibition of the other (Malliani *et al.* 1991, Montano *et al.* 1994, Spyer 1999). This brain-heart control enables second-to-second modulation of cardiac activity and vascular tone in response to physical activity, threats, stresses and emotional changes (Cheung & Hachinski 2003).

It is well documented that several groups of peripheral receptors contribute to the reflex control of circulation. These include the arterial baroreceptors and chemoreceptors as well as the receptors within the heart, airways and lungs (Spyer 1990). The arterial baroreceptors and the cardiopulmonary receptors with vagal afferents tonically inhibit the vasomotor centres, whereas the cardiopulmonary receptors with sympathetic afferents and the arterial chemoreceptors and ergoreceptors in the skeletal muscle stimulate these centres. The primary site of interaction of these afferents within CNS is at the level of NTS. As a consequence, the sympathetic activity is modified selectively to adjust appropriately the performance of the cardiovascular system. Sympathetic activation increases the heart rate and cardiac contractility, constricts the resistance vessels and

decreases capacitance in the splanchnic vascular bed. As a consequence, systemic vascular resistance and cardiac filling pressure are adjusted to maintain arterial BP at an appropriate level (Shepherd & Shepherd 1999). Cardiovascular baroreflexes provide beat-to-beat control of HR and short-term control of BP, whereas sympathetically activated renal regulation of blood volume provides long-term BP control (Joyner & Shepherd 1997). Apart from direct neural (sympathetic) control, the regulation of renal volume is also influenced by various endocrine and local factors, such as natriuretic peptides, vasopressin and the renin-angiotensin-aldosterone system (Cowley 1992).

In healthy individuals, HR at rest is dominated by parasympathetic innervation. Under circumstances where increased HR is required, as in exercise, the activity of the parasympathetic division is inhibited, while sympathetic activity is enhanced by the reflex mechanisms described above (Pocock & Richards 1999) and by direct central influences (Spyer 1990). Short-term periodic fluctuations in heart rate are also caused by the inhibitory effects of inspiration on cardiovagal motoneurons in the nucleus ambiguus and dorsal vagal nucleus. These oscillations in the R-R intervals within the frequency range of 0.15-0.4 Hz are called respiratory sinus arrhythmia, which is considered an important clinical index of vagal innervation of the heart (Eckberg 1983). Inspiration hyperpolarizes the cardiovagal motoneurons, decreasing their firing rate and sensitivity to central and reflex influences and hence resulting in acceleration of HR during inspiration (Richter & Spyer 1990).



**Fig. 2. Schematic diagram representing the connections between the nervous system to the heart. NTS = nucleus tractus solitarius, NA = nucleus ambiguus, DVN = dorsal vagal nucleus, IML = intermediolateral column, PVN = periventricular nucleus, PFC = prefrontal cortex.**

## **2.3 Measurement of autonomic nervous system function**

### ***2.3.1 General aspects***

Evaluation of ANS function includes assessments based on physiological, biochemical and pharmacological measurements. Various procedures for the evaluation of sudomotor, gastrointestinal, renal, urinary, sexual, respiratory and pupil functions are available, but the golden standard in the clinical testing of autonomic functions has been the measurement of cardiovascular reflexes. These tests, involving continuous HR, BP and respiratory monitoring to define circulatory responses under standardised conditions, provide information about both sympathetic and parasympathetic cardiovascular autonomic regulation (Mathias & Bannister 1999). Percutaneous microneurographic techniques provide direct information about sympathetic activity in skin and muscle (Wallin & Elam 1997, Macefield 2005), and scintigraphic methods using radiotracers, positron emission tomography and single photon emission tomography provide information about cardiac postganglionic sympathetic function (Courbon *et al.* 2003, Saiki *et al.* 2004, Richter *et al.* 2005). These methods are suitable for the study of sympathetic physiology in various conditions, but are not applicable to routine diagnostic work. Non-invasive and easily performed assessment of HR variability has now attained widespread use in diverse disciplines. With the advent of powerful desktop computers and the resulting ease with which cardiovascular signals can be acquired and processed digitally have resulted in an array of measures of HR variability (Karemaker 1997).

### ***2.3.2 Ambulatory ECG and heart rate variability analysis***

HR variability is a physiological phenomenon defined as variation in the normal-to-normal RR intervals during normal sinus rhythm. It reflects the effects of the autonomic nervous system and other physiological control mechanisms on cardiac function. It can be easily analysed from 24-hour electrocardiographic (ECG) recordings. The measurement of HR variability is non-invasive and has high intra- and interindividual reproducibility (Huikuri *et al.* 1999), which has led to its popularity in assessing neuroautonomic control of the heart. The traditional methods of HR variability analysis include time and frequency domain analysis, often referred to as linear methods. Since the genesis of HR variability also involves nonlinear mechanisms, several new methods have been developed to quantify complex HR dynamics. These nonlinear or dynamic measures have shown better prognostic value than the traditional measures of HR variability (Mäkikallio *et al.* 1999, Huikuri *et al.* 2000).

In the time domain measures of HR variability, HR fluctuation is assessed by calculating measures based on statistical operations (means and variance) on R-R intervals. The most widely used time domain measures are average HR and standard deviation of all normal-to-normal R-R intervals (SDNN) over a 24-hour period. These

measures are considered to reflect both parasympathetic and sympathetic influences on the heart (Myllylä *et al.* 2002).

Geometrical methods present R-R intervals in geometrical patterns. The two-dimensional Poincaré plots method provides a beat-to-beat analysis of R-R intervals, which can be interpreted both visually (Woo *et al.* 1994) and quantitatively. In the latter method, instantaneous beat-to-beat R-R interval variability (SD1) and the SD of continuous long-term R-R interval variability (SD2) are analysed (Huikuri *et al.* 1996, Tulppo *et al.* 1996). SD1 indicates the magnitude of beat-to-beat R-R interval variability, reflecting the vagal modulation of the heart. SD2 reflects the long-term R-R interval fluctuations.

The power spectrum of R-R intervals reflects the amplitude of HR fluctuations at different oscillation frequencies (Akselrod *et al.* 1981). Fast Fourier transformation and autoregressive analysis are the most commonly used methods providing frequency-specific information of HR behaviour. The spectrum is usually divided into three or four different frequency bands. The boundaries of the most commonly used bands are as follows; ultra low frequency (ULF),  $<0.0033$  Hz; very low frequency (VLF), 0.0033-0.04 Hz; low frequency (LF), 0.04-0.15 Hz; high frequency (HF), 0.15-0.4 Hz (Task force 1996). HF power is considered to reflect mainly cardiovagal modulation and the inspiratory inhibition of vagal tone, while LF power is affected by sympathetic activity. As a result, the ratio of LF power to HF power (LF/HF ratio) has been proposed as a specific measure of sympatovagal balance (Pagani *et al.* 1986, Malliani *et al.* 1991). Fluctuations in the VLF region have been suggested to reflect sympathovagal balance, thermoregulation and peripheral vascular resistance (Akselrod *et al.* 1981, Lindqvist *et al.* 1989, Dwain & Eckberg 1997, Pagani *et al.* 1997).

Methods of analysis derived from the nonlinear system theory provide information on the quality, scaling and correlation properties of the HR signal rather than the magnitude of variability assessed by the traditional HR variability measures. Several algorithms based on nonlinear dynamics and chaos theory have been introduced (Goldberger 1996, Francis *et al.* 2005, Perkiömäki *et al.* 2005) to detect abnormalities in R-R interval dynamics in various cardiovascular disorders (Mäkikallio *et al.* 1998, Huikuri *et al.* 2000, Mäkikallio *et al.* 2001, Vikman *et al.* 2005). Detrended fluctuation analysis (DFA) is a nonlinear method designed to determine the self-similar properties, i.e. fractal-like correlation properties, of the R-R interval signal (Peng *et al.* 1995). Healthy subjects have shown scaling exponent  $\alpha$  values close to 1, indicating fractal-like HR behaviour, and altered values have been reported in patients with cardiovascular diseases and advanced age (Mäkikallio *et al.* 1998, Mäkikallio *et al.* 1999, Pikkujämsä *et al.* 1999, Huikuri *et al.* 2000). Reduced values of scaling exponent  $\alpha$  have been shown to be associated with concomitant sympathetic and vagal activation (Tulppo *et al.* 2005) and an increased risk of mortality and life-threatening arrhythmias in patients with and without structural heart disease (Mäkikallio *et al.* 1997, Mäkikallio *et al.* 1998, Vikman *et al.* 1999, Mäkikallio *et al.* 2001, Tapanainen *et al.* 2002, Jokinen *et al.* 2003). The inverse power-law slope ( $\beta$ ) reflects the distribution of spectral power over the VLF and ULF frequency bands (Bigger *et al.* 1996). The power-law relationship has predicted mortality in the elderly (Huikuri *et al.* 1998) and in patients with impaired left ventricular function (Mäkikallio *et al.* 1999, Huikuri *et al.* 2000). The physiological background for the power-law slope is not well established, but ANS influences are probable, as the power-law slope is

especially steep in denervated hearts (Bigger *et al.* 1996). Approximate entropy (ApEn) is another non-linear method that quantifies the regularity and predictability of time series data (Pincus & Goldberger 1994). ApEn values have been reported to be reduced by vagal blockade (Penttilä *et al.* 2003). Reduced values of ApEn have been reported in sick neonates (Pincus & Viscarello 1992), in chronic liver failure patients (Fleischer *et al.* 2000) as well as in patients with complications after cardiac surgery (Fleischer *et al.* 1993). Decreases in ApEn and the scaling exponent  $\alpha$  have been shown to precede the spontaneous onset of atrial fibrillation in patients with no structural heart disease (Vikman *et al.* 1999).

## **2.4 Autonomic dysfunction in brain infarction**

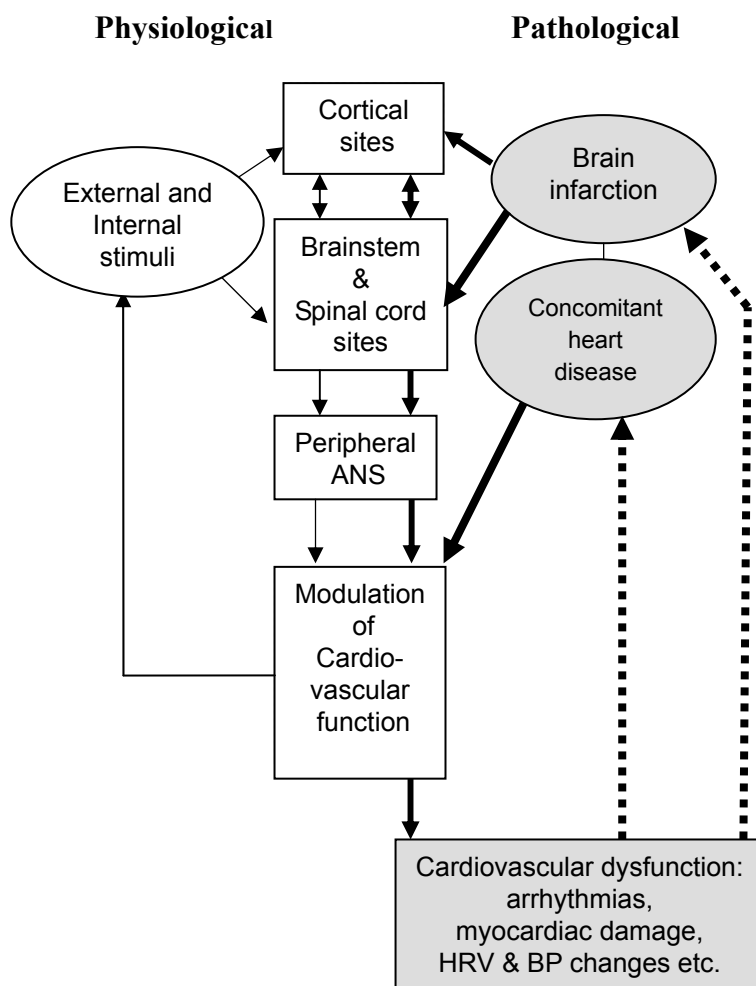
### ***2.4.1 General aspects***

Ischemic stroke is associated with various clinical manifestations of ANS dysfunction, including cardiovascular, gastrointestinal, urogenital, sudomotor and thermoregulatory disorders (Korpelainen *et al.* 1999). Cardiovascular disturbances have been recognised since the 1950's (Burch *et al.* 1954, Wasserman *et al.* 1956), but during the last decade, growing interest has also been attached to other manifestations. Hemispheric infarctions have been reported to result in contralateral hyperhidrosis (Korpelainen *et al.* 1993, Kim *et al.* 1995), decreased skin temperatures in the paretic limbs (Korpelainen *et al.* 1995, Wanklyn *et al.* 1995) as well as urinary and sexual disorders (Sakakibara *et al.* 1996, Korpelainen *et al.* 1998). Brainstem infarctions typically cause long-term ipsilateral hypohidrosis (Korpelainen *et al.* 1995), but excessive contralateral sweating has also been reported as a result of medullary and pontine brainstem infarctions (Naver *et al.* 1995, Rousseaux *et al.* 1996). Asymmetrical skin temperatures and Horner's syndrome have been observed in patients with brainstem infarction in addition to more severe cardiovascular abnormalities (Norrving *et al.* 1991, Korpelainen 1995, Cross 1997).

### ***2.4.2 Cardiovascular autonomic dysfunction in ischemic stroke***

Cerebrogenic, cardiovascular and autonomic disturbances in ischemic stroke are suggested to be prognostically unfavourable (Drislane & Samuels 1990, Ropper 1997) and to include electrocardiographic abnormalities, elevation of cardiac enzymes, cardiac arrhythmias, myocardial cytolysis and disturbances of BP regulation (Cheung & Hachinski 2004). Cardiovascular abnormalities caused by localised cerebral lesions are attributed to damage in the cerebral centres regulating cardiovascular functions, in the pathways connecting these centers and in the cerebral sites modulating cardiovascular reflexes and to increased intracranial pressure as well as neurohumoral factors. Clinical and experimental studies suggest that cortical and subcortical structures such as the insular cortex and amygdala play a pathogenic role (Cheung & Hachinski 2000, Cheung & Hachinski 2003, Cheung & Hachinski 2004, Colivicchi *et al.* 2004). The peripheral

mechanisms involve abnormal sympathetic activity (Oppenheimer *et al.* 1990), altered parasympathetic activity (Korpelainen *et al.* 1996, Naver *et al.* 1996) and elevated levels of circulating catecholamines (Cruickshank *et al.* 1974, Globus *et al.* 1989, Kantor & Krishnan 1996, Samuels 1999), whereas the central neurochemical mechanisms mediating the cerebrogenic cardiovascular disturbances are largely unknown. Especially the role of insular cortical involvement in poststroke cardiovascular disturbances has been emphasised. Insular cortex ischemia is known to be associated with QT prolongation, cardiac arrhythmias, nocturnal rise of BP (Cheung & Hachinski 2003), elevated plasma catecholamines (Strittmatter *et al.* 2003, Meyer *et al.* 2004) and poststroke hyperglycemia (Allport *et al.* 2004), reflecting cardiovascular and neuroendocrine dysregulation after an insular lesion. The amygdalar neurochemical change that follows a specific time course after experimental insular stroke has been proposed to mediate the cerebrogenic cardiovascular disturbances originating from insular cortex ischemia (Cheung *et al.* 1995, Cheung *et al.* 1997). In two studies of ischemic stroke patients, involvement of the insular cortex was associated with poor long-term outcome and an increased risk of sudden death (Tokgözoğlu *et al.* 1999, Sander *et al.* 2001). The proposed pathways involved in cerebrogenic cardiovascular disturbances are illustrated in Fig. 3.



**Fig. 3.** A schematic diagram summarizing the concepts of brain-heart control under physiologic conditions (left) and of cardiovascular dysfunction in brain infarction (right). Thin arrows represent influences and feedback interactions in physiologic conditions; thick arrows represent the proposed pathways of cerebrogenic cardiovascular disturbances; and dashed arrows represent the vicious cycle of the effects of cerebrogenic disturbances on the cerebral and cardiac functions. (Modified after Cheung & Hachinski 2003).

#### 2.4.2.1 *Electrocardiographic changes and myocardial damage*

The most common cardiovascular autonomic complications associated with cerebral lesions include changes in cardiac electrophysiology (Oppenheimer & Hachinski 1992,

Khechinashvili & Asplund 2002) as well as myocardial structural damage (Oppenheimer *et al.* 1991). The ECG changes often observed during the first few days after a brain infarction resemble abnormalities typical of myocardial ischemia or infarction and may therefore cause diagnostic and management dilemmas for clinicians. Repolarization changes, comprising flat, inverted or peaked T waves, ST segment elevation or depression and QT interval prolongation as well as a pathologic Q and U waves, are the most common ECG findings in stroke patients (Oppenheimer & Hachinski 1992, Cheung & Hachinski 2003). The ECG changes usually evolve for several days and disappear within 2 weeks, but QT prolongation and U waves may persist (Talman 1985). The ECG abnormalities may reflect the etiology of the stroke (e.g. embolism after an acute myocardial infarction, atrial fibrillation), but they are often direct consequences of the cerebral lesion or a manifestation of concomitant atherosclerotic coronary disease. A recent meta-analysis reported by Khechinashvili & Asplund (2002) showed that, among unselected patients with stroke, repolarization and ischemic-like ECG changes are the rule, being present in more than 90% of subjects. However, when patients with previously known cardiac disorders are excluded, the prevalence of ECG changes is considerably lower (approximately 30-40%), suggesting that the electrophysiological changes observed in ischemic stroke patients may be partly due to a concomitant heart disease. There is, however, powerful evidence to suggest that cerebral lesions may produce myocardial damage distinct from the true coagulative necrosis caused by myocardial ischemia (Samuels 1999, Tung *et al.* 2004). Such neurogenic myocardial damage that can be caused without coronary heart disease and in the absence of acute coronary ischemia (Cheung & Hachinski 2000) is called coagulative myocytolysis or contraction band necrosis, and it includes subendocardial petechial hemorrhages, interstitial mononuclear cell infiltration, edema formation and myofibrillar degeneration immediately adjacent to the cardiac nerve endings (Greenhoot & Reichenbach 1969, Samuels 1999). Animal experiments have shown that high levels of circulating catecholamines and various kinds of stress can induce neurogenic myocardial damage and exaggerate the ECG findings (Hachinski *et al.* 1986, Woolf *et al.* 1987, Oppenheimer *et al.* 1991). Nervous system stimulation at various locations such as the hypothalamus, limbic cortex and stellate ganglion may also produce cardiac lesions histologically indistinguishable from stress- and catecholamine-induced myocardial damage (Kaye *et al.* 1961, Attar *et al.* 1963, Todd *et al.* 1985).

#### 2.4.2.2 Arrhythmias

Cardiovascular autonomic dysregulation may also manifest as increased incidence of cardiac dysrhythmias in patients with ischemic stroke. The most common arrhythmias reported in patients with stroke are atrial fibrillation, supraventricular tachycardia, ectopic ventricular contractions, ventricular fibrillation and multifocal ventricular tachycardia (Oppenheimer *et al.* 1990a). Cardiac arrhythmias seem to be more frequent in patients with right-sided strokes (Lane *et al.* 1992, Orlandi *et al.* 2000), and concurrent right insular damage is associated with more complex arrhythmias (Colivicchi *et al.* 2004) and may explain the cases of sudden death that occur early after stroke (Oppenheimer *et al.*

1992c, Cheung & Hachinski 2000). In patients with acute stroke, the incidence of sudden arrhythmic death is approximately 6% (Oppenheimer *et al.* 1990b). The insular cortex has direct connections with brainstem autonomic control nuclei (Allen *et al.* 1991), and specific cardiovascular and other autonomic responses can be localised in this cortical location (Cechetto & Chen 1990, Yasui *et al.* 1991).

### 2.4.2.3 *Blood pressure changes*

Both systolic and diastolic BPs tend to elevate transiently immediately after ischemic stroke, but resolve spontaneously during the first week (Lip *et al.* 1997, Morfis *et al.* 1997). Reduction in BP levels is common already on the first minutes or hours after cerebral ischemia (Britton *et al.* 1986, Broderick *et al.* 1993). Acute BP elevation may occur due to pre-existing hypertension, which is aggravated by increased sympathetic discharge or stress, but it can also be a protective response to preserve cerebral perfusion. The latter notion was supported in a recent study assessing effects of intra-arterial thrombolysis on BP in patients with acute ischemic stroke. The study demonstrated an association between BP fall and vessel recanalisation (Mattle *et al.* 2005). Rapid resolving of BP levels in the acute phase have also been associated with milder strokes (Christensen *et al.* 2002) supporting the assumption that early reduction of BP might indicate vessel recanalisation and thus also better prognosis.

Paroxysmal neurogenic hypertension associated with sympathetic hyperactivity has been observed in hemispheric strokes, particularly when insular cortex lesions are involved (Sander & Klingelhofer 1996) and in brainstem infarctions with lesions in the NTS (Phillips *et al.* 2000). Acute distortion of the lower brainstem and edematous cerebellar infarctions may elicit a Cushing response involving hypertension, bradycardia and slow irregular breathing (Ropper 1997). Diurnal BP fluctuation is also known to be transiently reduced in the acute phase of ischemic stroke (Dawson *et al.* 1998, Jain *et al.* 2004).

### 2.4.2.4 *Heart rate variability in ischemic stroke*

HR variability reflecting autonomic cardiovascular dysfunction has been shown to be suppressed as a consequence of both hemispheric (Korpelainen *et al.* 1996a, Naver *et al.* 1996, Orlandi *et al.* 2000) and brainstem cerebral infarctions (Korpelainen *et al.* 1996b, Phillips *et al.* 2000, Meglic *et al.* 2001). This suppression involves all time and frequency domain HR variability measures and seems to lead to persistent autonomic dysfunction after hemispheric ischemic stroke (Korpelainen *et al.* 1996a). These abnormalities indicate the presence of complex derangement of autonomic balance, involving both the sympathetic and parasympathetic systems (Task force 1996). In the acute phase of stroke, the diurnal oscillation of HR variability is also transiently suppressed (Korpelainen *et al.* 1997), leading to a loss of relative nocturnal vagal dominance. Lesions at cerebral sites controlling cardiovascular functions are expected to cause cardioautonomic dysfunction, as has been reported in patients with strokes located in the insular cortex. Ischemic stroke

in the region of the insula, especially on the right side, has been shown to lead to suppressed HR variability and to an increased risk of sudden death (Tokgözüoğlu *et al.* 1999, Colivicchi *et al.* 2004). The prognostic significance of HR variability measures after stroke has not been studied. Non-linear HR variability methods have previously been used in stroke patients in only one study, which reported reduced randomness (ApEn) of HR variability in patients with left insular stroke (Oppenheimer *et al.* 1996).

## 2.5 Neurohumoral factors in acute ischemic stroke

### 2.5.1 Natriuretic peptides

NPs are cardiac vasoactive peptide hormones involved in volume homeostasis and cardiovascular remodelling (Levin 1998, Stein & Levin 1998). NPs relax vascular smooth muscle, causing arterial and venous dilatation and leading to reduced blood pressure and ventricular preload (Richards *et al.* 1988, Tonolo *et al.* 1989). They also function as neuromodulators affecting the autonomic cardiovascular control of the heart. They have central and peripheral sympathoinhibitory effects (Floras 1990, Brunner-LaRocca 2001) and augment parasympathetic vagal neurotransmission (Herring *et al.* 2001, Thomas & Woods 2003), although high concentrations of NPs have been shown to directly stimulate cardiac pacemaking (Herring *et al.* 2001). Moreover, NPs have cytoprotective effects in myocardial ischemia as well as anti-proliferative and anti-migratory effects on cardiac and vascular cells (D'Souza *et al.* 2004). They are suggested to regulate collagen scar formation and left ventricular remodelling after acute myocardial infarction (AMI) (Maggia *et al.* 2004). NPs and their receptors are actively modulated during the progression of coronary artery disease (CAD), suggesting that the NP system is involved in the pathobiology of atherosclerosis (Casco *et al.* 2002). NPs inhibit the activities of several other hormone systems, including the renin-angiotensin-aldosterone system (Stein & Levin 1998). The prime stimulus for the release of NPs is the cardiac myocyte stretch, but their synthesis is also augmented by tachycardia (Riddervold *et al.* 1991), glucocorticoids (Nishimoro *et al.* 1997) and other vasoactive peptides (Bruneau *et al.* 1997, Wiese *et al.* 2000) independently of the hemodynamic effects of these factors.

Especially the measurement of brain (B-type) NP (BNP) or its N-terminal pro-brain NP (N-BNP) has recently become valuable in the rapid diagnosis of heart failure (Maisel *et al.* 2002, Doust *et al.* 2004). In acute coronary syndromes it has been used in risk stratification (Ishii *et al.* 2002, Sabatine *et al.* 2002, Richards *et al.* 2003) and it has been found predictive of short-term mortality (Galvani *et al.* 2004). An association between elevated levels of NPs and increased mortality has been established in patients with heart failure (Cowie *et al.* 2002, Anand *et al.* 2003) and with acute coronary syndromes (Morrow *et al.* 2003, Galvani *et al.* 2004) as well as in the general population (McDonagh *et al.* 2001, Wang *et al.* 2004). The elevated levels of N-BNP have been associated with CAD (Jernberg *et al.* 2002, James *et al.* 2003) and parallel the clinical and angiographic severity of CAD (Kragelund *et al.* 2005, Ndrepepa *et al.* 2005). A large population-based study has also shown that plasma NP levels predict the risk of a wide

range of cardiovascular events, including the risk of cerebrovascular ischemia, suggesting that NP levels may be elevated even before the onset of clinically apparent cardiovascular disease (Wang *et al.* 2004).

The current information concerning the role of NPs in cerebrovascular diseases is limited. In the acute phase of stroke the plasma levels of atrial NP (ANP) (Estrada *et al.* 1994) and N-BNP (Etgen *et al.* 2005) have been reported to be elevated, and the levels of BNP have been shown to correlate with BP levels (Eguchi *et al.* 2002) and with poor functional outcome after 3 months (Etgen *et al.* 2005). Immunohistochemical studies suggest that cerebral ischemia induces ANP secretion in brain tissue (Giuffrida *et al.* 1992, Nogami *et al.* 2001), and the progression of cerebral ischemia has been reported to be associated with gradually rising BNP levels in patients with subarachnoid hemorrhage (Sviri *et al.* 2003). Polymorphisms of the gene for ANP (Rubattu *et al.* 2004) and elevated plasma levels of ANP were recently reported to predict the risk of stroke (Wang *et al.* 2004). The value of NPs as prognostic measures after cerebrovascular accidents has not been investigated previously.

### ***2.5.2 Cortisol, catecholamines and adrenocorticotrophic hormone***

The acute phase of ischemic stroke has been shown to involve increased activity of the hypothalamus-pituitary-adrenal (HPA) axis with elevated circulating cortisol level (Olsson *et al.* 1992, Fassbender *et al.* 1994, Slowik *et al.* 2002) and pathologic sympathetic activation with elevated catecholamine levels (Meyer *et al.* 1973, Myers *et al.* 1981, Sander & Klingelhöfer 1994, Sander *et al.* 2001). During the first week after stroke, a transient increase in adrenocorticotrophic hormone (ACTH) has been observed (Fassbender *et al.* 1994), suggesting an initial stress-induced activation of the hypothalamus, followed by cortisol-induced feedback suppression of ACTH levels. The elevated levels of cortisol have been associated with poor outcome, the size of the ischemic lesion (Feibel *et al.* 1977, Murros *et al.* 1993, Slowik *et al.* 2002, Smith *et al.* 2004) and death within 7 days after stroke onset (Christensen *et al.* 2004a). However, in patients with very large, space-occupying hemispheric strokes, cortisol and ACTH levels have been reported to be reduced (Schwartz *et al.* 2003). Recently, both elevated and reduced levels of cortisol in the acute phase were associated with increased one-year mortality (Marklund *et al.* 2004). In conclusion, it is still controversial whether the neuroendocrine changes in stroke are purely adaptive and beneficial, or whether they represent profound detrimental dysfunction.

### **3 Purpose of the study**

The main purpose of the study was to evaluate autonomic cardiovascular function in brain infarction by novel methods of investigation and to assess the prognostic value of these measures in predicting survival of ischemic stroke patients.

The specific aims of the individual studies were:

1. To evaluate the complexity and correlation properties of HR dynamics after ischemic stroke by using new methods of analysis based on nonlinear HR variability methods.
2. To evaluate nonlinear measures of heart rate behaviour as predictors of cerebrovascular mortality in a general elderly population.
3. To assess the ability of HR variability measures to predict mortality after ischemic stroke.
4. To assess the predictive value of natriuretic peptides after ischemic stroke.
5. To investigate the relations of natriuretic peptides and other neurohumoral factors such as cortisol, catecholamines and adrenocorticotrophic hormone during the first week after ischemic stroke and to assess their effects on long-term mortality.

## **4 Subjects and methods**

### **4.1 Subjects**

Brain infarction patients were recruited from the Department of Neurology at Oulu University Hospital in Oulu, Finland, during the years 1994-1999 (I, III, IV and V). The patient population consisted of 97 (age  $60\pm 11$  years) first-ever ischemic stroke patients admitted into the hospital within the first 24 hours of symptom onset. Patients older than 80 years of age and patients with unstable angina or non-sinus rhythm on ECG at recruitment or previous strokes, transient ischemic attacks, head trauma, other cerebral disease, severe psychiatric illness, alcoholism or malignancy in their medical history were excluded. In study I, subjects with medication known to affect the cardiovascular or autonomic nervous systems were excluded as well. The subjects for study II were recruited in connection with a large survey of the health status of the elderly in the city of Turku, Finland. A random sample of 480 people, aged 65 or older, living in the community was obtained from the register of Social Insurance Institution of Turku. Analyses of ambulatory ECG recordings were available for 325 subjects. No exclusion criteria other than living in an institution were used. The control populations (I, IV and V) consisted of healthy volunteers without any disease or medication known to affect the ANS. In study IV, another control population of AMI patients was used. The AMI patients were randomly selected from a larger population and matched in a 1-to-1 fashion for age and gender with the ischemic stroke patients. The clinical characteristics of the study and control populations are shown in Table 1.

The study was approved by the Ethics Committee of the Medical Faculty, University of Oulu, and informed consent was obtained from each patient or family member.

*Table 1. Clinical characteristics of the study populations (I-V).*

Study	Gender male/female	Age (mean±SD)	Follow-up	Mortality	Stroke localization right/left/brainstem
<b>I</b>					
Stroke patients (n=46)	33/13 (72%/28%)	52±11	6 months		19/12/15
Healthy controls (n=30)	21/9 (70%/30%)	52±11			
<b>II</b>					
General elderly population(n=325)	173/152 (53%/47%)	73±6	10 yrs	50.5% (164)	
<b>III</b>					
Stroke patients (n=84)	56/28 (67%/33%)	59±12	83±54 months	39% (33)	38/29/17
<b>IV</b>					
Stroke patients (n=51)	22/29 (43%/57%)	67±10	44±21 months	43% (22)	24/20/7
Healthy controls (n=25)	12/13 (48%/52%)	65±10			
AMI controls (n=51)	22/29 (43%/57%)	67±10	44±21 months		
<b>V</b>					
Stroke patients (n=51)	22/29 (43%/57%)	67±10	44±21 months	43% (22)	24/20/7

AMI; acute myocardial infarction.

## 4.2 Methods

### 4.2.1 Follow-up and endpoints

The stroke patients were followed up for 7 years (83±53 months) in study III and for nearly 4 years (44±21 months) in the studies IV and V. The population in study II was followed up for ten years. After the follow-up, the clinical outcome was assessed by reviewing the medical records supplemented by an interview of the patient or a family member. In the case of death, the hospital records and death certificates were reviewed to verify death. The endpoint was all-cause mortality (III, IV and V). Mortality statistics, hospital records, autopsy findings and death certificates were used to determine the mode of death and mortality in study II. Deaths were classified as sudden cardiac, nonsudden cardiac, cerebrovascular and due to other causes. The classification of deaths was performed blindly by the events committee before the analysis of HR variability (II). In study I, the ambulatory ECG recording was repeated after six months of follow-up.

## ***4.2.2 Clinical examination***

Past and present clinical history, including medication, was obtained by a personal interview of the patient or a family member in the case of aphasia or unconsciousness of the patient. Past medical history was also reviewed from the hospital records. The stroke patients underwent a comprehensive clinical examination and scoring by the Scandinavian Stroke Scale (SSS), Modified Ranking Scale (MRS), Barthel Index (BI) and Glasgow Coma Scale (GCS) within the first 12 hours after admission. SSS evaluates the neurologic deficit by assessing level of consciousness; eye movement; motor power of the arm, hand and leg; orientation; aphasia; facial paresis; and gait on a total score from 0 to 58 (maximum). MRS and BI assess functional disability. The MRS scores from 0 to 2 correspond to patients who are independent in their day-to-day activities, and scores from 3 to 6 refer to patients who are dependent or dead. BI evaluates the basic activities of daily living on a total score from 0 to 100 (independent functional level). GCS evaluates the level of consciousness by assessing eye opening, limb movement and speech responses on a total score from 3 to 15 (fully conscious). Standard ECG, chest X-ray, BP measurement, biochemical analyses and brain computed tomography were also performed on admission.

## ***4.2.3 Heart rate variability analysis (I - III)***

### ***4.2.3.1 ECG recordings***

The subjects were monitored for 24 hours with an ambulatory ECG recorder. The Del Mar Avionics, California (I) and Oxford Medilog, Oxford, UK (II, III) recording systems were used. The recordings were carried out for all subjects within the first week (I) and within 48 hours (III) of hospitalization. The data from the recordings were sampled digitally and transferred to a microcomputer for the analysis of HR variability by a special software package (Hearts, Heart Signal Co., Kempele, Finland). All RR interval tachograms were edited automatically, after which detailed manual editing was performed by visual inspection of the RR intervals to eliminate premature beats and noise. In the final analysis of HR variability, 24-hour measurements were divided into segments of 8000 RR intervals, and only segments with >85% sinus beats were included.

### ***4.2.3.2 Time domain and spectral analysis (I - III)***

The mean length of all RR intervals and the SDNN of all RR intervals were computed as time domain measures of HR variability. An autoregressive model was used to estimate the power spectrum densities of RR interval variability (Burg 1975, Kay & Marple 1981). The power spectra of HR variability were quantified by measuring the area in four frequency bands: <0.0033Hz (ULF), 0.0033-0.04Hz (VLF), 0.004- 0.15Hz (LF) and 0.15- 0.40Hz (HF).

#### 4.2.3.3 Poincaré plot analysis (I)

For quantitative, two-dimensional vector analysis, SD2 and SD1 were analysed and visually presented as Poincaré plot scattergrams, in which each RR interval of a tachogram is plotted as a function of the previous RR interval (Tulppo *et al.* 1996). The Poincaré plot gives a visual view of the RR data by representing qualitatively and as graphical means the kind of RR variations included in the recording. In the computerised analysis, the Poincaré plot is first rotated 45° clockwise, and the standard deviation of the plot data is then computed around the horizontal axis (axis 2), which passes through the data centre (SD1). The standard deviation of continuous long-term RR intervals is quantified by rotating the plot 45° counterclockwise (SD2) and by computing the data points around the horizontal axis (axis 1), which passes through the center of the data.

#### 4.2.3.4 Approximate entropy analysis (I)

ApEn analysis was used to measure the complexity of time series data. It quantifies the regularity or predictability of data and has been developed for time series. ApEn measures the logarithmic likelihood that runs of patterns that are close to each other will remain close in the next incremental comparisons. A greater likelihood of remaining close (high regularity) produces smaller ApEn values, and conversely, random data produce higher values (Pincus & Viscarello 1992, Pincus & Goldberger 1994, Myllylä *et al.* 2002).

#### 4.2.3.5 Detrended fluctuation analysis (I - III)

The DFA technique was used to quantify the fractal-like scaling properties of RR interval data. This method is a modified root-mean-square analysis of random walk, which quantifies the presence or absence of fractal correlation properties and has been validated for time series (Peng *et al.* 1995). In this method, the root-mean-square fluctuation of integrated and detrended time series is measured at each observation window and plotted against the size of the observation window on a log-log scale. HR correlations were defined for short-term (<11 beats,  $\alpha_1$ ) and long-term (>11 beats,  $\alpha_2$ ) correlations of RR interval data (Peng *et al.* 1995, Mäkikallio *et al.* 1997, Myllylä *et al.* 2002).

#### 4.2.3.6 Power-law relationship analysis (II, III)

The power-law relationship of RR interval variability is a spectral measure reflecting the distribution of the spectral characteristics of the RR interval oscillations in the region of the ULF and VLF bands by a previously described method (Bigger *et al.* 1992). The point power spectrum was logarithmically smoothed in the frequency domain, and the power was integrated into bins spaced 0.0167 log(Hz) apart. A robust line-fitting algorithm of log(power) on log(frequency) was then applied to the power spectrum between  $10^{-4}$  to  $10^{-$

<sup>2</sup>, and the slope of this line was calculated ( $\beta$ ). This specific frequency band was chosen on the basis of previous observations regarding the linear relationship between log (power) and log (frequency) in this frequency band in human HR time series data (Saul *et al.* 1991, Bigger *et al.* 1996).

#### ***4.2.4 Natriuretic peptides (IV, V)***

The blood samples for NPs were taken after bedrest on 7 am through an intravenous cannula into EDTA vacuum tubes between the days 2 and 7 after admission into hospital. The samples were placed on ice, centrifuged at 4°C for 10 minutes, and the plasma was stored at -70 °C. The amino-terminal fragments of NPs (NT-proANP and NT-proBNP) were measured directly from 25  $\mu$ l unextracted plasma with specific radioimmunoassays as described previously (Ala-Kopsala *et al.* 2004). Briefly, recombinant human NT-proANP or NT-proBNP standards and samples were incubated in duplicate overnight at +4 °C with specific goat antisera against NT-proANP(46-79) or NT-proBNP(10-29), and radioiodinated recombinant NT-proANP or NT-proBNP, respectively. The bound and free fractions were separated by double antibody precipitation and the radioactivity in the precipitates was measured with Wallac CliniGamma gamma counter.

#### ***4.2.5 Cortisol, catecholamines and adrenocorticotrophic hormone (V)***

Serum cortisol and ACTH concentrations were analysed from blood samples taken on the 2<sup>nd</sup> and 7<sup>th</sup> days after admission. Cortisol samples were taken on mornings and evenings. The analyses were performed by radioimmunoassays using reagent kits from Orion Diagnostica (Espoo, Finland) and Nichols Institute Diagnostics (San Juan Capistrano, CA), respectively. The concentrations of plasma epinephrine and norepinephrine were analysed by high-pressure liquid chromatography using kits from Bio-Rad Laboratories (Irvine, CA).

#### ***4.2.6 Statistics***

The data were analysed using the SPSS software (SPSS 11.0, SPSS Inc. Chicago, Illinois, USA). The Kruskal-Wallis test and the Mann-Whitney U-sample test were used to compare the values of the control subjects and those of the patients. Pearson's correlation coefficients were used to analyse the correlations between the various measures of HR variability in study I and between NPs and other neurochemical and clinical variables in study V. The baseline data were used as the explanatory variables in study II. Univariate comparisons of the baseline characteristics between the different patient and control groups were performed with the chi-square test for the categorical variables and with the 2-sample *t* test for the continuous variables (II, III, IV and V). To find the best cutoff points for all predictive variables, the dichotomous cutoff points that maximised the risk

ratio (RR) obtained from the Cox regression model were sought, with all-cause mortality as the endpoint. RR and 95% confidence intervals (CI) were calculated for each variable (II, III, IV and V). After adjustment for age, clinical variables and medication, the risk variables were included in a stepwise Cox proportional hazards regression analysis to estimate their independent predictive powers. Kaplan-Meier estimates of the distribution of times from baseline to death were computed, and log-rank analysis was performed to compare the survival curves between the groups (II, III and IV). Receiver operating characteristic curves showing sensitivity as functions of specificity were computed with the GraphROC software (Turku, Finland) for comparisons of the performance of HR variability measures (II). The value of  $p < 0.05$  was considered significant.

## **5 Results**

### **5.1 Clinical measures (I, III - V)**

Forty-three (44%) patients were diagnosed as having an ischemic stroke in the right hemisphere, 32 (33%) in the left hemisphere and 22 (23%) in the brainstem. The patients who died during follow-up had significantly lower SSS scores on the 2<sup>nd</sup> ( $25\pm 16$  vs.  $34\pm 12$ ,  $p<0.05$ ) and 7<sup>th</sup> days ( $27\pm 17$  vs.  $41\pm 13$ ,  $p<0.05$ ). MRS and BI scores on the 7<sup>th</sup> day were poorer in the patients who died (V). The patients who died during follow-up were more likely to have a myocardial infarction in their medical history (III), but otherwise the previous diseases and medications did not differ between the groups. The baseline clinical characteristics and previous diseases of the study populations are listed in the Table 2.

*Table 2. Baseline clinical characteristics, previous diseases and survival of stroke patients (studies III, IV, V).*

Variables	Survivors		Non-survivors	
	Study III (n=51)	Study IV and V (n=29)	Study III (n=33)	Study IV and V (n=22)
<b>Clinical variables</b>				
GCS (3-15)	14.1 ± 1.9	13.5±2.5	13.1 ± 2.4	12.1±3.8
SSS (0-58)	36 ± 10	34.2±11.8	27 ± 14*	25.1±15.7*
BI (0-100)	40.2±28.0	39.1±28.2	23.2±28.0	22.9±30.0
MRS (0-6)	3.4±1.2	3.5±1.3	4.0±1.0	4.2±1.0
Glucose (mmol/l)	7.2 ± 2.5	7.0±2.1	6.8 ± 2.2	7.1±2.0
CK-MB (U/l)	2.3 ± 2.0	2.7±2.3	2.2 ± 0.9	2.5±1.5
SBP (mmHg)	162 ± 23	173±35	176 ± 25	176±22
DBP (mmHg)	89 ± 14	94±18	92 ± 17	91±17
Current smoking	12 (24)	7 (24)	16 (48)	5 (23)
<b>Previous diseases</b>				
Diabetes	3 (6)	3 (10)	3 (9)	5 (23)
AMI	2 (4)	3 (24)	6 (18)*	6 (27)
CAD	4 (8)	7 (24)	9 (27)	9 (41)
Hypertension	9 (18)	15 (52)	11 (33)	13 (59)

Values are means ± SD or n (%). \*p < 0.05 compared to survivors; Abbreviations: GCS= Glasgow Coma Scale score, SSS= Scandinavian Stroke Scale score, BI=Barthel Index, MRS=Modified Ranking Scale, CK-MB= myocardium specific creatinekinase, SBP=systolic blood pressure, DBP=diastolic blood pressure, AMI=acute myocardial infarction, CAD=coronary artery disease.

## 5.2 Mortality of subjects (II – IV, V)

During the follow-up of nearly 7 years (83±54 months), 33 out of 84 (39%) brain infarction patients died (III). There were 14 (42%) cardiac deaths, 11 (33%) cerebrovascular deaths and 8 (24%) deaths from other causes. In the studies IV and V, the mortality of stroke patients was 43% (22/51) and the mortality of AMI patients in study IV was 12% (6/51) during the follow-up of 44±21 months. In study II, cerebrovascular mortality was 8% (25/325) during the 10-year follow-up.

### 5.3 Heart rate variability in ischemic stroke

#### 5.3.1 Time domain and spectral measures of heart rate variability

##### (I - III)

Concurrently with previous studies, the conventional time domain and spectral measures of HR variability showed reduced values in ischemic stroke patients. In the patients with hemispheric brain infarction as well as in the patients with medullary brainstem infarction, the values of SDNN ( $p < 0.001$ ), VLF (hemispheric,  $p < 0.001$ ; medullary,  $p < 0.01$ ) and LF (hemispheric,  $p < 0.001$ ; medullary,  $p < 0.05$ ) were lower than those of the control subjects in the acute phase of stroke. After 6-month follow-up, the values of SDNN ( $p < 0.001$ ), VLF ( $p < 0.01$ ) and LF ( $p < 0.05$ ) of the patients with hemispheric brain infarction were still impaired in comparison with those of the control subjects, but no differences were found between the values of the patients with brainstem stroke and those of the control subjects (I).

Time domain measures of HR variability (SDNN, HF, LF, VLF, ULF) did not differ significantly between the survivors of ischemic stroke and those who died during the 7 years' follow-up (III). In the general elderly population, no difference in SDNN was observed between the survivors and the subjects who succumbed to cerebrovascular death during the 10-year follow-up (II).

#### 5.3.2 Non-linear measures of heart rate variability (I - III)

Patients with ischemic brain infarction in study I were found to have lower SD2 values as compared to the healthy controls. The values of SD1 and the complexity and fractal measures of HR variability (ApEn,  $\alpha_1$  and  $\alpha_2$ ) of the patients and the control subjects were similar (I). The measures of HR variability in the acute phase in the ischemic stroke patients and healthy subject are shown in Table 3.

The HR variability scaling measures  $\alpha$  and  $\beta$  showed significant differences in the patients who died during the 7-year follow-up compared to the survivors. The long-term power-law slope  $\beta$  was steeper ( $p < 0.05$ ) and the short-term scaling exponent  $\alpha$  lower ( $p < 0.01$ ) among the patients who died during the follow-up than among the survivors (III). The long-term power-law slope was significantly steeper ( $p < 0.01$ ) in the elderly subject who died from cerebrovascular causes during the 10-year follow-up as compared to the survivors. The short-term scaling exponent  $\alpha$  did not differ between these groups (II).

*Table 3. Measures of HR variability in the acute phase in healthy subjects and in patients with hemispheric, medullary and pontine ischemic strokes (study I).*

Variables	Healthy (n=30)	Patients		
		Hemisphere (n=31)	Medulla (n=8)	Pons (n=7)
SDNN	161±34	109±113***	100±29***	163±48
VLF	2277±1262	1184±496***	946±669**	2520±1445
LF	980±704	494±298**	489±446*	1325±857
HF	860±1624	330±337	331±369	1120±1522
SD1	29±20	22±10	21±11	35±22
SD2	133±34	107±31**	93±26**	150±22
ApEn	1.10±0.19	1.11±0.18	1.03±0.24	1.08±0.25
$\alpha$ 1	1.23±0.19	1.20±0.19	1.16±0.18	1.20±0.23
$\alpha$ 2	1.08±0.09	1.05±0.10	1.07±0.07	1.04±0.09

Values are means±SD. Abbreviations: SDNN= standard deviation of all RR intervals, VLF= very low frequency, LF= low frequency, HF= high frequency, SD1= instantaneous beat-to-beat RR interval variability, SD2= long-term continuous RR interval variability, ApEn= approximate entropy,  $\alpha$ 1= short-term scaling exponent,  $\alpha$ 2= long-term scaling exponent. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 for comparisons between patients and healthy subjects.

### **5.3.3 Heart rate variability measures as predictors of mortality (II, III)**

Abnormal HR variability scaling measures  $\alpha$  and  $\beta$ , high age, prior myocardial infarction and poor SSS score were associated with an increased risk of death in univariate analysis. The power-law slope  $\beta$  showed the most significant prognostic power (p<0.001), followed by the short-term scaling exponent  $\alpha$  and prior myocardial infarction. In multivariate analysis, after adjustment for age, the power-law slope  $\beta$  was the only risk variable that showed independent prognostic value for mortality (p<0.001) (Table 4). The Kaplan-Meier survival curve of the power-law slope  $\beta$  is presented in Figure 4 (III). In the general elderly population, the power-law slope  $\beta$  predicted independently cerebrovascular mortality (p<0.001) (Table 5), whereas the short-term scaling exponent  $\alpha$  predicted sudden cardiac death, not cerebrovascular death (II).

*Table 4. HR variability risk variables as predictors of death after stroke (study III).*

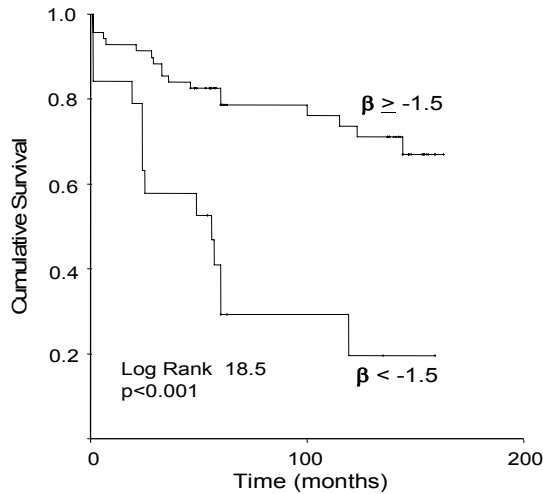
Analysis	Death Hazard ratio† (95% CI)
Univariate	
Age	3.1 (1.6-6.5)*
SSS (< 30)	2.8 (1.1-7.2)*
Prior AMI	3.4 (1.4-8.6)**
$\beta$ slope (<-1.5)	4.5 (2.2-9.5)***
$\alpha$ (<0.75)	5.2 (1.6-17.8)**
SDNN (<70 ms)	1.8 (0.6-6.3)
Multivariate	
SSS (< 30)	2.4 (0.9-6.1)
Prior AMI	2.1 (0.8-5.5)
$\beta$ slope (<-1.5)	3.8 (1.8-8.2)***
$\alpha$ (<0.75)	2.9 (0.9-10.5)

Abbreviations: CI= confidence intervals, others: see Tables 1 and 2. † Hazard ratio assessed by Cox regression analysis (multivariate adjusted for age). \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

*Table 5. Associations of HR variability variables with cerebrovascular mortality (study II).*

Analysis	Relative Risk (95% CI)
Univariate	
SDNN (< 120ms)	1.7 (1.1-2.4)*
$\alpha$ (< 1.0)	1.5 (0.9-2.4)
$\beta$ (< -1.5)	2.0 (1.4-3.0)***
Multivariate (Adjusted for all risk variables)	
SDNN (< 120ms)	1.6 (1.0-2.5)
$\alpha$ (< 1.0)	1.2 (0.7-2.2)
$\beta$ (< -1.5)	2.8 (1.7-4.8)***

Abbreviations: see previous tables. \*p<0.05 and \*\*\*p<0.001



**Fig. 4. Kaplan-Meier survival curve for the subjects with the power-law slope  $\beta$  less or more than -1.50. Estimated cumulative survival rate over a 7-year period was 67% in those with a slope over -1.50 and 20% in those with a slope under -1.50 (study III).**

## 5.4 Neurohumoral measures in ischemic stroke

### 5.4.1 Natriuretic peptides in the acute phase of stroke (IV and V)

The first week's average plasma concentrations of N-ANP were about 3-fold and those of N-BNP about 12-fold compared to the values of normal volunteers ( $p < 0.001$  and  $p < 0.01$ ). These levels remained significantly elevated on the days 2 and 7 (V). The plasma values of N-ANP and N-BNP in the ischemic stroke patients were equally high as the values in the AMI patients, with a tendency towards higher N-ANP values in the stroke patients ( $p = 0.07$ ). The healthy controls had significantly lower N-ANP ( $p < 0.001$ ) and N-BNP ( $p < 0.01$ ) values than the stroke or AMI patients (IV).

The N-ANP and N-BNP average plasma concentrations on the first week were significantly higher among the stroke patients who died during the follow-up than among the survivors ( $1491 \pm 1397$  pmol/l vs.  $630 \pm 400$  pmol/l,  $p < 0.01$  and  $1235 \pm 2018$  pmol/l vs.  $247 \pm 226$  pmol/l,  $p < 0.01$ , respectively). The differences in the NP values between the groups remained significant throughout the first week (V). Similarly, the plasma concentrations were higher among the AMI patients who died during the follow-up than among the survivors (IV).

### 5.4.2 Cortisol, catecholamines and adrenocorticotrophic hormone in the acute phase of ischemic stroke (V)

Serum morning cortisol concentrations were significantly higher on the days 2 and 7 in the patients who died than in the survivors (0.6, range 0.3-1.2  $\mu\text{mol/l}$  vs. 0.4, range 0.1-0.6  $\mu\text{mol/l}$ ,  $p < 0.05$  and 0.5, range 0.0-0.9  $\mu\text{mol/l}$  vs. 0.3, range 0.0-0.8  $\mu\text{mol/l}$ ,  $p < 0.05$ , respectively), as were the evening cortisol levels on the 2<sup>nd</sup> day and the ACTH levels on the 7<sup>th</sup> day. The BP values and other laboratory tests did not reveal significant differences between the stroke survivors and those who died. The serial first-week measurements of clinical variables and the survival of the patients are listed in Table 6.

Table 6. First-week measurements of clinical and chemical variables and survival of stroke patients.

Variables	Day 2		Day 7	
	Non-survivors (n=22)	Survivors (n=29)	Non-survivors (n=22)	Survivors (n=29)
N-ANP (pmol/l)	1501(180-5468)***	592(183-1554)	1611(283-5045)**	653(103-1576)
N-BNP (pmol/l)	1408(40-9910)***	244(50-1098)	1062(43-6650)***	241(50-1064)
norepi (nmol/l)	6.0(1.3-38.9)	2.0(0.7-3.7)	2.4(1.2-7.2)	1.9(0.9-3.1)
mcors ( $\mu\text{mol/l}$ )	0.6(0.3-1.2)*	0.4(0.1-0.6)	0.5(0.0-0.9)*	0.3(0.0-0.8)
ecors ( $\mu\text{mol/l}$ )	0.6(0.2-2.1)**	0.2(0.0-0.7)	0.3(0.0-0.7)	0.2(0.0-1.1)
ACTH (pmol/l)	7.4(1.9-19.4)	6.3(1.0-29.0)	6.9(3.5-14.0)*	4.3(1.0-14.4)
epi (nmol/l)	0.9(0.1-4.2)	0.2(0.1-0.6)	0.2(0.1-0.5)	0.3(0.1-0.8)
SSS (0-58)	25(2-51)*	34(12-50)	27(2-52)*	41(15-54)
MRS (0-6)	4.1(2-5)	3.5(1-5)	4.0(1-5)*	3.1(1-5)
BI (0-100)	23(10-90)	39(5-95)	28(10-100)*	58(5-100)
SBP (mmHg)	164(113-237)	150(101-200)	152(118-214)	152(116-203)
DBP (mmHg)	79(50-98)	85(60-115)	84(62-106)	87(67-125)

Values are means (range). Abbreviations: N-ANP= N-terminal-ANP, N-BNP= N-terminal-BNP, norepi= norepinephrine, epi= epinephrine, mcors= morning cortisol, ecors= evening cortisol, ACTH= adrenocorticotrophic hormone, BI= Barthel Index score, MRS= Modified Ranking Scale score, SSS= Scandinavian Stroke Scale score, SBP= systolic blood pressure, DBP= diastolic blood pressure. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$  and \*  $p < 0.05$  between survivors and non-survivors.

Table 7 presents the correlations between the various chemical and clinical measures. The N-ANP values correlated significantly with the morning and evening cortisol values on the days 2 and 7. Similar correlations were observed between the N-BNP and cortisol values. Significant correlations were also found between the 7th day NP values and the norepinephrine levels as well as between the N-ANP values and the SSS and BI scores. The cortisol values correlated significantly with N-ANP, N-BNP, norepinephrine, epinephrine, ACTH and the measures of neurologic deficit (SSS- and BI-scores).

Table 7. Pearson correlation coefficients for the first-week chemical and clinical variables (study V).

Variables	norepi	epi	mcors	ecors	ACTH	SSS	MRS	BI
ANP2	.199	.540**	.546***	.643***	-.118	-.265	.278	-.314*
BNP2	.284	.669***	.634***	.717***	-.048	-.240	.220	-.248
ANP7	.538**	.589**	.546**	.686***	.124	-.369*	.315	-.375*
BNP7	.774**	.810***	.641***	.818***	.095	.314	.252	-.279
norepi		.856***	.613**	.767**	.390	-.501**	.311	-.296
epi			.694***	.937***	.407*	-.496**	.355	-.349
mcors				.757***	.487**	-.191	.072	-.054
ecors					.489**	-.439**	.365*	-.313
ACTH						-.017	-.242	-.313

Abbreviations: ANP2= 2<sup>nd</sup> day N-ANP, BNP2= 2<sup>nd</sup> day N-BNP, ANP7= 7<sup>th</sup> day N-ANP, BNP7= 7<sup>th</sup> day N-BNP, others see table 5 (variables are 2<sup>nd</sup> day assessments). \*\*\* p<0.001, \*\* p<0.01 and \* p<0.05.

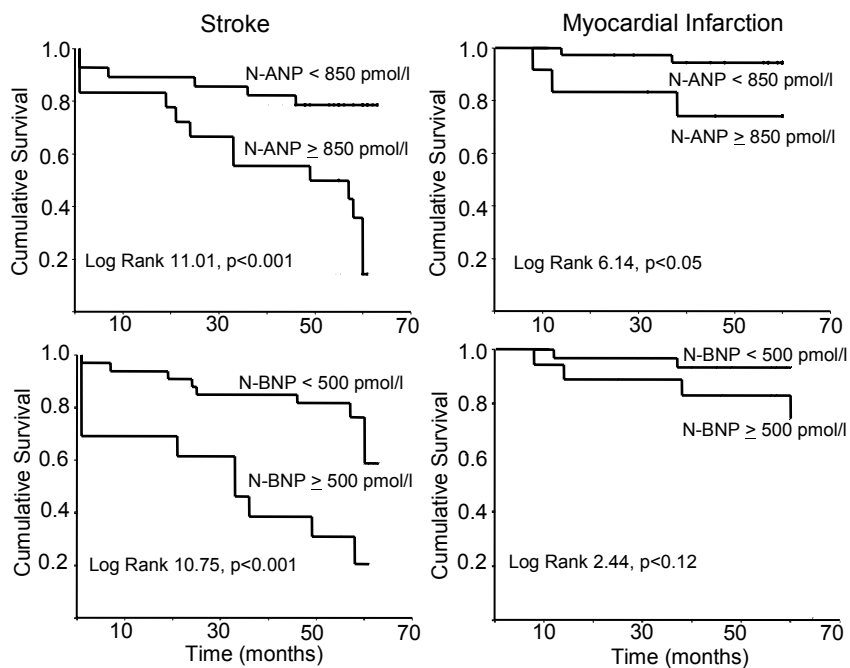
#### 5.4.3 Neurohumoral factors as predictors of mortality (IV, V)

Among the patients with stroke, both N-ANP and N-BNP had significant prognostic value. A low SSS score, high age and high cortisol levels also predicted death. In multivariate analysis, after adjustment for age, diabetes, CAD and usage of ACE-inhibitors and  $\beta$ -blockers, N-ANP, N-BNP and cortisol levels showed independent prognostic values for mortality, while SSS did not (Table 8). Among the AMI patients, N-ANP was also significantly prognostic, while N-BNP did not reach statistical significance. The Kaplan-Meier survival curves showed poor survival for the stroke and AMI patients with N-ANP higher than 850 pmol/l. The cumulative survival rate was 80% for the stroke patients with N-ANP lower than 850 pmol/l and 20% for those with higher values, estimated over the 4-year follow-up period. Similarly, the AMI patients with high N-ANP values had poorer survival than those with lower N-ANP levels (Figure 5).

*Table 8. Clinical risk variables as predictors of death after stroke (studies IV and V).*

Analysis	Death (n=22)
Univariate	Risk ratio (95% CI)
Age	7.5 (1.0-56.3)*
SSS (< 30)	2.5 (1.1-6.1)*
N-ANP (>850pmol/l)	4.3 (1.6-11.2)**
N-BNP (>500pmol/l)	3.9 (1.6-9.4)**
mcors (>0.5µmol/l)	5.9 (1.3-26.8)*
ecors (>0.3µmol/l)	7.7 (2.1-29.0)**
Multivariate †	
SSS (< 30)	1.2 (0.5-3.3)
N-ANP (>850pmol/l)	3.9 (1.2-12.6)*
N-BNP (>500pmol/l)	3.7 (1.4-10.0)*
mcors (>0.5µmol/l)	5.4 (1.3-32.7)*
ecors (>0.3µmol/l)	5.8 (1.5-40.1)*

Abbreviations: CI, confidence interval; †, risk ratio assessed by Cox regression analysis (adjusted for age, diabetes, CAD, medications); others: see previous tables \*\* p<0.01 and \* p<0.05



**Fig. 5. Kaplan-Meier survival curves for stroke and acute myocardial infarction patients with N-ANP plasma concentrations under or over 850 pmol/l and N-BNP concentrations under or over 500 pmol/l. The estimated cumulative survival rate over a 4-year period was 80% for the stroke patients with N-ANP lower than 850 pmol/l and 20% for those with N-ANP higher than 850 pmol/l.**

## 6 Discussion

### 6.1 General aspects

Autonomic cardiovascular disturbances have been detected in brain infarction patients for a long time (Talman 1985, Oppenheimer *et al.* 1990). Pathologic changes in the myocardium, fluctuations in BP and ECG changes are well known, but they are often neglected in clinical practice because they are not well described in the literature and textbooks and do not assist in the diagnosis of the cerebral lesion. Reduction of HR variability is also a well documented phenomenon in stroke patients (Korpelainen *et al.* 1996a, Naver *et al.* 1996, Orlandi *et al.* 2000, Phillips *et al.* 2000, Meglic *et al.* 2001), but its clinical or prognostic significance is largely unknown. A stress response consisting of increased levels of cortisol and catecholamines in the first weeks after acute stroke has been known since the 1950s (Oka 1956, Feibel *et al.* 1977, Meyer *et al.* 1973, Myers *et al.* 1981), but information concerning its prognostic significance is still limited and the role of other neurohormonal systems in stroke also needs to be clarified. More attention to this topic is warranted because these disturbances can be serious or even fatal and may worsen the prognosis of the primary condition.

Previous methods for assessing the autonomic cardiovascular function have been either non-practical for bedside use or unable to offer reliable prognostic information. In this study, we wanted to test new methods for the evaluation of cardiovascular autonomic regulation in ischemic stroke patients and wanted to find out if these methods predict survival in stroke patients equally efficiently as they have previously been shown to do in patients with cardiac diseases (Huikuri *et al.* 1998, Mäkikallio *et al.* 1999, Morrow *et al.* 2003, Galvani *et al.* 2004, Kragelund *et al.* 2005, Ndrepepa *et al.* 2005). The main finding of this study was that an autonomic cardiovascular dysfunction exists in the acute phase of ischemic stroke involving activation of the neurohormonal system, including elevated levels of natriuretic peptides, cortisol and catecholamines, and a disturbance in the long-term dynamics of HR. These disturbances predict survival in stroke patients.

Methods to predict the survival after stroke are of limited value in the management of individual patients and can be harmful if they are used to determine access to treatment. They do, however, have a role in predicting the outcome for groups of patients, as in

adjusting for differences in baseline characteristics in multicentre trials involving different treatments. Prognostic information is also important for the patient and the family and may improve their compliance with the offered treatments. This study introduces new candidate methods for the assessment of survival in ischemic stroke. More importantly, the new findings we have obtained will raise several questions and open up new perspectives for future research work to further elucidate the mechanisms attributable to the onset of the cerebrovascular disease causing a stroke and the subsequent series of events leading to death or recurrence rather than recovery. Therapeutic interventions will emerge once better understanding of the pathobiology of the disease processes is achieved. This study aims to increase that understanding. Possible mechanisms explaining the findings as well as perspectives for future studies and clinical implications will be discussed in the following paragraphs.

## 6.2 Heart rate dynamics in ischemic stroke

Autonomic cardiovascular dysfunction has previously been evaluated by conventional linear measures of HR variability. Suppressed values of all time and frequency domain measures of HR variability as a consequence of both hemispheric (Korpelainen *et al.* 1996a, Naver *et al.* 1996, Orlandi *et al.* 2000) and brainstem cerebral infarctions (Korpelainen *et al.* 1996b, Phillips *et al.* 2000, Meglic *et al.* 2001) have been observed, indicating the presence of derangement of both the sympathetic and parasympathetic systems (Task force 1996). Non-linear measures of HR variability have been developed to quantify complex HR dynamics and are suggested to show patterns of RR interval behaviour not easily detected by the commonly used methods based on moment statistics of HR variability (Mäkikallio *et al.* 1996). The prognostic significance of HR variability measures after ischemic stroke has not been studied previously, and non-linear HR variability methods have previously been used in only one study. Oppenheimer *et al.* (1996) reported decreased randomness (ApEn) of HR variability in patients with left insular lesion in a study of six insular stroke patients and one patient with an insular tumor. This study is therefore the first study using nonlinear HR variability measures in a larger ischemic stroke population and the first to demonstrate the value of these methods in the prediction of mortality in stroke.

The first major finding in this study was that the disturbance in the long-term behaviour of HR dynamics is related to poor survival of stroke patients. The long-term continuous variability from Poincaré plots (SD2) was first observed to be impaired after hemispheric and medullary ischemic strokes (study I), and later another measure of long-term HR dynamics, power-law slope  $\beta$ , was used and found particularly effective in the prediction of mortality (studies II and III). What was interesting, however, was the finding that the long-term behaviour of HR dynamics (power-law slope  $\beta$ ) was altered both before a deadly cerebrovascular insult (study II) and after an ischemic stroke with a poor survival (study III), suggesting that the disturbance of HR dynamics may not only be caused by the cerebral lesion, but may also have existed before the cerebral insult and may even have been related to the causative factor of ischemic stroke. Alterations in the long-term behaviour of HR dynamics have also previously been related to

cerebrovascular accidents (Huikuri *et al.* 1998), whereas short-term HR dynamics is more closely related to cardiac causes of death (study II, Mäkikallio *et al.* 1999, Huikuri *et al.* 2000, Mäkikallio *et al.* 2001).

### ***6.2.1 Possible pathophysiological mechanisms for abnormal long-term HR dynamics***

The physiology of long-term HR dynamics is largely unknown, but the observation of significantly steeper power-law slopes in denervated hearts suggests that it is at least partly influenced by the autonomic input into the heart (Bigger *et al.* 1996). The slope has also been shown to be steeper in elderly subjects (Saul *et al.* 1987, Bigger *et al.* 1996), suggesting that the autonomic modulation of long-term HR behaviour is altered with advancing age. The age-related changes in long-term HR dynamics may arise from degenerative changes in various organs and regulatory systems, which may, by interacting with each other, impair the function of cardiovascular autonomic regulation. In this study, a disturbance in long-term HR dynamics was observed even before the cerebrovascular accident, and it was found to have deleterious effects on the long-term outcome, which also supports the suggestion that the long-term behaviour of HR dynamics must be attributable to a persistent condition. One such explanatory factor could be atherosclerosis. Since the disturbances of long-term HR dynamics seem to be particularly related to cerebrovascular rather than cardiac diseases, the most progressive atherosclerosis in these patients is assumed to be located in the precerebral or cerebral arteries.

Some investigators have suggested that long-term fluctuations of HR may be explained by thermoregulatory mechanisms (Akselrod *et al.* 1981, Lindqvist *et al.* 1989, Dwain & Eckberg 1997, Pagani *et al.* 1997, Kinugasa *et al.* 1999). ANS dysfunction manifested as thermoregulatory disturbances is common in ischemic stroke. An asymmetric sweating pattern has been observed in the majority of both brainstem (Korpelainen *et al.* 1995, Naver *et al.* 1995, Rousseaux *et al.* 1996) and hemispheric ischemic stroke patients (Korpelainen *et al.* 1993a, Korpelainen *et al.* 1995, Kim *et al.* 1995, Wanklyn *et al.* 1995). Interestingly, the degree of thermoregulatory dysfunction has also been associated with the severity of the neurologic deficit (Korpelainen *et al.* 1995), which is a powerful predictor of poor outcome (Sumer *et al.* 2003), paralleling our finding of the association between increased mortality and altered long-term HR dynamics.

Another proposed mechanism for long-term HR fluctuations is the ability of the renin-angiotensin system (RAS) (Kinugasa *et al.* 1999) to regulate long-term BP levels and HR fluctuations. RAS is acknowledged to play an important role in the pathophysiology of cardio- and cerebrovascular diseases (Dahlöf 1995, Fyhrquist *et al.* 1995, Booz & Baker 1998, Willenheimer *et al.* 1999, Daugherty *et al.* 2000, Fogo 2000, Barone *et al.* 2001), and its activation elevates BP and increases the secretion of NPs (Booz & Baker 1996, Lijnen & Petrov 1999, Baltatu *et al.* 2000, Hankey 2003). Therefore, the next step in our study on cardiovascular autonomic regulation in ischemic stroke was to evaluate the role of NPs in it.

## 6.3 Neurohormonal disturbances in ischemic stroke

### 6.3.1 *Natriuretic peptides in ischemic stroke*

The second main finding in this study was that the elevated levels of NP in the acute phase of ischemic stroke predict long-term mortality after stroke. In the acute phase of stroke, the plasma levels of NPs have been reported to be elevated (Etgen *et al.* 2005, Estrada *et al.* 1994), and elevated levels have been shown to correlate with poor functional outcome at 3 months (Etgen *et al.* 2005), but the value of NPs as predictors of mortality after cerebrovascular accidents has not been investigated before this study. An association between elevated levels of NPs and increased mortality was first established in patients with heart failure (Cowie *et al.* 2002, Anand *et al.* 2003) and with acute coronary syndromes (Galvani *et al.* 2004, Morrow *et al.* 2003). Recently, elevated levels of N-BNP have been associated with stable CAD (Jernberg *et al.* 2002, James *et al.* 2003), and NP values were found to correlate with the clinical and angiographic severity of CAD (Kragelund *et al.* 2005, Ndrepepa *et al.* 2005). Plasma NP levels have also been reported to predict the risk of cardiovascular events, including the risk of cerebrovascular ischemia, suggesting that the NP system may be activated even before the onset of clinically apparent cardiovascular disease (Wang *et al.* 2004). Further evidence was provided by Rubattu *et al.* (2004), who found that polymorphisms of the gene for ANP and abnormality of the gene encoding type A NP-receptor (NPRA) were associated with the risk of ischemic stroke. Previously, the same polymorphism has been found to be associated with higher circulating levels of ANP in essential hypertensives (Widecka *et al.* 2002) and with nonfatal myocardial infarction and the extent of CAD in coronary angiography (Gruchala *et al.* 2003). Evidence from animal studies has documented that abnormalities of the NPRA gene are involved in the pathogenesis of hypertension, as shown by the NPRA abnormalities seen in the brains of hypertensive rats even before the development of hypertension (Woodart *et al.* 2003). Also, a study using human hearts showed that NPs and their receptors are actively modulated during the progression of CAD (Casco *et al.* 2002). Concluding from the experimental and clinical evidence, it is suggested that the NP system is involved in the pathobiology of hypertension and atherosclerosis, and that pathological activation of this neurohormonal system is prognostically unfavourable.

In study IV, the NP values in the acute phases of ischemic stroke and AMI were compared, and the stroke patients were observed to have equally high or even higher NP plasma values than the AMI patients, although an acute cardiac event was ruled out. This suggests that neurohormonal activation exists in both conditions, but the mechanisms for elevated NP levels must be distinct in cerebral and myocardial ischemia. It was surprising to discover high NP levels in acute cerebral ischemia, because markedly higher NP levels were expected in AMI patients based on the knowledge that acute myocardial ischemia is associated with an NP elevation proportional to the severity of ischemia (Sabatine *et al.* 2004). High NP levels in stroke may be caused by an increased secretion of NPs from various extracardiac sites, such as the brain and the vascular wall, in addition to secretion from the myocardium. Based on immunohistochemical studies, NP release has been

suggested to be associated with the intensity of brain ischemia, reflecting increased biosynthesis and secretion from ischemic brain tissue (Giuffrida *et al.* 1992, Nogami *et al.* 2001). In concordance with this suggestion, the acute phase NP values of the stroke patients correlated significantly with the severity of neurologic deficit assessed by SSS, BI and MRS. However, as predictors of survival, NPs surpassed the measures of neurological deficits, indicating that NPs provide prognostic information incremental to that obtained from measures assessing merely the clinical severity of brain injury.

### **6.3.2 Renin-angiotensin system in stroke**

The importance of neurohormonal activation in the course of cardiovascular disease is well known, and RAS plays a central role in this (Lindpaintner & Ganten 1991, Dzau 1992, Dahlöf 1995). The role of RAS in cerebrovascular disease has come under scrutiny as a result of evidence suggesting that pharmacological therapies affecting the RAS and the NP systems can reduce the risk of stroke and improve the prognosis after a stroke (The Heart Outcomes Prevention Evaluation Study Investigators 2000, PROGRESS Collaborative group 2001, Bosch *et al.* 2002, Sleight *et al.* 2001, Schrader *et al.* 2005). Activation of RAS is known to elevate BP and is assumed to induce atherogenesis and thrombosis by several mechanisms (Hankey 2003). The end product of RAS, angiotensin II, exerts a number of harmful effects on the cardiovascular system and potentiates the activity of other neurohormonal systems, including the NP system (Jilma *et al.* 1997, Willenheimer *et al.* 1999). Angiotensin II has been proposed as a specific risk factor for stroke based on the results of clinical trials, indicating that measures inhibiting the formation or action of angiotensin II prevent stroke and suggest that a substantial proportion of the effect may be independent of BP lowering (The Heart Outcomes Prevention Evaluation Study Investigators 2000, PROGRESS Collaborative group 2001, Bosch *et al.* 2002, Sleight *et al.* 2001, Schrader *et al.* 2005). Natriuretic peptides oppose the vasoconstrictory, hypervolemic and sympathoexcitatory effects of angiotensin II (Benarroh 1999, Hall 2004), and elevated NPs can therefore be seen as an indicator of RAS activation. In the acute phase of stroke, the increased activity of SNS (Meyer *et al.* 1973, Talman 1985, Myers *et al.* 1981, Sander *et al.* 2001) activates RAS by stimulating the release of renin from the juxtaglomerular cells of the kidney, which in turn leads to elevated levels of angiotensin II (Feingold *et al.* 1990). Furthermore, angiotensin II is suggested to have direct excitatory effects on the sympathetic pre- and postsynaptic nerve endings (Ohlstein *et al.* 1997), further aggravating neurohumoral activation. These mechanisms provide us with another plausible explanation of the elevated natriuretic peptide values seen during the first week after a stroke.

Local production of angiotensin II is suggested to explain the beneficial effects of RAS inhibitors on cardiovascular diseases even in the absence of angiotensin II plasma levels that directly increase BP (Danser 1996, Dhang *et al.* 1999). The role of brain RAS in the central control of cardiovascular homeostasis and pathophysiology is well documented (Fink *et al.* 1987, Luft *et al.* 1989, Fink *et al.* 1991, Gorbea *et al.* 1994). Complementing and interacting with endocrine RAS, brain RAS is postulated to contribute to the pathology of hypertension and cardiovascular diseases (Fink *et al.*

1997). Progress in genetic and transgenic technology will increase our understanding of brain RAS in the future, but based on the contemporary results from animals studies (Baltatu *et al.* 2004), it seems logical to assume that cerebral diseases, such as brain infarction, would cause alterations in the brain RAS functions and therefore disturbances in the central control of cardiovascular function.

### ***6.3.3 Other neurohormonal factors in ischemic stroke***

Previous studies have indicated increased activity of the hypothalamus-pituitary-adrenal (HPA) axis with elevated circulating cortisol levels (Olsson *et al.* 1992, Fassbender *et al.* 1994, Slowik *et al.* 2002) and pathologic sympathetic activation with elevated catecholamine levels (Meyer *et al.* 1973, Myers *et al.* 1981, Sander & Klingelhöfer 1994, Sander *et al.* 2001) in the acute phase of ischemic stroke. Similar neurohormonal activation was also seen in this study, although the limits of normal reference ranges were not exceeded, as has also been reported in other studies (Fassbender *et al.* 1994, Christensen *et al.* 2004a). However, cortisol levels were found to be significantly higher in the stroke patients who died during the follow-up compared to the survivors, and elevated cortisol levels were found to be predictive of mortality even independently of other risk factors (study V). In ischemic stroke patients, elevated levels of cortisol have previously been associated with stroke severity and death in the short term (Christensen *et al.* 2004a, Marklund *et al.* 2004). In this study, a similar association between cortisol levels and the measures of neurologic deficit (assessed by SSS, MRS and BI) reflecting the severity of the cerebral lesion was observed, and elevated cortisol levels were, for the first time, associated with a poor long-term prognosis (study V).

During the first few days after a stroke, an initial rise in ACTH and cortisol and a subsequent, rapid decline of ACTH have been shown (Fassbender *et al.* 1994, Marklund *et al.* 2004), suggesting initial stress-induced activation of the hypothalamus, followed by cortisol-induced feedback suppression of ACTH levels. This pattern was observed in this stroke population among the survivors, but the patients with a poor survival showed a less obvious ACTH decline, providing another piece of evidence for possible HPA axis dysfunction in the acute phase of ischemic stroke in patients with a poor survival.

Glucocorticoids are known to stimulate NP synthesis in cardiac myocytes (Nishimori *et al.* 1997) and NP release in healthy subjects (Brotman *et al.* 2005). This study is the first to show an association between elevated levels of cortisol and NPs in patients with acute ischemic stroke, suggesting that the neurohormonal disturbance in acute ischemic stroke involves the HPA axis, catecholamines and the NP system.

#### ***6.3.3.1 Mechanisms for increased post-stroke mortality***

The neurohumoral disturbance involving the HPA axis and catecholamines may lead to poor survival of stroke patients by several mechanisms. The association between cortisol levels and the measures of neurologic deficit (assessed by SSS and MRS) reflecting the severity of stroke observed in this study supports the hypothesis implying a deleterious

effect of high cortisol levels on the cerebral lesion. The severity of the cerebral damage assessed by SSS also predicted long-term mortality, but its predictive power was weaker than that of NPs and power-law slope  $\beta$  (studies III and IV). This suggests that the severity of stroke proposes poor survival, but NPs and long-term HR dynamics are better predictors of mortality and presumably describe prognostically relevant phenomena that are not directly related to the size of the primary lesion.

Glucocorticoids have been demonstrated to exacerbate the ischemic injury to neurons (Sapolsky & Pulsinelli 1985, Sapolsky 1996), especially hippocampal neurons (Antonawich *et al.* 1999). As the hippocampus is suggested to have an important role in the feedback regulation of the HPA axis (Seckl & Olsson 1995), its dysfunction may further exacerbate the existing brain damage, causing a vicious circle and a poor outcome.

Some researchers have suggested that the association between high stress hormone levels and less favourable outcome could be related to cardiac abnormalities resulting from the elevated levels of glucocorticoids and catecholamines (Feibel *et al.* 1977, Myers *et al.* 1981). There is convincing evidence to indicate that stroke causes myocardial cell damage related to stroke severity and may contribute to poststroke cardiac morbidity and mortality (Oppenheimer *et al.* 1990b, James *et al.* 2000, Christensen *et al.* 2004b). High levels of circulating catecholamines and various kinds of stress with elevated cortisol levels can induce this cerebrogenic myocardial damage and the ischemic-like ECG changes observed in the acute phase of stroke (Hachinski *et al.* 1986, Woolf *et al.* 1987, Oppenheimer *et al.* 1991). Since the myofibrillar damage is predominantly subendocardial (Greenhoot & Reichenbach 1969, Samuels 1999), it may involve the cardiac conducting system, thus predisposing to cardiac arrhythmias. The cardiac lesion, combined with the propensity of catecholamines to produce arrhythmias even in a normal heart may well give rise to a risk of serious arrhythmia (Zaza *et al.* 1991, Grassi *et al.* 2003). The myocardial injury is suggested to be responsible for the frequently encountered elevated levels of cardiac enzymes in the acute phase of stroke. Cardiac troponin I levels were recently reported to be related to elevated cortisol levels in acute stroke, which further supports this hypothesis (Christensen *et al.* 2004b). Cerebral damage at a specific location, particularly in the insular cortex, has been shown to result in an increase in the circulating levels of catecholamines (Smith *et al.* 1986, Strittmatter *et al.* 2003, Meyer *et al.* 2004) as well in poor long-term outcome and increased mortality (Tokgözoğlu *et al.* 1999, Sander *et al.* 2001), suggesting this as a mechanism for the cardiac complications associated with stroke. Unfortunately, because of the lack of brain MRI information and the limited size of the population, the relations between the location of the cerebral lesion, the cardiovascular autonomic and neurohumoral disturbances and the different modes of death could not be resolved in this study. However, the changes in cardiac enzymes and electrophysiology are usually reversed fast after the acute phase (Talman 1985), suggesting that the myocardial damage is a transient condition and, as such, cannot be responsible for the increased long-term mortality observed in this study. Therefore, the poor long-term outcome of stroke patients is more probably due to a persistent condition reflected by the neurohormonal disturbances in the RAS and NP systems discussed earlier, or it may also reflect the severity of the primary cerebral lesion possibly leading to functional disability, permanent institutionalisation and, therefore, an

increased risk of death (Sacco *et al.* 1994, Sacco *et al.* 1997, Hankey *et al.* 2000, Kimura *et al.* 2005).

## 6.4 Future perspectives

Despite what we have learned about cardiovascular autonomic disturbances in cerebrovascular diseases from clinical observations and experimental studies in the past two decades, there are still wide gaps in our knowledge. This study has shown that prognostically unfavourable cardiovascular autonomic disturbances exist in ischemic stroke, involving an alteration in the long-term HR dynamics that seems to be a specifically stroke-related phenomenon and an extensive neurohormonal disturbance involving the NP system, HPA axis and catecholamines. These findings are, however, only preliminary, and larger studies to confirm the results are warranted. The physiological background of the long-term HR fluctuations and NP elevation in ischemic stroke needs to be further elucidated, to better understand their role in the disease process. Also, larger populations should be studied to find out if these cardiovascular disturbances predict cerebrovascular and cardiac deaths differently and if they also predict poststroke functional disability? Larger sample size would also permit an assessment of relations between cardiovascular disturbances and lesions in different cerebral locations. Further studies are also needed to study the role of NPs in ischemic brain. Do they aggravate the cerebral damage by causing excessive vasodilatation and edema? Or do their effects on systemic BP or cerebral perfusion mediate their effects? Furthermore, the possible therapeutical implications need to be tested. The NP system can be manipulated by treatments with angiotensin-converting enzyme inhibitors and angiotensin II blockers, thus making NP-guided therapy possible. Evidence from patients with heart failure has shown promising results on the effects of NP-guided therapy on outcome. Patients whose therapy was adjusted according to serial measurements of N-BNP had a better outcome compared to patients treated with conventional therapies (Troughton *et al.* 2000, Morimoto *et al.* 2004). The implication of a corresponding procedure for stroke patients as a secondary preventive treatment would seem logical, knowing the beneficial effects of these regimens on the outcome of patients with cardiovascular diseases and on stroke patients in particular.

## 7 Conclusions

Cerebral infarction involves disturbances in the autonomic cardiovascular regulation manifested as low traditional HR variability measures, abnormal HR dynamics and neurohumoral activation involving the NP system, HPA axis and catecholamines. These disturbances were found to be prognostically unfavourable.

1. An acute cerebral infarction located either at the hemispheric level of the brain or in the medulla oblongata seems to alter the regulation of HR dynamics. The traditional time and frequency domain measures of HR variability and the long-term continuous variability of Poincaré plots were impaired in patients with acute ischemic stroke compared to healthy subjects.
2. Assessment of long-term HR dynamics with a nonlinear HR variability measure, power-law slope  $\beta$ , provides a specific risk marker of cerebrovascular mortality, whereas short-term HR dynamics was found to be predictive of cardiac death.
3. Power-law slope assessed in the acute phase of ischemic stroke predicted survival better than any other assessed risk variable, the risk of dying being 4-fold among the patients with abnormal power-law slope values. HR dynamics may have some value in the risk stratification of stroke patients.
4. Elevated levels of natriuretic peptides in the acute phase of ischemic stroke predict mortality after ischemic stroke.
5. Activation of the hypothalamus-pituitary-adrenal axis in the acute phase of ischemic stroke is associated with elevated levels of natriuretic peptides. High cortisol and natriuretic peptide values predict long-term mortality after ischemic stroke, suggesting that this profound neurohumoral disturbance is prognostically unfavourable.

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