Baroreflex regulation of the peripheral circulation

Ph.D. thesis

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ABBREVIATIONS

BRS= baroreflex sensitivity

HR= heart rate

BP= blood pressure

CVR= cutaneous vascular resistance

HUT= head-up tilt

ECG= electrocardiogram

SBF= skin blood flow

SkT= skin temperature

MAP= mean arterial pressure

HRV= heart rate variability

BPV= blood pressure variability

FFT= fast Fourier transformation

HF= high-frequency

LF= low-frequency

TF= total-frequency

CPAP= continuous positive airway pressure

CONTENTS

PAPERS ATTACHED TO THIS THESIS

I. Torok T, Bari F, Paprika D, Rudas L, Kardos A, Gingl Z. Short-term monitoring of the vascular resistance of the human skin microvasculature. *Acta Physiol Hung.* 1997;85:153-62.

II. Torok T, Bari F, Kardos A, Paprika D, Rudas L. Isometric handgrip exercise-induced muscarinic vasodilation in the human skin microvasculature. *Acta Physiol Hung.* 1997;85:193-8.

III. Torok T, Rudas L, Kardos A, Paprika D. The effects of patterned breathing and continuous positive airway pressure on cardiovascular regulation in healthy volunteers. *Acta Physiol Hung.* 1997;85:1-10.

IV. Lengyel C, Torok T, Varkonyi T, Kempler P, Rudas L. Baroreflex sensitivity and heart-rate variability in insulin-dependent diabetics with polyneuropathy. *Lancet.* 1998;351:1436-7.

V. Szili-Torok T, Gingl Z, Kardos A, Halmai L, Rudas L. Windowed FFT- a time variant spectral analysis: applicability during the head-up tilt test. *J Clin Basic Cardiol.* 1999;2:241-4.

VI. Szili-Torok T, Rudas L, Dibo G, Paprika D, Kardos A. Abnormal cardiovascular autonomic regulation in Parkinson's disease. *J Clin Basic Cardiol.* 1999;2:245-7.

VII. Kovacs L, Torok T, Bari F, Keri Z, Kovacs A, Makula E, Pokorny G. Impaired microvascular response to cholinergic stimuli in primary Sjogren's syndrome. *Ann Rheum Dis.* 2000;59:48-53.

VIII. Szili-Torok T, Kalman J, Paprika D, Dibo G, Rozsa Z, Rudas L. Depressed baroreflex sensitivity in patients with Alzheimer's and Parkinson's disease. *Neurobiol Aging.* 2001;22:435-8.

1. INTRODUCTION

1.1. Background

There is growing interest in cardiovascular autonomic regulation of the peripheral circulation. The regulation of HR, BP and pulse regulation are in the center of this renewed interest, which was facilitated and turned into a new direction by development of noninvasive methods[1,2]. Observations were made regarding deteriorated cardiovascular reflex regulation in cardiovascular and non-cardiovascular diseases such as ischemic heart disease, heart failure, depression etc.[2-4]. Deteriorated cardiovascular reflex regulation may be associated with life threatening and non-life threatening arrhythmias[3,5]. However the potential value of these findings in risk stratification remains questionable despite promising results of recent large-scale studies[5]. Recent advancements in interventional cardiac electrophysiology allow better understanding of sudden cardiac death and more importantly may lead to the improvement of prevention. In the new era of prophylactic treatment of such life threatening arrhythmias -by implanting therapeutic devices- the methods providing prognostic and risk related information become more interesting. In order to implement recent observations into these devices basic physiological questions should still be answered. Furthermore, baroreflex regulation in non-cardiovascular diseases and baroreflex regulation of the peripheral microcirculation including the skin microcirculation are new fields, which may influence the direction of future investigations.

1.2. Aims

The experiments, including the methods, results and discussion of the data are reported in detail in the Papers attached. This essay is to provide a broad background and a perspective for our research.

The studies included in this thesis diverged into two directions. During the first group of experiments our primary goal was to assess the cardiovascular autonomic regulation in patients with non-cardiovascular diseases including Parkinson's disease, Alzheimer's disease. We realized that one of the major disadvantages is the fairly static nature of the available methods. Therefore, we aimed to develop a novel method -based on a new mathematical transformation- for detecting dynamic changes in cardiovascular reflex regulation. The feasibility of this method was tested during tilt table testing. Second, we aimed to evaluate the baroreflex regulation of the skin microvasculature. A new method was developed for testing microvascular changes of the human skin based on a combination of laser-Doppler flowmetry and continuous non-invasive BP monitoring. We observed and hypothesized a new mechanism regarding the regulation of skin microvasculature during static exercise. Furthermore, using this method regulation of the skin microvasculature was tested in Sjogren's syndrome.

2. METHODS

2.1. Study patients/volunteers and study protocols:

2.1.1. Assessment of cardiovascular reflex regulation

2.1.1.1. Effects of the breathing pattern and CPAP on baroreflex regulation in healthy volunteers

Healthy subjects were recruited into this study. The study group consisted of 9 male volunteers. They were studied in a supine rest position. All subjects gave written informed consent prior to the experiments. Three sets of measurement were taken: during spontaneous, then during patterned breathing (6/min) without CPAP and during patterned breathing with CPAP. HRV and BRS parameters were calculated off-line.

2.1.1.2. BRS in diabetic patients

Twelve patients (6 women) were included into this study. Autonomic function was assessed by standard autonomic reflex tests and was scored to express the severity of diabetic impairment. BRS and HRV values were measured, and compared with control subjects.

2.1.1.3. Windowed FFT: a time variant spectral analysis

HUT was performed in 29 patients. All subjects had been referred to the arrhythmia service because of unexplained syncope. Cardiac cause of the syncope was excluded in each case. HUT test was performed in a quiet room with low light and always between 10 and 12 a.m. After 30 min in a supine resting position, patients were tilted to 70° for 40 min. A table with a footboard support was utilized, and the tilt position was reached in 30 s. Baseline ECG and BP recordings were made for 10 min prior to tilting, and were continued throughout the study. Syncope was defined as a transient loss of

consciousness, accompanied by a loss of postural tone. Warning symptoms of syncope include nausea, vomiting, impaired vision, the hearing of distant sounds, a slow response to verbal commands, and a partial loss of postural tone. Dizziness accompanied by one or more of the above symptoms was defined as presyncope.

2.1.1.4. Measurement of BRS in patients with Parkinson's disease and Alzheimer's disease

BRS was assessed in 23 patients with PD and 24 patients with AD, who were recruited from the Parkinson's Clinic and the Dementia Clinic of the Neurology and Psychiatry Departments at Albert Szent-Györgyi Medical University, Szeged, Hungary. Patients without history of any cardiovascular disease, who under physical examination showed no abnormalities and had a normal 12-lead ECG were selected for this study. Patients were excluded with any other medical condition, which was likely to affect baroreceptor regulation. 76% of the PD patients were on L-DOPA, 63% on an MAO inhibitor, 25% on an anticholinergic drug, 67% on amantadine and 31% on bromocryptine. 36% of the patients took antidepressants and 15% beta-blockers. AD patients stopped all of their medication 5 half-life time before the measurements. There was a control group of age and sex matched healthy individuals.

2.1.2. Assessment of the skin microcirculation:

2.1.2.1. Monitoring of the vascular resistance of the humans skin microvasculature

Twelve healthy subjects were included into this study. The subjects were studied in the supine rest position. Two sets of measurements were (Valsalva maneuver and cold pressor test) were taken. Each test was done in duplicate. SBF, BP were measured and CVR was calculated.

2.1.2.2. Exercise induced muscarinic vasodilation in the human skin microvasculature

Nine healthy subjects were recruited into this experimental study. The subjects were studied in the supine rest position. BP and SBF were measured and CVR was calculated off-line. To block muscarinic receptors, atropine was applied to the skin of the non-exercising forearm 30 minutes before recording. Each individual performed isometric handgrip exercise. The subjects were studied in the supine rest position. Two sets of measurements (Valsalva maneuver and cold pressor test) were taken. Each test was done in duplicate. SBF, BP were measured and CVR was calculated at the treated and control sites.

2.1.2.4. Skin microcirculation in Sjogren's syndrome

Twenty-two primary SS patients (20 female and 2 male) were enrolled in the study. All were diagnosed as having SS by the European Community criteria15 at the Division of Autoimmune Diseases at the 1st Department of Internal Medicine, Albert Szent-Györgyi Medical University, Szeged, Hungary. The average age of the patients was 50.6±13.2 years (ranging: 33-60). The average time since the first symptom had appeared was 11.5±5.2 years (ranging: 4-24), while the average time since the establishment of the diagnosis was 9.1±6.7 years (ranging: 3-20). In 18 of the 22 patients, a minor salivary gland biopsy had been performed. In 17 of these 18 patients, histological examination revealed focal lymphocytic sialadenitis, meeting the histological criteria of SS, while in one patient a negative result was obtained. In this latter patient and in the four patients on whom no biopsy was performed, the appropriate number of other criteria for the diagnosis of SS was met. All patients were regularly followed up. To exclude other factors that might possibly influence the microcirculatory physiology, SS patients older than 60 years, those who had hypertension or clinical evidence of arteriosclerosis

(coronary heart disease, arteriosclerotic cerebral disease or arteriosclerosis in other organs) or peripheral neuropathy, and those who regularly took -adrenergic blockers, calcium channel blockers, pentoxyphylline, or other drugs with vasodilatory or anticholinergic properties, were regarded as not eligible for the study. For the same reason, the use of non-steroid anti-inflammatory drugs was suspended at least five days before the examinations. Twelve healthy, age and sex matched people were examined as controls. None of these subjects had any known illness, or were taking any regular medication. SBF was measured with a laser-Doppler flowmeter. ECG was monitored continuously, as was the BP by means of a photoplethysmographic BP monitor. Then 0.1 ml of carbachol (Miostat, Alcon, USA), a muscarinic receptor agonist, was injected intracutaneously into the forearm skin. As control, almost simultaneously, 0.1 ml of 0.9% saline solution was injected similarly into the forearm skin at approximately 10-cm from the other injection site. SBF was measured simultaneously at the two injection sites for another 10 minutes and the highest deviations from the baseline flow values were designated SBF final.

2.1.2.4. Skin microcirculation after axillary blockade

The study group consisted of 11 patients (age ranging from 19 to 52 years) who underwent below wrist hand surgery. BP, HR, SBF, SkT, respiratory frequency and the respiratory depth were continuously monitored. Axillary blockade was performed as a routine analgesic procedure for below -wrist hand surgery, by inserting a stimplex cannula and injecting 30ml Lidocain (1%) and 30 ml Marcain. After instrumentation and five minutes baseline data collection patients were requested to perform a Valsalva maneuver. Each subject was asked to maintain a column of mercury at 40 to 50 mm Hg for 15 seconds with forced expiration, and then to resume normal expiration. A small air

leakage was allowed to prevent closure of the glottis. During the test three characteristic points were selected for further assessment. V1 is the early strain, V2 is the late strain and V3 indicates the overshoot phase of the Valsalva maneuver (paper #I. Figure 1).

2.2. Data acquisition and analysis

2.2.1. BP and HR monitoring

BP was monitored with a photoplethysmograph (Finapres 2300, Ohmeda) The ECG and the breathing were continuously recorded with a Sirecust 730 (Siemens) monitor. The BP and ECG signals were transmitted through an amplifier, filter and analogue-digital converter into an IBM-AT-compatible computer. Data were stored and analyzed off-line by means of a program developed in our laboratory. With our system the precision of RR interval detection is 2 ms, and that of BP detection is 1 mm Hg.

2.2.2. HRV measurement

Power spectrum analysis was performed on 2-min segments of the ECG and BP records by using FFT over two frequency bands. The low-frequency band (LF) of the HRV and systolic BPV was defined at 0.04-0.15 Hz, and the high-frequency component (HF) at 0.15-0.4 Hz [4]. The short-term measures of time domain indexes as the average normal R-R intervals (mean R-R), the standard deviation of the consecutive normal R-R intervals (SD), % of consecutive normal R-R interval differences >50ms (pNN50) and the root mean square successive differences (rMSSD) were calculated.

2.2.3. BRS assessment:

The cardiotachogram and trend-grams of the BP values were continuously recorded on-line. BRS was characterized by the spontaneous sequences method[6]. It has been established that 3 or more cardiac cycles of unidirectional BP increase or decrease with the corresponding lengthening or shortening of the interbeat intervals to form spontaneous "up" or "down-sequences". These sequences are analogous to those induced by pharmacological maneuvers, and a spontaneous BRS could be determined as an average of several individual slopes. In this study, all of the changes in systolic BP (delta SBP) were paired with the changes in the subsequent RR intervals (delta RR). This method is referred as the lag1 technique[6]. Oscillations in HR (dHR) and BP (dBP) were calculated over the same 60-minute period.

2.2.4. Measurement of SBF:

Relative blood cell perfusion was measured with a double channel skin perfusion monitor (Perimed, Stockholm, Sweden). This method uses the frequency shift of laser light (2 mW helium-neon laser source of 632.8-nm wavelength.) induced by reflection on moving red blood cells to measure red blood cell flux. Skin laser Doppler flow values cannot be expressed in conventional physiological units unless certain specific conditions are fulfilled. In vivo, the readings are therefore expressed in perfusion units related to the Brownian motion in motility standard emulsion provided by the manufacturer. At standard temperature the emulsion produces a motility of 250 perfusion units. This corresponds to 2.5 V at the analogue perfusion output. The measurement field of the laser Doppler skin probe is restricted to 1 mm^3 area of the skin

microvasculature. The laser Doppler probes and probe holders were attached to the ventral side of the treated forearm. Usually, the control site was at a corresponding site to the region of the untreated arm. The SkT probe was placed close to the laser Doppler probe holders, on both sites.

2.3. Calculations

2.3.1. Windowed FFT

Windowed FFT is a narrow time Fourier transformation. Briefly, the method is based on recalculation of the FFT with a variable shifting of the initial complexes in time, allowing the generation of 3D representations of spectral changes. The principles of the mathematical assessment were formulated by Gábor in 1946 [7]; For w(t), the Hamming window is used with 2-min durations. FFT was performed to calculate the power spectrum for all frequencies for this time range. Shifting the window in time yields the time-dependent power spectrum. In this study, the method was applied for both the RR interval and BP records. The time course of HR and systolic BP spectral changes was analyzed in each case, and power spectrum values of certain characteristic tilt stages were determined. Values calculated from the last sequence prior to tilting were defined as baseline. Post-tilt maximum and minimum values in the LF and HF power bands for both HR and systolic BP were determined and compared with the baseline. For those exhibiting syncope, the corresponding values were also determined at the time of fainting[8].

2.3.2. Calculation of CVR:

CVR was determined as the ratio of MAP and SBF. The percentage change in cutaneous vascular resistance (dCVR) were expressed as follows:

$$dCVR = (CVRx - CVRbase) / CVRbase$$

where x denotes characteristic points of the test

2.4. Statistical analysis

Normally distributed data were compared by using the one way repeated measurement ANOVA. To isolate which group is differing from the others we used a multiple comparison procedures. (Bonferroni t test). Non-gaussian data were analyzed by using the Friedman repeated measurement ANOVA on ranks test. Student- Newman- Keuls method was performed as all pairwise multiple comparison method. The level of statistical significance was set at $p<0.05$.

3. Results:

3.1 Cardiovascular reflex regulation

3.1.1. The effects of patterned breathing and CPAP on cardiovascular autonomic regulation

The mean of the R-R intervals did not show any changes between the three different situations. The standard deviation of the R-R intervals showed significant increment between the patterned (6/min) and spontaneous breathing. The other time domain parameters such as pNN50 and rMSSD did not change significantly between the spontaneous and patterned breathing. There were no significant, just tendentious

differences between the time domain parameters measured at metronomic ventilation and during the application of CPAP with the same rhythm of respiration. Thus the time domain indexes of the HRV showed significant differences only between the spontaneous respiration and patterned breathing with CPAP. The frequency domain parameters of the HRV showed significant elevation on transition from spontaneous to patterned mode. Similar differences were seen between the spontaneous and CPAP mode in the total and low frequency range. In the high frequency range the HRV did not exhibit any significant modification throughout the study. The mean and the standard deviation of the systolic BP remained unchanged during the study. The application of 6/min metronomic ventilation did not alter the BPV compared to spontaneous respiration. The application of CPAP caused marked increment in the power of the total and low frequency ranges of the BPV compared to both the baseline parameters and to those recorded during patterned breathing. The BP power in the HF range did not change during the three different situations. The oscillation amplitude of the R-R intervals showed significant increase during 6/min breathing. The application of CPAP did not cause further increment in the oscillation of R-R intervals. In contrast, the oscillation amplitude of the systolic BP increased during patterned breathing, and showed further significant elevation due to the application of CPAP. BRS showed significant elevation on transition from spontaneous to patterned breathing. No further changes were detected while breathing with CPAP (Table 1, paper #III.).

3.1.2. BRS in IDDM patients

The mean autonomic score of the patients was 5.7±2.4 and all had raised perception thresholds. In patients with IDDM the BRS was lower than in the controls. SDNN was depressed in the IDDM group (Table, paper #IV).

3.1.3. HUT induced autonomic changes- Results of windowed FFT

Twenty-two subjects exhibited no abnormal reactions during HUT (tilt-negative cases). Syncope developed in 7 subjects (tilt-positive cases), 7 to 30 min after tilting. The tilt-positive subjects displayed a "mixed response", characterized by bradycardia and a variable degree of hypotension. Upon tilting, there were immediate fluctuations in HR and systolic BP power spectral components (Fig 1 and 2, paper #V.). TFBPV and LFBPV decreased in both groups and leveled at lower-than-maximum values for several minutes. The minimum TFBPV and LFBPV values after tilting in this phase remained significantly higher than the baseline in the tilt-negative group (Table 2, paper #V.). The same tendency was seen in the tilt-positive group. TFHRV and LFHRV increased significantly in response to tilting, and then declined quickly to post-tilt minimum values, but these were still significantly higher than the baseline in both groups. A different response was seen in HFHRV. In the tilt-negative group, HFHRV decreased immediately on tilting and underwent no subsequent changes, whereas a significant increase in this parameter was seen in the tilt-positive group. This increase reached its peak within 60 s after tilting, and HFHRV then quickly returned to a lower-than-baseline level (Table 3, paper #V.). The syncopal episode itself in the tilt-positive group was characterized by a diminution of all HRV spectral bands, though this decline reached statistical significance for only the TFHRV and LFHRV bands as compared with the

baseline. There was a similarly marked reduction in the TFBPV and LFBPV components in association with syncope.

3.1.3. Cardiovascular reflex regulation in Parkinson's disease and Alzheimer's disease

The average R-R interval length was shorter in both of the PD and AD groups than in the healthy volunteers. The systolic blood pressure, however, was very similar in all three groups. BRS was markedly reduced in the AD and PD groups compared with controls. There was no statistically significant difference between the AD and PD group in BRS values. Patients with AD and PD showed significantly decreased dR-R, and increased dBP compared with controls. There was no statistical difference between AD and PD patients in dR-R and dBP (Table, paper #VIII., Table paper #VI.).

3.2. Regulation of skin blood flow

3.2.1. Monitoring of the CVR

Skin blood flow responses followed a pattern of four phases that corresponded to the four blood pressure phases of the Valsalva maneuver (Table 1, Figure 1, paper #I.). The initial phase of the Valsalva maneuver resulted in a significant increase in MAP and SBF, however the CVR remained unchanged. Towards the end of the strain phase there was a drop in MAP below baseline. At this point there was also a marked decrease in SBF. The CVR showed significant elevation. Cold immersion of the contralateral hand resulted in significant increase in MAP. The SBF at the same time exhibited a significant reduction. As a consequence the CVR showed a marked – more than double-fold – elevation (Table 3, paper #I.).

3.2.2. Exercise induced muscarinic vasodilation in the human skin microvasculature

MAP increased continuously during handgrip exercise. On termination of isometric exercise, MAP returned to baseline level. SBF increased significantly in the control area and a rapid post-exercise recovery was observed. SBF did not change significantly in the atropine-treated region throughout the. CVR decreased significantly at the control site. In contrast, no significant CVR changes were seen in the region treated with atropine. The maximum changes in CVR differed significantly between the atropine-treated and control areas (paper #II.).

3.2.3. Damage of skin muscarinic vasodilation in Sjogren's syndrome

In the event of a positive vascular reaction, the blood flow started to increase within 30 seconds after the injection of carbachol and reached a new steady state approximately 4-7 minutes later, then remaining unchanged until the end of the examination. In the controls, the average dSBF was significantly higher than the average dSBF in SS patients. In the controls, the dSBF values reflected a 1.66-7.6-fold increase in microcirculatory blood flow after the injection of carbachol (Table 2, paper #VII.). However, in a relatively high proportion of the SS patients, the reaction to the administration of carbachol was small or virtually absent, while in other patients a marked vasodilatation was observed. We defined a positive microvascular reaction to carbachol in the SS patients as a dSBF value higher than the smallest dSBF value in the group of healthy controls. Following this definition, exactly half of the SS patients (11 out of 22) could be considered to be non-responders that is, producing a less than 1.66-fold increase in the microcirculatory blood flow while the other half of the patients were

regarded as responders to carbachol on the basis of the pronounced vasodilatation (a more than 1.66-fold increase in SBF).

3.2.4. CVR changes after axillary blockade

Reflexes were studied at constant ambient temperature. There was no significant difference in SBF and CVR values between the two sides before the axillary blockade was performed (SBF: axillary blockade side 534±195 a.u., control side: 559±215 a.u., p=NS; CVR: axillary side 0.16±0.8, control side 0.17±0.2 a.u., p=NS) After the axillary blockade became effective SBF and CVR did not show significant change between the treated and control regions (SBF: axillary blockade side 636±272 a.u., control side: 547±197 a.u., p=NS; CVR: axillary side 0.16±0.9, control side 0.17±0.1 relative units, p=NS). There was only a tendency in increase of SBF on the side of the axillary blockade. CVR did not change after axillary blockade.

The SBF and the CVR remained unchanged throughout the maneuver. Towards the end of the strain phase there was a drop in mean arterial pressure below baseline. At this point there was no observable change in SBF. Therefore, the calculated CVR did not show significant change, too. In the overshoot phase SBF and CVR remained unchanged.

4. Discussion

Cardiovascular risk stratification, especially arrhythmic risk stratification, is an issue that has still not been wholly addressed in modern clinical cardiology[9-12]. In the past 10 years, arrhythmic risk stratification has been approached mainly by evaluating frequency

and complexity of premature ventricular contractions, detected on Holter monitoring, often in association with determination of ejection fraction. This methodology has been proven to be limited and fallacious. Conversely, in some institutions, risk stratification in post-AMI patients has been performed by electrophysiology study, without any previous noninvasive arrhythmic risk stratification, in all post-AMI patients. In recent years, many other noninvasive parameters, such as late potentials (signal-averaged electrocardiography), heart rate variability, baroreflex sensitivity, and, more recently, T-wave alternance, have been shown to be useful, but they are associated with a low specificity in the noninvasive identification of patients at high risk for arrhythmic mortality[3,5]. Today, the most convincing approach seems to be the one combining both noninvasive risk stratification parameters followed by a further arrhythmic risk stratification, obtained through electrophysiologic study. Although, several published and ongoing trials that utilize various arrhythmic risk stratification techniques as part of their protocol are reviewed, the physiological basis of the methods used in this setting is not completely understood. The aim of the present series of studies was to add additional information in a broader spectrum of patient population regarding the above mentioned tools, which may lead on a long run to develop a tool in risk stratification with better specificity.

4.1. Cardiovascular autonomic reflex regulation in physiological conditions and in movement disorders

The modulation of cardiac interbeat intervals has been subjected to extensive studies. A certain degree of consensus regarding the spectral assessment of HRV has already been reached[3]. However, when FFT is used to assess HRV, a minimum period 2-3 of

min. is required. This is somewhat of a paradox: during a maneuver in which the autonomic tone can change faster or the significant changes in autonomic tone are completed. Thus, the spectral approach focuses on discrete sections of the events and there is no appropriate tool to characterize the dynamics of the spectral fluctuations. We introduced an alternative means of assessing the dynamics of the spectral HRV and BPV[8]. As HUT results in characteristic changes in the cardiovascular autonomic tone, we tested the applicability of our suggested method, windowed FFT, by analyzing the records obtained from patients during HUT. Since the literature data relating to the modification of HRV and BPV during HUT are controversial we also aimed to describe the HRV and BPV changes during HUT focusing on the dynamics of the changes. The assessment of HRV by spectral analysis is gaining increasing use in clinical cardiology[3]. A few publications related to time- dependent spectral assessment [13,14], but the proposed methods are not yet widely accepted. Thus, the spectral analysis currently remained as a possibility for the description of stationary states. Dynamic changes were then usually expressed as differences between two stable conditions, without any analysis of the process of transition between the two states. The effect of upright tilting on HRV is typically characterized by comparing the supine resting condition with that in a given post-tilt period. This post-tilt transient is defined either in a given time interval after the beginning of or prior to termination of the tilt maneuver [15-17], or at a fixed time preceding the syncopal event [18]. Our results indicate complex fluctuations in the HRV and BPV spectral parameters upon tilting. The major finding in this study is that LFHRV and LFBPV increase markedly and promptly after the tilt position is reached. If FFT is used without time variant analysis, this effect can not be observed, because it occurs in the first 30 s of the post-tilt period. At the time of presyncope, when the sympathetic tone

is declining, these components are greatly decreased. Stationary analysis would yield a quite different spectrum in an early assessment starting with the tilt procedure, as compared with a delayed analysis performed even only 2 min post-tilting. Our observations suggest that certain discrepancies in the literature tilt test results might have been due to the different timings of the assessment. Theodorakis et al. generated a series of adjoining spectral segments to characterize the tilt-induced responses[19], and presented first documented time-dependent spectral fluctuations. Pagani et al. selected "stationary sections of data both at rest and during tilting" for assessment, however, the exact timing of their post-tilt data acquisition was not reported[20]. Montano et al. reported on a study in which they applied different extent of angle tilting for periods of 10 min. From the continuous recordings, stationary segments devoid of arrhythmia [200 to 500 RR intervals] were analyzed, but the timing of data acquisition in relation to the onset of tilting was not stated[21]. Boulos et al. determined spectral changes by comparing the baseline values with those for the last 256 consecutive beats of a 15-min tilt test[17]. Bootsma et al. reported on the effects of different angle tilting, but they excluded from their analysis the first minute of recordings in each position: min 2 to 5 were used for computation of HRV spectra [15]. Mizumaki et al. established tilt-induced spectral responses by comparing supine resting values with those recorded during the last 200 beats until 1 min before the end of the tilt [18]. Bloomfield et al. performed comparisons between data acquired in a supine position and those recorded during the last 5-min of a 13-min tilting [16]. Our findings are in general agreement with those of previous studies, but we found that these changes are most pronounced within the first 2-min post-tilting. Nevertheless, the subsequent equilibrium, which is basically maintained until the end of tilting in tilt-negative subjects, is still characterized by a predominance of LFHRV

markers. Furthermore, the continuous assessment allowed us to detect a short-lasting increase in HFHRV among tilt-positive subjects very early after tilting. This temporary increment in HFHRV among syncope subjects is strikingly different from the immediate HFHRV diminution detected in the group of tilt-negative patients, and may indicate parallel changes in the vagal regulation. Some of the previous studies demonstrated and increased HFHRV just prior to an episode of syncope [17,19], but no early post-tilt peak of HFHRV has been reported previously. The significance of this finding is not clear and demands further studies. During the phase of equilibrium, i.e. after the first 2 min of tilting, no apparent differences in spectral trends were seen between the two groups. The spectral constellation among negative subjects remained unchanged until the end of the study (Fig. 1, paper #V.). In contrast, a second phase of LFHRV and HFHRV elevation was seen among tilt-positive subjects prior to syncope (Figs 2 and 3, paper #V.). The LFHRV component presumably represents sympathetic activation; indeed, studies on presyncopal catecholamine levels and muscle sympathetic nerve activity recordings documented transient pre-syncopal elevations[22,23]. The mechanism of presyncopal elevation in HFHRV is not clear. The increase may represent the intense vagal stimulation preceding the onset of syncope. However, we have observed a presyncopal increase in HFBPV as well (Figs 2 and 3, paper #V.), a phenomenon difficult to explain in terms of any vagal mechanism. We hypothesize that this HFBPV peak is a consequence of the presyncopal decrease in venous return, which results in central hypovolemia and in increased breathing-related fluctuations in the stroke volume. Alternatively, altered depth and frequency of breathing may explain this phenomenon. The increased HFBPV may in turn contribute to the genesis of the HFHRV peak via baroreflex mechanisms. One limitation of our study is that the magnitude and timing of

the presyncopal spectral peaks remained undefined. It is well known, that the duration of presyncope varies widely in these patients. Thus, a stationary analysis in a fixed time interval prior to the full-blown syncope may be inadequate for a representation of the presyncopal constellations. Individual characterization of the presyncopal peaks would be desirable.

Measurement of BRS in patients with AD and PD: The examination of spontaneous BRS involves a noninvasive procedure that does not need the active cooperation of the patients and it can therefore be used with disabled or weak patients too. Our results demonstrate that the procedure is suitable for screening autonomic involvement among patients with PD and AD. Basal HR of the patients with AD and PD was higher than that of healthy volunteers, suggesting diminished vagal tone. It seems plausible, that the decreased vagal tone in these subjects is related to their abnormally low BRS. Vagal tone however could be generated by both baroreflex-dependent and baroreflex-independent mechanisms[24]. Increased basal HR may also indicate, that the resting point on the sigmoid baroreflex response curve is located close to the saturation region (i.e. the upper flat portion of the relationship). BRS methods based on spontaneous blood pressure fluctuations do not describe the whole sigmoid baroreflex curve[25]. This relationship could have been depicted only by the neck chamber technique, a method extremely unsuited to these patient populations. The pharmacological baroreflex methods are often regards as gold standard techniques, however recent evidence points to considerable limitations in their use[26]. Nevertheless, the baroreflex gains yielded by the different methods are closely related, and the method of spontaneous sequences may gain further acceptance in the future[26].

Mechanism of depressed BRS in patients with AD and PD: Early morphological studies have indicated damage to important blood pressure and baroreflex regulatory centers in AD and PD. Recently, damage to autonomic-related cortices, which can contribute autonomic imbalance often associated with AD was described in detail [27]. However, the reason of the autonomic imbalance remained unknown in patients with such neurological disorders. The determination of the BRS by a spontaneous sequences method [6] is based on the measurement of respiratory sinus arrhythmia. The genesis of this complex mechanism is a result of an interplay between the most important blood pressure regulatory mechanisms such as central cardiorespiratory coupling, reflexes from pulmonary stretch receptors, arterial baroreflexes, reflexes from the cardiac (low pressure) receptors. The relative roles of these mechanisms remain unclear. Therefore damage to any of these reflexes may influence the measured value of BRS. Patients with AD showed decreased heart rate variability and this was associated with increased acethyl-choline esterase activity. Although the involvement of afferent nerves can not be excluded, these results suggest that the peripheral efferent autonomic system may play a role in the presence of cardiac autonomic dysfunction superimposed by significant damage to central autonomic structures[27]. Decreased BRS may reflect cholinergic deficits in the cardiac autonomic nerves as well as in the central nervous system[28]. In contrast with previous findings, the diminished HR oscillation during normal breathing (indicated by decreased dRR) was associated with increased BP oscillation in both groups in this study[29]. BRS in PD was first examined by Appenzeller and Goss. They concluded that BRS was pathological in some PD patients[30]. Our studies confirm this and demonstrate more general aspects of this early observation[31,32]. The function of the HR changes is commonly known to buffer changes in BP. Accordingly, it is not

surprising that the amplitude of BP surge increases with decreasing HR fluctuation. The significance of the abnormalities in cardiovascular regulation among AD and PD patients is not yet fully known. It is possible that the imbalance of the sympathetic and the parasympathetic tone is connected with the ischemic heart muscles. The pathological BRS that signaled an autonomic imbalance in the acute phase of myocardial infarction was a sensitive predictor of ventricular arrhythmias and sudden death[5]. The connection between autonomic dysregulation and arrhythmia-related death has recently been considered in other, non-cardiovascular diseases, such as depression[4]. The known central abnormalities in AD and PD would be consistent with the deficient baroreflex regulation in these patients originating centrally. Large scale clinical trials indicate that a decreased baroreflex gain is a predictor of adverse outcome in congestive heart failure and in acute myocardial infarction[5], but the significance of the abnormal baroreflex gain in neurological disorders is unclear. Analogously, we can presume a connection between the autonomic imbalance and the high mortality among Parkinson's disease patients. Increased mortality in Parkinson's disease patients due to ischemic heart disease was reported recently[33,34]. Increased cardiovascular mortality has also been reported in patients with Alzheimer's dementia associated with a tendency to lower blood pressure[35]. The mortality of Parkinson's disease patients is almost twice that for age and sex-matched healthy control groups[33,34]. The 20-year follow-up study by Ben-Shlomo and Marmot suggested that this increased mortality is connected with an increase in heart ischemia related deaths[34]. However, this study did not distinguish sudden cardiac deaths, and thus the real importance of arrhythmia-related deaths could not be assessed. Hence, the possible predictive value of centrally based depression of baroreflex sensitivity necessitates further studies.

4.2. Baroreflex regulation of the human skin microcirculation

The importance of this subject stems from animal studies demonstrated that vessels of the skin could be targets for important powerful homeostatic barereceptor reflexes[36]. On the other hand in humans the concept was developed that arterial baroreceptors have only a minor influence on limb vascular resitance[37,38]. In contrast these data, Edfeldt and colleges have demonstrated in their studies using lower body negative pressure that deactivation of the arterial baroreceptors significantly contributes to the regulation of forearm resistance, which involves active participation of the skin microvasculature[39-41]. In a recent study Bernardi and colleges presented that laser Doppler flowmetry fluctuations were responsive to sinusoidal neck suction, indicating response to sympathetic modulation. Thus the changes in SBF reflect the modifications determined by autonomic activity[42]. In our studies Valsalva maneuver was used to stimulate the arterial baroreceptor system. The BP response to Valsalva maneuver is in concordance with previous reports. Initially, arterial pressure increases steeply for a few seconds and this is attributed mainly to the effects of the imposed pressure on the intrathoracic and intraabdominal arteries. The pressure then falls due to decreased venous return. Towards the end of the strain phase blood pressure recovers as HR and vascular resistance increase to compensate for the lower cardiac output. Direct microneurographic methods proved that the second phase of the Valsalva maneuver is accompanied by increased sympathetic activation[43,44]. On release of the Valsalva maneuver, MAP transiently falls, then increases to above control. We found, that Valsalva maneuver induces a significant change in CVR during the strain phase. This indicates, that active mechanism is participating in this change. The above findings lend

support to previous reports suggesting that sympathetic vasomotor fibers of the human skin microvasculature can exert a very potent control of vascular resistance under thermoneutral conditions. This control must partly ascribed to reflexes originating from the low-pressure cardiopulmonary receptors, however, indirect evidence suggests that the high-pressure arterial receptor system also contributes to a significant extent. Although sympathetic blockade is widely used in clinical practice, the reliable assessment of its efficacy is not developed yet. Previously the temperature of the treated region was assessed by physical examination. In contrast with this daily routine, Sherman et al. has demonstrated elegantly, that the painful limb can be cooler but sometimes warmer than the other limb[45]. Furthermore, the relative coolness is not proportional to the pain intensity[45]. There is also inconsistency between pain localization and the greatest thermal asymmetry assessed by video thermography[45,46]. Oberg and Nilsson reported a technique, which uses the frequency shift of the laser light[1,47-49]. Laser Doppler flowmetry is a feasible and non-invasive method for the measurement of skin perfusion[48]. Recently, numerous papers reported the feasibility of laser Doppler flowmetry in the measurement of SBF after sympathetic blockade[50-52]. Increased SBF was reported after sympathetic blockade using laser Doppler flowmetry. An additional possibility is that to use a novel system called laser Doppler perfusion imager for similar purposes[53]. Although the absolute blood flow increased after sympathectomy in these studies, this method is accurate only for short-term monitoring, because the laser Doppler method is very sensitive to external stimuli, such as for thermal changes[54]. More importantly, there are a large number of factors like signal processing, choice of bandwidth, motion artifacts, and instrument calibration which can seriously affect the interpretation of laser Doppler signals[54]. These factors are often not considered during

routine use. The detection and the interpretation of the signal are even more difficult in a routine clinical setting. Our results suggest that the absolute blood flow and a cutaneous resistance do not necessary change after sympathetic blockade. The decreased adrenergic tone can be compensated by some other factors including the release of local vasoconstrictor factors such as several forms of endothelins[55,56]. The cutaneous cholinergic vasodilator system could also be deactivated by axillary blockade resulting in a new set point in the sympatho-cholinergic balance[56-58]. To avoid the above-mentioned possible pitfalls of measuring absolute flow values we postulated that the assessment of a reflex response may provide more sophisticated monitoring. Since the cutaneous microvasculature is involved in reflex regulation, we tested the responsiveness of the adrenergic vasoconstrictor fibers to baroreflex stimuli. One of the most unique observation of ours is that the responses to baroreflex stimuli are basically different at the side of the blockade and the control side. A great number of skin disorders and various clinical situations are accompanied by reduced skin perfusion. In peripheral nerve injuries, atherosclerosis induced ischemia and connective tissue disorders the skin microcirculation is diminished[50-53,58]. The increase of blood perfusion of the cutaneous tissues may help in the management of many of these conditions. After skin transplantation, increased SBF can also efficiently prevent the rejection of the graft[59]. As the human skin microvasculature is richly innervated by adrenergic vasoconstrictor nerve fibers, the reduction of sympathetic vasoconstrictor tone may result in increased SBF. Theoretically, activation of the cutaneous vasodilator system may serve as an additional tool to improve the regional circulation in the skin[57]. Whether this system could be activated for this purpose is a feature that remains to be clarified. Further observations were made in a patients where the cholinergic innervation of various

organs is definitely altered. This seemed to be a good model for conducting experiments in this patient population. We found that the average increase in cutaneous microcirculatory blood flow in response to carbachol was significantly smaller in the SS patients than in the healthy controls. In half of the SS patients, the reaction to a potent parasympathomimetic drug was virtually absent or markedly diminished. As vasodilatation is characterized physiologically by a decrease in vascular resistance, the CVR was also calculated by dividing the mean arterial pressure by SBF. As the arterial pressure remained virtually unchanged in all the examined persons during the examinations, an increase in SBF always reflected cutaneous vasodilatation in this experimental setting. The above finding lends support to previous results suggesting a disturbance in the parasympathetic autonomic nervous system in SS[60-62]. As our experiments involved the administration of a muscarinic receptor agonist directly to the examined target organ, the detected unresponsiveness favors the hypothesis that a dysfunction may exist at a receptorial or post-receptorial level.

5. CONCLUSIONS

1. The major finding regarding the methodology of BRS is that alteration of baroreflex regulation plays central role in different HR and BP responses to slow patterned breathing. This is the first report, which demonstrates the interplay between the breathing pattern and BRS of spontaneous sequences.
2. Windowed FFT is a feasible method to detect dynamic HRV and BPV changes.

3. Baroreflex regulation is markedly depressed in Alzheimer's dementia and Parkinson's disease. This is characterized by a significant decrease in BRS. Diminished BRS was demonstrated in patients with IDDM. Spontaneous sequences BRS technique may replace traditional reflex tests for screening autonomic neuropathy. Neuropathy associated with diminished BRS may be related to the poor prognosis of these patients.

4. Several observations were inferred from the studies on the autonomic regulation of the skin microcirculation. The major finding regarding methodology is that combined SBF and BP monitoring is a feasible tool for assessment of microvascular responses in the human skin. Furthermore, using this technique we demonstrated that the human skin is involved into muscarinic vasodilation during static exercise. In addition, our findings indicate that baroreflex regulation involves the human skin microvasculature. Clinical ramification of this finding is that monitoring of skin microvascular responses allows assessment of the effectiveness of topical sympathetic blockade.

REFERENCES

1. Almond NE, Jones DP, Cooke ED. Noninvasive measurement of the human peripheral circulation: relationship between laser Doppler flowmeter and photoplethysmograph signals from the finger. *Angiology*. 1988;39:819-29.
2. Hainsworth R. Non-invasive investigations of cardiovascular reflexes in humans. *Clin Sci (Colch)*. 1990;78:437-43.
3. Camm J, Malik M. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur Heart J*. 1996;17:354-81.
4. Guinjoan SM, Bernabo JL, Cardinali DP. Cardiovascular tests of autonomic function and sympathetic skin responses in patients with major depression. *J Neurol Neurosurg Psychiatry*. 1995;59:299-302.
5. La Rovere MT, Bigger JT, Jr., Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet*. 1998;351:478-84.
6. Parlow J, Viale JP, Annat G, Hughson R, Quintin L. Spontaneous cardiac baroreflex in humans. Comparison with drug-induced responses. *Hypertension*. 1995;25:1058-68.
7. Gabor D. Theory of communications. *J Inst Elect Eng*. 1946;93:429.

8. Szili-Torok T, Gingl Z, Kardos A, Halmai L, Rudas L. Windowed FFT- a time variant spectral analysis: applicability during the head-up tilt test. *J Clin Basic Cardiol.* 1999;2:241-4.

9. Sarasin FP, Louis-Simonet M, Carballo D, Slama S, Rajeswaran A, Metzger JT, Lovis C, Unger PF, Junod AF. Prospective evaluation of patients with syncope: a population-based study. *Am J Med.* 2001;111:177-84.

10. Leier CV, Alvarez RJ, Binkley PF. The problem of ventricular dysrhythmias and sudden death mortality in heart failure: the impact of current therapy. *Cardiology.* 2000;93:56-69.

11. Friedman PA, Glikson M, Stanton MS. Defibrillator challenges for the new millennium: the marriage of device and patient-making and maintaining a good match. *J Cardiovasc Electrophysiol.* 2000;11:697-709.

12. Windhagen-Mahnert B, Kadish AH. Application of noninvasive and invasive tests for risk assessment in patients with ventricular arrhythmias. *Cardiol Clin.* 2000;18:243-63, vii.

13. Petrucci E, Mainardi LT, Balian V, Ghiringhelli S, Bianchi AM, Bertinelli M, Mainardi M, Cerutti S. Assessment of heart rate variability changes during dipyridamole infusion and dipyridamole-induced myocardial ischemia: a time variant spectral approach. *J Am Coll Cardiol.* 1996;28:924-34.

14. Kuo TB, Chan SH. Continuous, on-line, real-time spectral analysis of systemic arterial pressure signals. *Am J Physiol.* 1993;264:H2208-13.

15. Bootsma M, Swenne CA, Van Bolhuis HH, Chang PC, Cats VM, Bruschke AV. Heart rate and heart rate variability as indexes of sympathovagal balance. *Am J Physiol.* 1994;266:H1565-71.

16. Bloomfield DM, Bigger JT, Jr., Pavri BB, Han J, Fleiss JL, Rolnitzky LM, Steinman RC. Vagal modulation of RR intervals during head-up tilt and the infusion of isoproterenol. *Am J Cardiol.* 1995;75:1145-50.

17. Boulos M, Barron S, Nicolski E, Markiewicz W. Power spectral analysis of heart rate variability during upright tilt test: a comparison of patients with syncope and normal subjects. *Cardiology.* 1996;87:28-32.

18. Mizumaki K, Fujiki A, Tani M, Shimono M, Hayashi H, Inoue H. Left ventricular dimensions and autonomic balance during head-up tilt differ between patients with isoproterenol-dependent and isoproterenol- independent neurally mediated syncope. *J Am Coll Cardiol.* 1995;26:164-73.

19. Theodorakis GN, Kremastinos DT, Stefanakis GS, Iliodromitis EK, Avrambos GT, Livanis EG, Karavolias GK, Toutouzas PK. The effectiveness of beta-blockade and its influence on heart rate variability in vasovagal patients. *Eur Heart J.* 1993;14:1499-507.

20. Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga E, et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res.* 1986;59:178-93.

21. Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation.* 1994;90:1826-31.

22. Lipsitz LA, Mietus J, Moody GB, Goldberger AL. Spectral characteristics of heart rate variability before and during postural tilt. Relations to aging and risk of syncope. *Circulation*. 1990;81:1803-10.

23. Jardine DL, Ikram H, Crozier IG. Autonomic control of asystolic vasovagal syncope. *Heart*. 1996;75:528-30.

24. Kollai M, Jokkel G, Bonyhay I, Tomcsanyi J, Naszlady A. Relation between baroreflex sensitivity and cardiac vagal tone in humans. *Am J Physiol*. 1994;266:H21-7.

25. Rudas L, Crossman AA, Morillo CA, Halliwill JR, Tahvanainen KU, Kuusela TA, Eckberg DL. Human sympathetic and vagal baroreflex responses to sequential nitroprusside and phenylephrine. *Am J Physiol*. 1999;276:H1691-8.

26. Peveler RC, Bergel DH, Robinson JL, Sleight P. The effect of phenylephrine upon arterial pressure, carotid sinus radius and baroreflex sensitivity in the conscious greyhound. *Clin Sci (Colch)*. 1983;64:455-61.

27. Chu CC, Tranel D, Damasio AR, Van Hoesen GW. The autonomic-related cortex: pathology in Alzheimer's disease. *Cereb Cortex*. 1997;7:86-95.

28. Medina A, Bodick N, Goldberger AL, Mac Mahon M, Lipsitz LA. Effects of central muscarinic-1 receptor stimulation on blood pressure regulation. *Hypertension*. 1997;29:828-34.

29. Linden D, Diehl RR, Berlit P. Sympathetic cardiovascular dysfunction in long-standing idiopathic Parkinson's disease. *Clin Auton Res*. 1997;7:311-4.

30. Appenzeller O, Goss JE. Autonomic deficits in Parkinson's syndrome. *Arch Neurol*. 1971;24:50-7.

31. Szili-Torok T, Rudas L, Dibo G, Paprika D, Kardos A. Abnormal cardiovascular autonomic regulation in Parkinson's disease. *J Clin Basic Cardiol.* 1999;2:245-7.

32. Szili-Torok T, Kalman J, Paprika D, Dibo G, Rozsa Z, Rudas L. Depressed baroreflex sensitivity in patients with Alzheimer's and Parkinson's disease. *Neurobiol Aging.* 2001;22:435-8.

33. Bennett DA, Beckett LA, Murray AM, Shannon KM, Goetz CG, Pilgrim DM, Evans DA. Prevalence of parkinsonian signs and associated mortality in a community population of older people. *N Engl J Med.* 1996;334:71-6.

34. Ben-Shlomo Y, Marmot MG. Survival and cause of death in a cohort of patients with parkinsonism: possible clues to aetiology? *J Neurol Neurosurg Psychiatry.* 1995;58:293-9.

35. Guo Z, Viitanen M, Fratiglioni L, Winblad B. Low blood pressure and dementia in elderly people: the Kungsholmen project. *Bmj.* 1996;312:805-8.

36. Shepherd JT. *Circulation to skeletal muscle.* Bethesda, Maryland: American Physiological Society; 1983.

37. Walker JL, Abboud FM, Mark AL, Thames MD. Interaction of cardiopulmonary and somatic reflexes in humans. *J Clin Invest.* 1980;65:1491-97.

38. Mancia G, Mark AL. *Arterial baroreflexes in humans.* Bethesda, Maryland: American Physiological Society; 1983.

39. Lundvall J, Edfeldt H. Sympathetic control of vascular resistance in skeletal muscle and skin of man. *Acta Physiol Scand.* 1991;143:359-60.

40. Lundvall J, Edfeldt H. Much more potent baroreflex sympathetic control of vascular resistance in the resting human limb than previously believed. *Acta Physiol Scand.* 1993;147:185-93.

41. Edfeldt H, Lundvall J. Sympathetic baroreflex control of vascular resistance in comfortably warm man. Analyses of neurogenic constrictor responses in the resting forearm and in its separate skeletal muscle and skin tissue compartments. *Acta Physiol Scand.* 1993;147:437-47.

42. Bernardi L, Hayoz D, Wenzel R, Passino C, Calciati A, Weber R, Noll G. Synchronous and baroceptor-sensitive oscillations in skin microcirculation: evidence for central autonomic control. *Am J Physiol.* 1997;273:H1867-78.

43. Pryor SL, Lewis SF, Haller RG, Bertocci LA, Victor RG. Impairment of sympathetic activation during static exercise in patients with muscle phosphorylase deficiency (McArdle's disease). *J Clin Invest.* 1990;85:1444-9.

44. Matsukawa T, Sugiyama Y, Watanabe T, Kobayashi F, Mano T. Baroreflex control of muscle sympathetic nerve activity is attenuated in the elderly. *J Auton Nerv Syst.* 1998;73:182-5.

45. Sherman RA, Karstetter KW, Damiano M, Evans CB. Stability of temperature asymmetries in reflex sympathetic dystrophy over time and changes in pain. *Clin J Pain.* 1994;10:71-7.

46. Sherman RA, Woerman AL, Karstetter KW. Comparative effectiveness of videothermography, contact thermography, and infrared beam thermography for scanning relative skin temperature. *J Rehabil Res Dev.* 1996;33:377-86.

47. Oberg PA, Nilsson GE, Tenland T, Holmstrom A, Lewis DH. Use of a new laser Doppler flowmeter for measurement of capillary blood flow in skeletal muscle after bullet wounding. *Acta Chir Scand Suppl.* 1979;489:145-50.

48. Nilsson GE, Tenland T, Obert PA. A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Trans Biomed Eng.* 1980;27:12-9.

49. Almond NE, Jones DP, Cooke ED. High quality photoplethysmograph signals from a laser Doppler flowmeter: preliminary studies of two simultaneous outputs from the finger. *J Biomed Eng.* 1988;10:458-62.

50. Lantsberg L, Goldman M, Khoda J. Should chemical sympathectomy precede below knee amputation? *Int Surg.* 1996;81:85-7.

51. Lantsberg L, Goldman M. Lower limb sympathectomy assessed by laser Doppler blood flow and transcutaneous oxygen measurements. *J Med Eng Technol.* 1990;14:182-3.

52. Lantsberg L, Goldman M. Laser Doppler flowmetry, transcutaneous oxygen tension measurements and Doppler pressure compared in patients undergoing amputation. *Eur J Vasc Surg.* 1991;5:195-7.

53. Sorensen J, Bengtsson M, Malmqvist EL, Nilsson G, Sjoberg F. Laser Doppler perfusion imager (LDPI)--for the assessment of skin blood flow changes following sympathetic blocks. *Acta Anaesthesiol Scand.* 1996;40:1145-8.

54. Obeid AN, Barnett NJ, Dougherty G, Ward G. A critical review of laser Doppler flowmetry. *J Med Eng Technol.* 1990;14:178-81.

55. Bull HA, Dowd PM. Edothelin-1 in human skin. *Dermatology.* 1993;187:1-5.

56. Burnstock G, Ralevic V. New insights into the local regulation of blood flow by perivascular nerves and endothelium. *Br J Plast Surg.* 1994;47:527-43.

57. Torok T, Bari F, Kardos A, Paprika D, Rudas L. Isometric handgrip exercise-induced muscarinic vasodilation in the human skin microvasculature. *Acta Physiol Hung.* 1997;85:193-8.

58. Kovacs L, Torok T, Bari F, Keri Z, Kovacs A, Makula E, Pokorny G. Impaired microvascular response to cholinergic stimuli in primary Sjogren's syndrome. *Ann Rheum Dis.* 2000;59:48-53.

59. Barisoni DM, Veall N. Effect of thymoxamine on circulation in skin flaps and in denervated skin. *Lancet.* 1969;1:400-1.

60. Bacman S, Sterin-Borda L, Camusso JJ, Arana R, Hubscher O, Borda E. Circulating antibodies against rat parotid gland M3 muscarinic receptors in primary Sjogren's syndrome. *Clin Exp Immunol.* 1996;104:454-9.

61. Fox RI, Maruyama T. Pathogenesis and treatment of Sjogren's syndrome. *Curr Opin Rheumatol.* 1997;9:393-9.

62. Mandl T, Jacobsson L, Lilja B, Sundkvist G, Manthorpe R. Disturbances of autonomic nervous function in primary Sjogren's syndrome. *Scand J Rheumatol.* 1997;26:401-6.

APPENDIX