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Short-term monitoring of the vascular resistance of the human skin microvasculature

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Objective and method: Laser Doppler flowmetry is a non-invasive tool in assessing the temporary changes of skin microcirculation. Another non-invasive equipment the Finapres 2300 finger blood pressure monitor provides a continuous blood pressure signal. The combination of the two devices allows short-term monitoring of the changes in the resistance of skin microvasculature. In order to assess the role of skin blood vessels in physiological responses to complex reflex tests Valsalva manoeuvre was performed by 12 healthy volunteers. For comparison a thermal stimulation (cold pressor) test was also done.

Results: The two tests resulted in skin blood flow responses of similar magnitude. The changes in calculated regional peripheral resistance (dRPR) indicated that both responses involved active vasoconstrictor mechanisms. It is of importance that the active vasoconstriction could be documented only at the late strain phase (V2) but not in the early strain phase (V1) of the Valsalva manoeuvre (%dRPR in V1=0.14 vs. V2=0.96, $p<0.05$).

Conclusions: In conclusion our findings support the theory that changes in the tone of the skin blood vessels parallel the changes in systemic vasculature in response to complex reflex tests. This is the first report which documents the feasibility of the continuous monitoring of the regional peripheral resistance.

Keywords: laser Doppler flowmetry, Valsalva manoeuvre, cold pressor test, regional vascular resistance

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Laser Doppler flowmetry (LDF) provides a continuous non-invasive quantitative measurement of tissue blood flow with high spatial and temporal resolution of the microcirculation [1, 2, 3, 4]. The laser Doppler method used to measure perfusion in discrete sections revealed several categories of rhythmic variations in the flow of human skin [5, 6, 7]. There are also indications that skin is involved in microcirculatory responses induced by cardiovascular reflexes in normal and pathological conditions [8, 9]. The technique, which primarily monitors flow in the arteriolar-capillary network quantifies the frequency shift of laser light caused by moving blood cells in the vessels. The LDF is highly sensitive to movement, positioning, and myriad other factors that can alter skin perfusion. The LDF technique has limitations and can be modified by several uncontrollable factors. The best application of LDF is therefore to determine the relative changes in microcirculatory blood flow to certain characterized stimuli instead of the determination of absolute blood flow [8, 9]. As skin blood flow can be measured simply, non-invasively, simultaneously and continuously it provides several advantages in autonomic reflex tests. The reduction in skin blood flow in response to the various stimuli can be used as an index of arteriolar tone, which in turn is determined by sympathetic nerve traffic.

The traditional laboratory reflex tests are accompanied by rapid blood pressure fluctuations. Formerly these blood pressure swings could be detected only by intra-arterial monitoring. The invasive technique however has several limitations. Technical, legal and ethical considerations have so far prevented its widespread use. More importantly the intravascular instrumentation itself may significantly alter the autonomic regulation [10]. Most recently however a continuous non-invasive blood pressure monitor, the Finapres has become commercially available. The reliability of this device has been already tested during laboratory conditions with satisfactory results [11]. The combination of the laser Doppler flowmetry and continuous blood pressure monitoring allows continuous determination of the local peripheral vascular resistance. The resistance changes in turn may help in differentiation of active and passive blood flow responses.

The aim of the present study therefore was to assess the feasibility of continuous monitoring of the cutaneous vascular resistance in healthy volunteers. Our further goal was to characterise passive and active local alterations of the cutaneous vascular resistance during autonomic reflex tests.

Methods

Healthy subjects were recruited to participate in this study. The study group consisted of 12 (6 male and 6 female) healthy subjects, who ranged from 18 to 28 years of age.

The subjects were studied in supine rest position, in the afternoon, 3 to 4 hours after meal. For our recordings, the study was not begun until stable baseline of blood pressure and skin blood flow signals for each subject was obtained. Two sets of measurements (Valsalva manoeuvre and cold pressor test) were taken on each subject. Each test was done in duplicate. The measured parameters returned to the baseline within 3 minutes following the tests. Another test was not performed until stability had been regained.

Regional peripheral resistance (RPR) was calculated in the strain (V1 point) and at the late strain phase (V2 point) of the Valsalva manoeuvre (Fig. 1) For characterisation of vasoconstrictor response to cold pressor test the lowest value of skin blood flow was used.

Measurement of skin blood flow

Relative blood cell perfusion was measured with a Periflux PF 3 LD 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 a 2.5 V at the analogue perfusion output. The measurement field of the laser Doppler skin probe is restricted to 1 mm^3 of the skin microvasculature [12].

The laser Doppler probe and probe holder were attached to the index finger pad of the left hand. In preliminary studies, we examined skin vasomotor reflexes at different sites including the forehead, forearm and index finger pad. Responses of these sites were fairly uniform, therefore we continued the measurements at the index finger pads, which contain only vasoconstrictor fibers. Skin temperature was found to modify the vasoconstrictor responses substantially. At a skin temperature of less than 28 °C, skin blood flow was very low and vasomotor responses were often unobtainable. At skin temperature of more than 40 °C blood flow was very high, and skin vasomotor reflexes were attenuated. Responses seemed to be optimal at 34 to 36 °C.

Continuous blood pressure monitoring

The blood pressure was measured continuously with Finapres 2300 non-invasive blood pressure monitor developed by Wesseling and coworkers. The measurement is based on the Penaz principle. The blood volume under an inflatable finger cuff is measured with an infrared photoplethysmograph, which is mounted inside the cuff. The blood volume as seen by the plethysmograph is clamped to setpoint value by appropriately adjusting cuff pressure in parallel with intraarterial pressure by means of an electropneumatic servosystem. The volume clamp setpoint is regularly adjusted to keep the pressure difference across the arterial wall, the transmural pressure, at zero. At zero transmural pressure, cuff pressure equals intraarterial pressure, which is then determined indirectly by measuring cuff pressure [13, 14].

Valsalva manoeuvre

Each subject was asked to maintain a column of mercury at 40 to 50 mm for 15 seconds with forced expiration, and then to resume normal expiration. A small air leakage was allowed to prevent closure of the glottis.

The Valsalva manoeuvre is commonly used for assessing autonomic function in humans. The effects of the Valsalva manoeuvre on arterial blood pressure is well documented. [15] 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. Toward the end of the strain phase blood pressure recovers as heart rate and vascular resistance increase to compensate for the lower cardiac output. On release of the Valsalva manoeuvre, pressure transiently falls, then increases to above control. The overshoot phase is accompanied by rebound bradycardia. In our experiments initial blood pressure increase set the V 1 point, and the deepest point of the second phase on blood pressure recording set the V2 point (Fig. 1) [15].

Cold pressor test

The subjects were asked to immerse their right hand (the hand contralateral to the laser Doppler velocimeter) into a basin of ice cold water (0 Celsius degree) to the level of the right wrist for 20 seconds. Immersion of part of the body in ice cold water causes an increase in arterial blood pressure and efferent sympathetic activity (Fig. 2)[16].

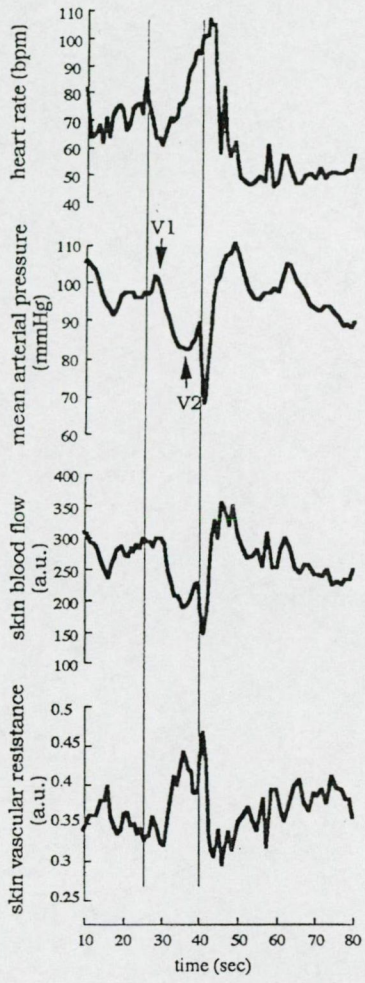

Fig. 1. The heart rate, mean arterial blood pressure, skin blood flow, and skin vascular resistance changes are presented during Valsalva manoeuvre. The lines indicate the onset and the release of the manoeuvre. The initial blood pressure increase (V1) and the deepest point of the strain phase (V2) on the blood pressure trend curve are set

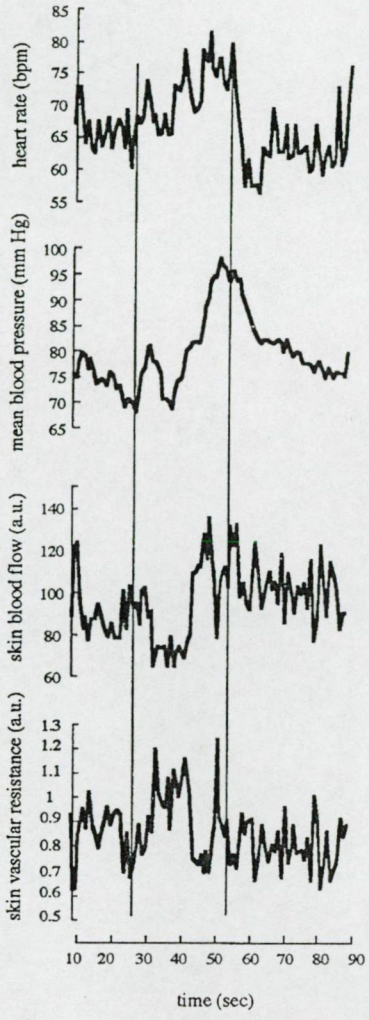

Fig. 2. The heart rate, blood pressure, skin blood flow and regional peripheral resistance during the cold pressor test are presented. The lines indicates the onset and the end of the ice water immersion

Calculation of the regional peripheral resistance

The skin blood flow (SBF) and mean arterial pressure (MAP) were used to calculate the regional peripheral resistance

$$RPR = MAP/SBF$$

The skin blood flow (SBF) and mean arterial pressure (MAP) were also used to express the relative change in regional peripheral resistance (dRPR %) during the examined reflexes according to the following formula:

$$dRPR = (RPRx - RPRbase)/RPRbase$$

where x means any points of the reflex tests.

For characterization the vasomotor responses in our study we used the previously defined V1 and V2 points during the Valsalva manoeuvre. For characterisation of vasoconstrictor response to cold pressor test the maximal change in skin blood flow was used.

Statistical analysis

Normally distributed data were compared by using the one-way repeated measurement ANOVA. To isolate which group is differ from the others we used a multiple comparison procedure (Bonferroni's *t*-test). Non-Gaussian data were analyzed by using the Friedman repeated measurement ANOVA test. Student-Newman-Keuls method was performed as all pairwise multiple comparison method.

Results

Valsalva manoeuvre (Tables I and III)

The typical skin blood flow and blood pressure responses to Valsalva manoeuvre is shown in Fig. 1. Skin blood flow responses followed a pattern of four phases that corresponded to the four blood pressure phases of the Valsalva manoeuvre. Comparison of skin blood flow, mean arterial pressure and calculated regional peripheral resistance at the baseline, V1 point and at V2 point levels are summarized in Table I. The initial phase of the Valsalva manoeuvre resulted in a significant increase in mean arterial pressure and skin blood flow, however the calculated regional peripheral resistance remained unchanged. Toward the end of the strain phase there was a drop in mean arterial pressure below baseline. At this point there was also a marked decrease in skin blood flow. The calculated regional peripheral resistance showed significant elevation.

Table I

The mean arterial pressure, the skin blood flow and the regional peripheral resistance at the three characteristic points of the Valsalva manoeuvre

	Baseline	V1	V2
MAP (mmHg)	77.595 ± 8.3	104.79 ± 9.2*	70.96 ± 9.29*
SBF (PU)	546.55 ± 275.22	655.195 ± 222.62*	329.27 ± 294.42*
RPR	0.18 ± 0.096	0.178 ± 0.062	0.37 ± 0.23*

MAP = mean arterial pressure; SBF = skin blood flow; RPR = regional peripheral resistance * = $p<0.05$ vs baseline

Table II

The mean arterial pressure, the skin blood flow and the regional peripheral resistance responses to cold pressor test

	Baseline	Cold pressor test
MAP (mmHg)	70.33 ± 7.17	96.396 ± 10.48*
SBF (PU)	524.25 ± 252.92	390.709 ± 246.53*
RPR	0.168 ± 0.083	0.36 ± 0.228*

MAP = mean arterial pressure; SBF = skin blood flow; RPR = regional peripheral resistance * = $p<0.05$ vs baseline

Table III

The extent of the mean percentage changes in regional peripheral resistance (dRPR%) at different points of the cardiovascular reflex tests

	dRPR%
V1	0.139
V2	0.956
Cold pressor test	0.962

dRPR% = (RPRx – RPRbase)/RPRbase

Cold pressor test (Tables II and III)

Cold immersion of the contralateral hand resulted in significant increase in mean arterial pressure. The skin blood flow at the same time exhibited a significant reduction. As a consequence the calculated regional peripheral resistance showed a marked – more than double-fold – elevation. In Table III we summarized the extent of the percentage change in regional peripheral resistance at different points in our reflex tests.

Discussion

According to the traditional view changes in the resistance of skin vessels are governed mainly by thermoregulatory reflexes [17, 18]. Thus the relative changes in skin blood flow during complex reflex tests such as Valsalva manoeuvre could be regarded as reflections of the changes in systemic blood flow. Recently however Low et al. have documented that these changes in skin blood flow are results of active local regulating mechanisms [19]. Our goal was to assess the responses of the skin vasculature to thermal and baroreflex changes. Changes were characterized by calculating relative resistance from the continuously recorded blood flow signals. Our findings are in concordance with that of Low et al. The thermal and the baroreflex responses involved comparable temporary fluctuations in skin blood flow. Physiological responses to cold exposure are well documented [16, 17, 18]. It is of importance that the initial strain phase of the Valsalva manoeuvre was parallelled by proportional changes in the skin blood flow and finger blood pressure. Thus the calculated skin resistance remained unchanged. Toward the end of the strain phase however the decrease in blood pressure was accompanied by an inproportionally marked decrease in blood flow, signalling increasing skin resistance. The same tendency has been already documented in the changes in systemic vascular resistance during the Valsalva manoeuvre [20, 21]. Cutaneous blood flow shows a parallel behaviour with blood pressure modification under Valsalva manoeuvre while it clearly decreases when blood pressure rises during cold pressore test. The opposite relationship between cutaneous blood flow and blood pressure behaviour during the two stimuli is probably determined by different reflexogenic modifications triggered by the Valsalva manoeuvre and cold pressor test. The response to the cold exposure certainly involves similar active vasoconstriction. The extent of the active vasoconstriction characterised by the percentage changes of regional peripheral resistance was very similar during the two tests.

Low et al. were the first to suggest a possibility to calculate skin vascular resistance [19]. In their study they utilized traditional blood pressure measurements with mercury sphygmomanometer, which allows only infrequent sampling. Owing to the continuous blood pressure measurements our findings are more accurate then the previous data. Indeed the continuous monitoring of the skin vascular resistance with our system seems to be realistic. The feasibility of this monitoring however is not yet evaluated, and merits further studies.

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II

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Isometric handgrip exercise-induced muscarinic vasodilation in the human skin microvasculature

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The existence of an active vasodilator system in the human skin microvasculature is well documented, but its physiological role and the underlying mechanisms are not completely understood. Cutaneous blood flow increases during isometric handgrip exercise. To examine whether this response is mediated by active vasodilation, isometric handgrip exercise testing was performed in nine healthy subjects. Local iontophoresis of atropine was applied to the forearm skin. Skin blood flow (SBF) monitoring by means of laser Doppler flowmetry was combined with continuous noninvasive blood pressure monitoring. SBF monitoring was performed at a site pretreated with atropine and an adjacent control area. Mean arterial pressure (MAP) was recorded noninvasively. Cutaneous vascular resistance (CVR) was calculated as MAP/SBF for the atropine treated and the control areas. Changes in CVR were expressed as percent deviation from the baseline (dCVR). Isometric handgrip exercise resulted in a marked reduction in CVR at the control site (dCVR: –66±4%). In contrast, the CVR was not significantly altered at the atropine-treated site (2.4±7%). It is concluded that isometric exercise induces an atropine-sensitive vasodilation which is mediated by muscarinic receptors in the human skin.

Keywords: cholinergic, vasodilation, cutaneous vascular resistance, static exercise

Cutaneous vasodilation may result from a withdrawal of the sympathetic adrenergic activity and/or activation of an independent vasodilator system. Active vasodilation plays an important role in thermoregulatory responses [5]. In contrast, the mechanism of cutaneous vasodilation during static exercise is controversial [3, 4, 9]. The onset of the static exercise is accompanied by vasodilation, mediated by

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sympathetic cholinergic nerve fibers in the muscles [8]. Cholinergic vasodilation may also be operative in the skin during isometric handgrip exercise.

Methods

Nine healthy male volunteers, ranging in age from 19 to 32 years, were recruited to participate in the study. All subjects provided written informed consent. Blood pressure (MAP), heart rate, skin blood flow (SBF), skin temperature were continuously monitored. Since the responsiveness of the skin microvasculature may vary with the ambient temperature [5], we studied the reflex responses at constant skin temperature (see below). To block muscarinic receptors, atropine was applied to a 0.6 cm^2 circular area of the non-exercising forearm skin by means of iontophoresis 30 min before the onset of recording [5]. The subjects were studied in a supine resting position, in the afternoon, 3 to 4 h after food intake. The subjects were instrumented for measurements of heart rate, finger blood pressure, skin temperature and SBF. The recording was started when stable baselines for blood pressure, heart rate and SBF were observed. Subsequent to a 5-min period of baseline data collection, isometric handgrip exercise was performed by each individual. Post-exercise data acquisition was continued until all parameters had returned to baseline values.

Relative blood cell perfusion was measured by means of a double-channel skin perfusion monitor (Perimed). The laser Doppler probes and the probe holders were attached to the ventral side of the treated (non-exercising) forearm. SBF was determined in the atropine-treated and in control areas. The control area was approximately 5 cm proximal to the treated region. The temperature probe was placed in between the two laser Doppler probe holders. Blood pressure was measured continuously on the non-exercising side by means of a Finapres 2300 (Ohmeda) noninvasive blood pressure monitor. The ECG was monitored by a Siemens Sirecust 730 ECG monitor. The R wave of the electrocardiogram and the plethysmographic signals were fed via an amplifier, a filter and analog to digital converter into an IBM–AT compatible computer.

All subjects performed isometric handgrip exercise (IHG) at 30% maximal voluntary contraction for 2 min with an exercise dynamometer. Maximum voluntary contraction was determined for each subject just before the beginning of the experimental session. Subjects were instructed to avoid a Valsalva manoeuvre during IHG.

Cutaneous vascular resistance (CVR) was determined as the ratio of MAP and SBF.

The percentage change in CVR (dCVR) was expressed as follows:

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

where x denotes the maximum change during the test.

Statistical analysis

Data with normal distribution were compared by using the one-way ANOVA for repeated measures. Non-Gaussian data were analyzed using the Friedman' test. An alfa level of $p<0.05$ was considered to be significant.

Results

Skin temperature (mean±S.E.M) of the volunteers was 33.9±1.2 °C; no significant fluctuations were detected during static exercising.

The SBF and MAP responses to IHG are shown in Figures 1 and 2. MAP increased continuously during the manoeuvre (baseline: 91±4 mm Hg, maximum: 122± 4 mm Hg, $p<0.05$). On termination of isometric exercise, MAP returned to baseline level. SBF increased significantly in the control area (baseline: 335±88 a.u., maximum: 1396±286 a.u., $p<0.05$) and a rapid post-exercise recovery was observed. SBF did not change significantly in the atropine-treated region throughout the test (baseline: 509±38 a.u maximum: 531±132 a.u., p=NS). CVR decreased significantly at the control site (–0.66±0.1 a.u., $p<0.05$ vs. baseline). In contrast, no significant CVR changes were seen in the region treated with atropine (0.02±0.06 a.u., p=NS). The maximum changes in CVR differed significantly between the atropine-treated and control areas.

Discussion

The objective of the present study was to determine microvascular responses of the non-exercising forearm skin during contralateral isometric exercise and to characterize the underlying mechanisms. A cholinergic cutaneous vasodilation response was hypothesized to accompany static exercise. We therefore utilized a self-controlled study design, comparing atropine-treated and control skin areas. The major finding of our study is that isometric exercise-induced human skin vasodilation is cholinergically mediated. This result is at variance with previous reports [7]. Roddie et al. reported vasodilation in the forearm tissues during body heating. This response was not prevented by intraarterial administration of atropine, but was abolished the sweating reaction effectively. A non-cholinergic, non-adrenergic vasodilator nerve activity was postulated as the probable mechanism [7].

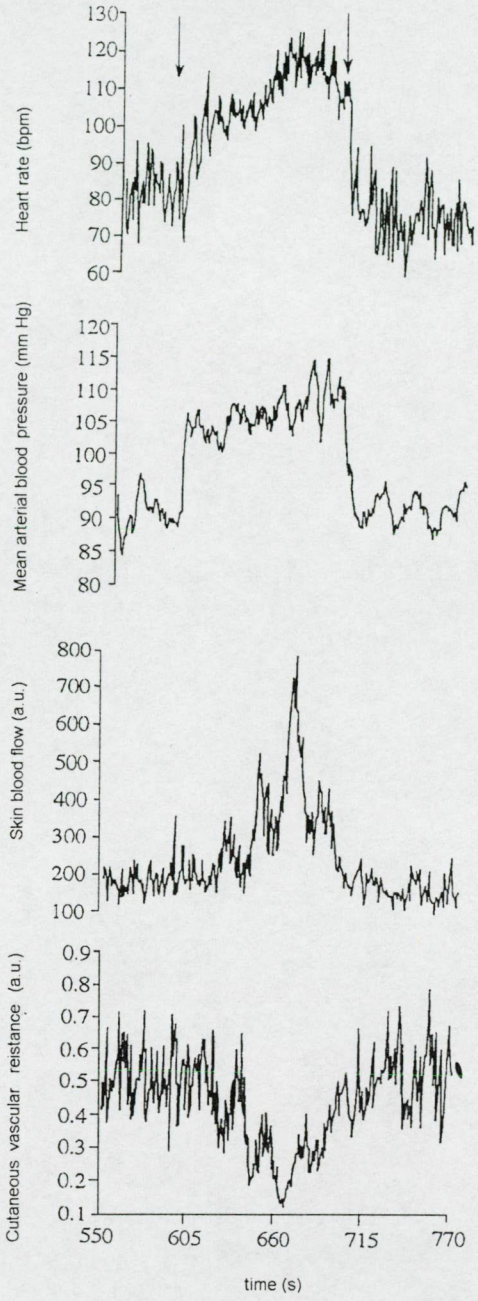

Fig. 1. Typical heart rate, MAP, and SBF response to 2-min IHG at the control site. MAP increases continuously during the maneuver. On termination of IHG MAP returns to the baseline level. SBF increases, and a rapid post-exercise recovery period can be observed. The change in CVR shows active vasodilation

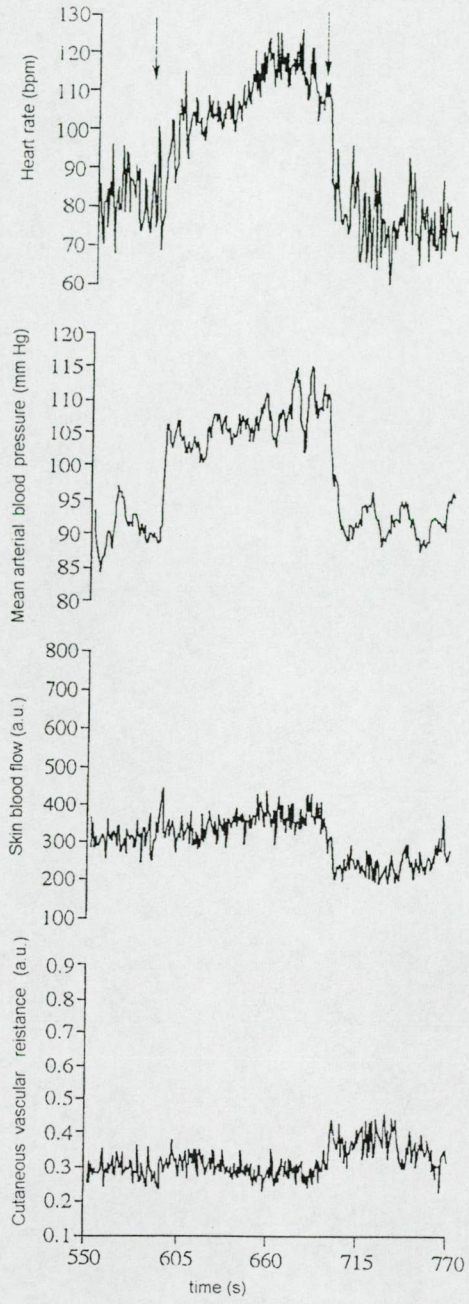

Fig. 2. Typical heart rate, MAP, and SBF response to 2-min IHG at the atropine-treated site. No significant SBF and CVR changes can be seen in that region

Sanders et al. found an active vasodilation in the non-exercising forearm muscles at the beginning of isometric handgrip exercise, despite the well-known activation of sympathetic vasoconstrictor fibers. They have documented that this response is primarily mediated by cholinergic mechanisms. Withdrawal of the adrenergic vasoconstrictor tone and the activation of β2 receptors could both be excluded [8]. It has been reported that an acethylcholine-mediated active vasodilation is operational in the skin microvasculature [2, 6]. Our present findings further extend the observation of these studies. The cutaneous cholinergic vasodilation system could be triggered by isometric exercise.

Acethylcholine induced vasodilation is currently attributed to the release of endothelium derived relaxing factors including nitric oxide [1]. By analogies drawn from this study, there is a possibility of nitric oxide-mediated dilation of the human skin microvasculature as well.

In conclusion, our results provide further evidence in favour of the presence of a cutaneous cholinergic vasodilator system which can be activated by isometric exercise.

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III

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The effects of patterned breathing and continuous positive airway pressure on cardiovascular regulation in healthy volunteers

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1. Although the increased heart rate variability in healthy subjects in association with slow patterned breathing and continuous positive airway pressure is well documented, there is no general agreement regarding the underlying mechanism. The arterial baroreceptor stimulation due to greater blood pressure variability, the stimulation of pulmonary stretch and low pressure baroreceptors can play important role in this phenomenon.

2. In order to assess the interplay between blood pressure and heart rate changes we have studied nine healthy volunteers (mean age was 22 yrs. range 19–24), by applying 6/min patterned breathing, and continuous positive airway pressure of 10 cm of water. ECG and finger blood pressure (Finapres 2300) was continuously recorded. The oscillation amplitude of R–R intervals were analysed as well as the time and frequency domain indexes of heart rate variability. The oscillation amplitude and the corresponding frequency domain components of systolic blood pressure were also calculated.

3. The forced deep breathing caused significant increase in heart rate variability as indicated by time and frequency domain analysis of R-R intervals (LF HRV ms^2: spontaneous: 777.40±526.1, patterned breathing 6828.00±5468.0). The application of CPAP in the same rhythm during patterned breathing resulted in further enhancement in heart rate variability (LF HRV ms^2: 9052.00±4533.0). The analysis of the same frequency domain components of systolic blood pressure showed marked elevation of the total and low frequency power during patterned breathing. (LF BPV mm Hg^2: spontaneous: 8.24±6.2, patterned breathing: 16.22±9.7). Applying CPAP with the same breathing pattern elicited further significant increment in systolic blood pressure fluctuation (LF BPV mm Hg^2: deep breathing+CPAP: 27.11±9.8). The baroreflex sensitivity as calculated from spontaneous HR and BP sequences was 11.66±2.9 at baseline and increased to 17.66±6.1 and changed to 15.22±3.2 with the addition of patterned breathing and CPAP, respectively.

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4. Our findings indicate that the heart rate and blood pressure responses to slow patterned breathing may be interpreted as consequences of an altered baroreflex sensitivity. In contrast the active breathing with CPAP exerts mechanical effects which in turn present an augmented systemic baroreflex trigger, however, the baroreflex sensitivity remains unchanged.

Keywords: spectral power, heart rate variability, blood pressure variability, continuous positive airway pressure, autonomic nervous system

It has been long known that respiration is associated with heart rate and blood pressure changes [1, 2]. The respiratory sinus arrhythmia involves inspiratory heart rate acceleration, and expiratory (or postinspiratory) heart rate deceleration [3]. It has also been realized that the extent of the pulse rate fluctuations is related to the frequency and depth of breathing, the highest amplitude changes being reached around the breathing frequency of 6/min [4, 5]. The breathing rate also bears influence on the blood pressure responses. The amplitude of the blood pressure swings increases with decreasing respiratory rate, with a maximum again around the 6/min. respiratory frequency [3]. It has been noted by Dornhorst et al. that the relation between the blood pressure variation and the respiratory phase is a function of the respiratory rate [3]. More recently Laude et al. documented that systolic blood pressure decreases during inspiration with a time delay which increases as breathing frequency decreases [6].

The effects of the positive pressure ventilation and continuous positive airway pressure on the circulation and the cardiovascular autonomic regulation are well recognized, however the exact mechanisms remain subject of controversy [7, 8]. Continuous positive airway pressure (CPAP) exerts mechanical effects in healthy subjects by increasing the left ventricular filling pressure and the right atrial pressure, the downstream pressure for venous return [9]. The effect of active and passive breathing with CPAP may induce different responses [10], and the role of the breathing frequency while applying CPAP is not known.

Monitoring the oscillations of hemodynamic parameters is a new field in anesthesiology. Since the magnitude of blood pressure oscillation caused by the mechanical effect of positive pressure ventilation is dependent on the volume status of the patients, a number of recent studies focused on this relationship [11]. Another approach, the spectral assessment of heart rate variability, has already gained different applications [12, 13]. Although the hemodynamic effects of both components have been extensively studied their interactions remains unclear. In order to assess the additional effects of slow patterned breathing and continuous positive airway pressure a series of studies was performed on 9 healthy volunteers.

Methods

Subjects

Healthy subjects were recruited to participate in this study. The study group consisted of nine male volunteers who ranged from 19 to 24 years in age (mean: 22 years). Their assessment revealed no pulmonary, cardiovascular or neurologic disease. None were taking medications. The subjects were studied in supine rest position, in the afternoon, 3 to 4 hours after meal. For our recordings, the study was not begun until the most stable baseline of blood pressure and heart rate signals for each subject was obtained. The subjects were non smokers. All subject gave their written informed consent prior to the experiments.

Measurements and calculation

The electrocardiogram was continuously recorded with a Siemens Sirecust 730 monitor. Blood pressure was measured noninvasively with a Finapres 2300 (Ohmeda) device, which has been referred to reflect blood pressure spectral changes reliably. The R wave of the electrocardiogram and the plethysmographic signals were fed through an amplifier, filter and analog-digital converter into an IBM–AT compatible computer. Data were stored and analysed by a self-developed program written in Microsoft C/C++ 7.0. The computer measured the interval between successive R waves with a precision of 2 ms. The accuracy of ECG signals detection was 40 microvolts and 1 mm Hg. The cardiotachogram and trend-grams of the blood pressure values were continuously recorded on-line. The oscillation amplitude of R–R intervals and systolic blood pressure were analysed. The spectral analysis of R–R interval and systolic blood pressure variability were computed by using fast Fourier transformation, using the Hanning window over two frequency bands: low frequency power (0.04 to 0.14 Hz) and high frequency power (0.15 to 0.4 Hz). We considered a total frequency power range from 0.01 to 0.4 Hz. 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 >50 ms (pNN5O), root mean square successive differences (rMSSD) were also calculated.

Baroreflex sensitivity (BRS) was characterised by the spontaneous sequences method as described earlier [14, 15]. It has been stated that 3 or more cardiac cycles of unidirectional BP increase or decrease with the corresponding lengthening or shortening of the interbeat intervals form spontaneous "up-" or "down-sequences" [15]. These sequences are analogous to those induced by pharmacological manoeuvres, and a spontaneous baroreflex sensitivity could be determined as an average of several

individual slopes [14]. In this study each step changes in systolic blood pressure (delta SBP) were paired with the changes in the subsequent RR intervals (delta RR). This method is referred as lag 1 technik [15].

Protocol

The subjects were studied in supine rest position. The subjects were asked to close their eyes, relax not to cough during the measurements. The R–R intervals and the finger blood pressure were monitored and recorded continuously over ten minutes baseline period followed by 30 minutes of study period. Three sets of measurements were taken: during spontaneous, then during patterned breathing (6/min) without CPAP, and finally during 6/min patterned breathing while applying CPAP. High flow system was used to keep CPAP level constant throughout the respiratory cycle, avoiding flow dependency of the airway pressure. Ten cm of water CPAP was applied through a mouthpiece using the Veolar (Hamilton) ventilator while the subject's nose was clamped. Subjects were instructed not to change the depth of breathing with the addition of CPAP. The magnitude of applied pressure was monitored by the Leonardo graphical computer program attached to the ventilator.

Statistical analysis

Sheffe's test was used to compare the three independent variables. For data showing non-normal distribution, and the skewness coefficient was >1 the statistical tests were done only after logarithmic transformation. We considered the differences statistically significant when p was less then 0.05. Data are given as means and standard errors.

Results

The effect of patterned breathing and CPAP on HRV (Fig. 1, Table I)

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 pNN5O 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 heart rate variability 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.

Table I

Heart rate and blood pressure variability and baroreflex sensitivity during the three different situations

	Spontaneous breathing	Scheffe F test	Metronom breathing	Scheffe F test	Metronom breathing+CPAP
R–R mean (ms)	847.5±34.7	0.85	798.6±26.1	0.51	786.7±14.0
SBP mean (mm Hg)	116.1±6.0	0.065	113.2±5.5	0.125	117.1±5.2
R–R oscill (ms)	80.7±10.1	12.9*	238.1±26.72	1.63	294.1±24.9
SBP oscill (mm Hg)	6.83±1.16	7.32*	14.5±1.36	4.24*	20.3±1.66
SD RR (ms)	46.4±4.3	6.61*	90.6±11.87	0.81	106.2±7.8
pNN50%	25.3±5.03	0.436	31.1±4.5	0.702	38.4±3.36
rMSSD (ms)	44.1±4.3	1.48	61.3±9.4	0.34	69.6±6.4
TFhrv (ms^2)	1703±256.7	3.87*	8059.8±2232.4	0.526	10402.1±1664.6
LFhrv (ms^2)	777.4±175.3	4.87*	6828±1822	0.65	9052.4±1511
HFhrv (ms^2)	595.8±173.8	0.52	1000±422.7	0.029	904.3±155.3
TFbpv (mm Hg^2)	15.46±3.2	0.85	23.3±4.79	3.53*	39.3±4.6
LFbpv (mm Hg^2)	8.24±2.07	1.85	16.22±3.25	3.46*	27.11±3.2
HFbpv (mm Hg^2)	2.37±0.91	0.28	1.63±0.38	1.324	3.23±0.61
BRS spont (ms/mm Hg)	11.6±1.2	4.12*	17.6±2.06	0.649	15.2±1.09

CPAP=continuous positive airway pressure, SBP=systolic blood pressure, hrv (bpv)=heart rate (blood pressure) variability, BRS=baroreflex sensitivity, TF, LF, HF=total, low, high frequency power, *significant at 95%

The effects of the patterned breathing and CPAP on the blood pressure variability (Fig. 1)

The mean and the standard deviation of the systolic blood pressure 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 blood pressure variability compared to both the baseline parameters and to those recorded during patterned breathing. The blood pressure power in the high frequency range did not change during the three different situations.

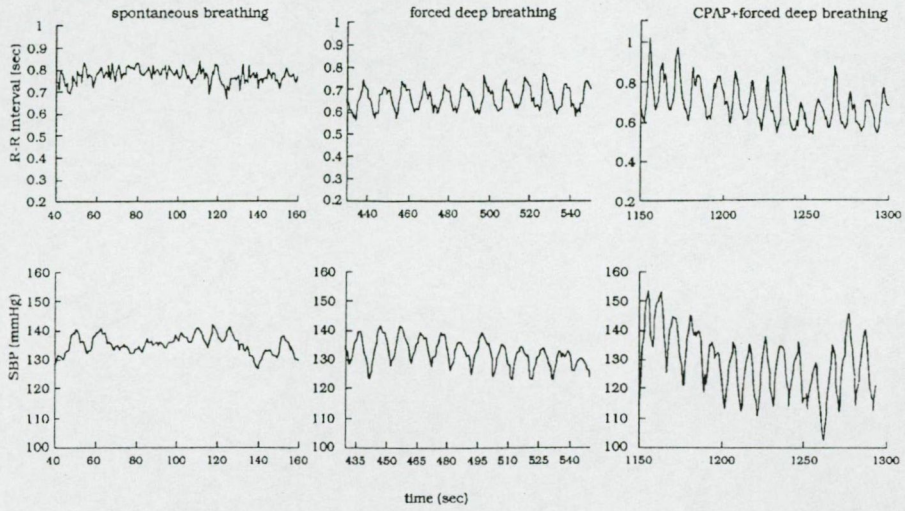

Fig. 1. The R–R intervals and systolic blood pressure (SBP) trendgrams are shown during the three breathing conditions in a single subject. CPAP: continuos positive airway pressure

The oscillation amplitude of the R–R intervals and the systolic blood pressure (Fig. 2)

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 blood pressure increased during patterned breathing, and showed further significant elevation due to the application of CPAP.

Analysis of baroreflex sensitivity and baroreceptor gain

The baroreflex sensitivity determined by the method of spontaneous sequences showed statistically significant elevation on transition from spontaneous to patterned breathing. No further changes were detected while breathing with CPAP.

Discussion

The genesis of respiratory sinus arrhythmia is complex. The following mechanisms have been implicated: 1. Central cardiorespiratory coupling. 2. Reflexes from pulmonary stretch receptors. 3. Arterial baroreflexes. 4. Reflexes from the cardiac

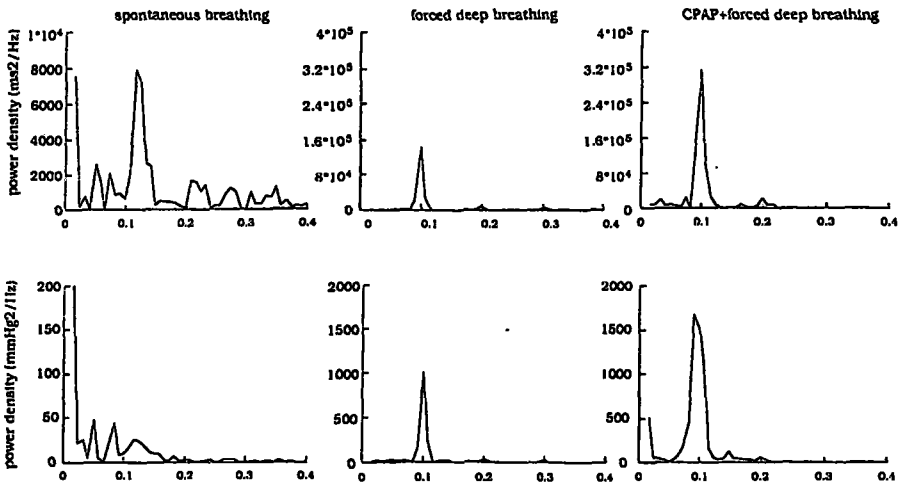

Fig. 2. The power spectral densities of R–R intervals and systolic blood pressure (SBP) are shown during the three breathing conditions in a single subject. CPAP: continuos positive airway pressure

(low pressure) baroreceptors [16]. The relative roles of these mechanisms remain unclear. The mechanism of augmented heart rate fluctuation at slow breathing rates – a proposed marker of cardiac vagal integrity – is also subject of debate. In our study, in concordance with previous publications, the patterned breathing with the frequency of 6 cycles/min. elicited a significant increase in the amplitude of both HR and BP fluctuations [3, 6]. The same effect has been reflected by the increment in the BPV and HRV total power. The marked respiratory effect appeared in the low frequency spectral band, corresponding to the slow breathing rate. There is a superposition of the breathing induced hemodynamic changes on the other low frequency oscillations governed mainly by baroreflex mechanisms [17]. This amplification, or resonating baroreflex control loop mechanism explains the accentuated 0.1 Hz BPV and HRV peaks. The efferent mechanisms of this regulations involve both parasympathetic influences on the heart rate, and sympathetic influences on the heart rate and peripheral vascular tone [17]. Thus the designation of this peak in the given setting as "sympathetic" or "parasympathetic" is meaningless. Although the amplitude of HR changes in response to the slow patterned breathing is widely used to characterize cardiac parasympathetic tone [18], there have also been controversies regarding this marker. Kollai and Mizsei demonstrated that respiratory sinus arrhythmia is a limited measure of cardiac parasympathetic control in man [19]. One of our most interesting results is that the baroreflex sensitivity was substantially higher during slow deep

breathing than during spontaneous breathing. The increased BRS during 6/min breathing could be expressed as a 50% greater HR change in response to a given rise or drop in blood pressure. The mechanism of the exaggerated baroreflex responsiveness might have been related to the altered activity of cardiopulmonary baroreceptors. Vatner et al. documented a progressive reduction of the arterial baroreflex sensitivity with acute volume loading [20], and Shi et al. found similar tendency using lower body positive pressure [21]. In contrast Bevegard et al. [22], Takeshita et al. [23], and Eiken et al. [24] reported that physiological variation of central venous pressure do not influence sinus node responses to arterial baroreceptor stimulation in man.

Toska and Eriksen first documented that the blood pressure changes during spontaneous breathing are related to the changes in stroke volume [25]. They found that the normal heart rate fluctuation exerts a buffering effect on mean blood pressure changes [25]. The anti-oscillatory role of HRV was further substantiated by Saul and Triedman [26], and Kardos et al. [27]. Beaussier et al. recently reported similar causative relationship between stroke volume and blood pressure transients during positive pressure ventilation [11]. There is a respiration related fluctuation of the preloads and afterloads of the ventricles, and pulmonary vascular capacitance. As Dornhorst pointed out that the changes in the filling of the left ventricle are determined but lags behind that of the right [3]. In addition there is a possible ventricular interdependence mediated by the displacement of the ventricular septum, and by the pericardial pressure. All of these factors may contribute to the stroke volume changes, and in turn to the blood pressure oscillations during spontaneous and positive pressure mechanical ventilation. Although application of PEEP causes marked changes in these parameters, Innes et al. [28], Yi-Hankala et al. [29], and Taha et al. [10] reported reduction of respiratory sinus arrhythmia at the time of positive pressure mechanical ventilation. Yi-Hankala et al. [29], and Taha et al. [10] also reported a phase shift of HR swings compared to spontaneous breathing. The explanation for this paradox was presented by Taha et al who demonstrated an altered profile of systolic blood pressure during passive positive pressure ventilation in comparison with spontaneous breathing. Thus, in turn the different responses were mediated by the systemic baroreflex. In our present experimental design we utilized spontaneous breathing with additional positive end expiratory pressure. Therefore the blood pressure fluctuation pattern remained normal during the test. The actual amplitude of these swings with CPAP were greater than during spontaneous and patterned breathing without positive end expiratory pressure. Thus the amplified HR oscillation with the application of CPAP could be explained by increased trigger of the systemic baroreflex. The role of low pressure baroreceptors again could not be excluded. Nevertheless the spontaneous BRS showed no further alterations compared to that of slow patterned breathing.

In conclusion our findings indicate that the well-known effects of slow patterned breathing may be interpreted as consequences of an altered baroreflex sensitivity. The exact mechanism of the phenomenon is not known and necessitates further studies. In contrast the active breathing with CPAP exerts mechanical effects which in turn present an augmented systemic baroreflex trigger. The involvement of low pressure baroreceptors and pulmonary stretch receptors remains unclear.

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IV

because of the increased number of spontaneous losses before procedures at that stage.

We agree with Konje et al about the denominator used for calculations, and that it would be inappropriate to include those lost to follow-up in the denominator for table 2. 2183 and 2185 in table 2 represent the numbers randomised, but those lost to follow-up were not included in calculation of the total loss percentage.

Konje and colleagues comment that the intent-to-treat evaluation would underestimate the frequency of various complications. We agree that various denominators can be used to present these data but we could present the data in numerous ways. Although there may be merits to the various comparisons, there were 2183 patients randomised to the early group, and 1916 amniocenteses were done in the early gestational window with 11 lost to follow-up, 150 had spontaneous and therapeutic abortions before delivery, and the remaining 2022 were born. In the 2022 babies born, there were 28 that had a talipes-like deformity, which is a marginally higher percentage than we reported.

The apparent discrepancy between the figures in table 3 and the denominators for the talipes analysis is a reflection of the different measures of estimated gestational age at amniocentesis. Table 3 represents gestational age calculated on the basis of the dating ultrasound at the time of randomisation. If the estimated age at randomisation was 60 days and the amniocentesis was done 20 days later, the calculated age at amniocentesis as shown in table 3 would be 80 days. The alternative measure of the estimated gestational age at amniocentesis was to use the crown-rump length as obtained by ultrasound on the day of amniocentesis. This crown-rump length measurement was used for the presentation of the talipes data to make it comparable with the data presented by Sudenberg and colleagues.[1]

The CEMAT group used the total fetal loss rate as a primary outcome for this trial because it was felt to be the most unbiased comparison between treatment groups. This trial was not designed to evaluate the excess fetal loss rate after early amniocentesis because the control group for that study would have had no procedure. We emphasise that although the CEMAT study showed a significant difference in total losses between the two groups, it is the other findings that provide strong evidence against the use of early amniocentesis for routine first-trimester prenatal diagnosis.

In response to Bernd Eiben and colleagues, the CEMAT trial randomised 2183 and 2185 women to early and midtrimester amniocentesis, respectively. The strength of the study is the fact that only 11 and 23 women were lost to follow-up in these groups, respectively; complete follow-up (99·2%) was available for 2172 and 2162 pregnancies, respectively. The Eiben study was not a randomised trial and fetal outcome was reported to be available in about 90% of the early-amniocentesis group. We cannot assume that adverse outcomes will be reported to the physician because on many occasions patients and physicians may not make the connection of a foot abnormality and early amniocentesis. Since foot abnormalities are fairly common (1–3 per 1000 livebirths[2]), complete follow-up is the only mechanism for appropriate comparison.

In CEMAT, the difference in total losses in the two groups was 1·7%, with one-sided CIs up to 2·98%: this is a significant difference for the early group and cannot be completely explained by physiological spontaneous losses. The spontaneous postprocedure loss rates (2·6% *vs* 0·8%) are not comparable because of different gestational ages at procedure.

The amount of fluid removed at the time of amniocentesis might be important, but both the CEMAT trial and Sudenberg's study[1] (which did not remove any fluid) had similar foot abnormality rates at 1·3% and 1·7%, respectively.

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Conflicting brain images

Sir—Miguel Viana Baptista and co-workers (Feb 7, p 414)[1] present an interesting case of a patient who underwent carotid endarterectomy due to unilateral internal-corotid-artery stenosis. Brain images taken after clinical deterioration showed that interstitial oedema appeared not only in the whole white matter (including posterior portion) of the ipsilateral side, but also in posterior portion of the white matter contralateral to the endarterectomy.

Reversible posterior leukoencephalopathy syndrome (RPLS) with encephalopathy is usually reversible and associated with hypertension, renal failure, and eclampsia, as well as the administration of immunosuppressants.[2,3] The cause of this syndrome is usually an asymmetric lesion in the white matter dominant in the posterior portion of the brain, regarded as a vasogenic oedema due to leakage from the capillary.[2,3]

Because perfusion magnetic-resonance imaging showed that the hyperperfused brain had a normal blood flow, Baptista and colleagues propose a nomenclature of "reperfusion syndrome" instead of "hyperperfusion syndrome" for their case. Here, the term RPLS involves the consequences of internal-carotid-artery endarterectomy. That RPLS can occur with such asymmetry is also noteworthy.

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Baroreflex sensitivity and heart-rate variability in insulin-dependent diabetics with polyneuropathy

Sir—The ATRAMI investigators (Feb 14, p 478)[1] show that measurement of baroreceptor reflex sensitivity (BRS) is an appropriate way to identify patients after myocardial infarction who were at a high risk of cardiac death or a non-fatal cardiac arrest because of documented ventricular fibrillation. Autonomic imbalance, however, may be asociated with an increased risk of sudden death, even without myocardial infarction.

In a 5-year follow-up study, the mortality rate of diabetics with abnormal autonomic reflexes was above 50%.[2] A meta-analysis of data on 1203 patients in nine studies revealed that the mortality rate after 5·8 years of diabetes with autonomic neuropathy was 29%, whereas the rate was 6% in diabetics with a normal autonomic function.[3] The MIDAS Study Group[4] found that myocardial

	IDDM patients	Controls	p
Autonomic score	5·7 (2·4)	0	<0·001
BRS (ms/mm Hg)			
Supine position	3·7 (2·1)	13·3 (12·7)	<0·001
Standing up	2·2 (2·1)	5·7 (2·6)*	0·002
SDNN (ms)			
Supine position	17·9 (9·1)	40·7 (24·7)	0·002
Standing up	18·5 (10·0)	43·9 (16·3)	<0·001
Peripheral sensory function (Hz)			
Median nerve			
2000 Hz	4·1 (2·1)	2·7 (0·6)	0·010
250 Hz	3·3 (3·5)	1·0 (0·4)	0·040
5 Hz	2·3 (3·3)	0·5 (0·2)	0·030
Peroneal nerve			
2000 Hz	6·6 (3·1)	3·5 (0·7)	0·019
250 Hz	5·0 (3·8)	1·6 (0·5)	0·007
5 Hz	3·0 (3·3)	0·7 (0·2)	0·002

*<0·05 (BRS after standing up vs BRS in supine position within the control group). Values are means (SD).

Neuropathy indices in patients with IDDM and controls

infarction in diabetic patients led to an increased mortality rate in diabetics, both in hospital and during 3 years of follow-up; they also found that diabetes mellitus was an independent predictor of mortality, and the relative risk for individuals aged 30–49 years was 1·87.

We analysed the BRS and heart-rate variability (SDNN: standard deviation of normal-to-normal beats) in the resting supine position and after standing up in patients with long-standing insulin-dependent diabetes mellitus (IDDM) with autonomic and sensory neuropathy.

We enrolled 12 patients (six women, six men, mean age 47·8 [SD 15·9] years, duration of IDDM 26·8 [13·4] years, HbA1c 9·1 [1·4]%, body-mass index 26·1 [5·0]) and 12 healthy controls with similar sex, age, and weight distributions. We excluded patients with coronary heart disease and those with any other disorders or on any medication likely to affect the investigated indices. Autonomic function was assessed by five standard cardiovascular reflex tests. A score (0–10) was used to express the severity of the autonomic impairment. Peripheral sensory nerve function was characterised by current perception thresholds measured by a neuro-selective diagnostic stimulator on the peroneal and median nerves, which permits transcutaneous testing at three sinusoidal frequencies (2000, 250, and 5 Hz) of electrical stimulus. We measured blood pressure continuously by means of a non-invasive photo-plethysmographic device.[4] The continuous electrocardiogram signal was fed through an analogue-digital converter and the data were analysed off-line.

BRS was calculated in the supine position and after standing up by spontaneous sequences method. We confirmed that three or more cardiac cycles of unidirectional increase or decrease in blood pressure, together with the corresponding lengthening or shortening of the interbeat intervals, form spontaneous up sequences or down sequences. These sequences are similar to those induced by drugs, and a spontaneous BRS could be determined as an average of several individual slopes. All changes in systolic blood pressure were paired with the changes in the subsequent RR intervals (lag1 technique).

The mean autonomic score of the IDDM patients was 5·7 [2·4] and all had raised current perception thresholds. In the IDDM group, BRS was lower in the resting supine position and after standing up than in the controls (table). SDNN was depressed in the IDDM patients compared with the controls. In the control group, BRS was lower after standing up than in the resting position. In the IDDM group, there was no significant difference in the BRS values measured in the supine or standing positions. Thus, the IDDM patients with polyneuropathy displayed a severely impaired cardiovascular adaptation mechanism. The decreases in heart-rate variability and BRS probably contribute to the poor prognosis of acute myocardial infarction in diabetic patients. Screening for an autonomic dysfunction in diabetics could identify patients at increased risk of cardiac death.

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Isolation of measles virus from infants in Lusaka

Sir—N S Wairagkar and colleagues (Feb 14, p 495)[1] confirm measles infections in infants younger than 4 months in India by isolation of measles virus. Since 1992, we have been isolating measles virus from children admitted to hospital in Lusaka, Zambia. Between 1992 and 1995, 1670 children in the paediatric ward of the University Teaching Hospital had a clinical diagnosis of measles. 398 (23·8%) children were younger than 9 months, the recommended age of measles vaccination in Zambia, 192 (11·2%) were aged 6 months or less, and 44 (2·6%) were 4 months or less.

Isolation of measles virus was carried out by throat swabs and peripheral-blood mononuclear cells by B95-a cells which are highly sensitive for isolation of clinical measles strains.[2] We also tested serum samples for measles-specific IgM antibodies by ELISA.[2] One of nine throat swab samples collected from infants aged 4 months was positive for the virus isolation, and six strains each were isolated from infants aged 5 and 6 months. IgM antibodies were detected in two of four cases aged 4 months, and all samples from those aged 5 or 6 months were positive. The low rate of virus isolation might reflect the delay of the sample collection after the onset of clinical symptoms.

Measles infection before the recommended vaccination age is an important issue for measles control in developing countries.[4] In Lusaka, measles infection in infants younger than 6 months is still common, and our laboratory results also confirmed measles infections in this age-group. Since measles vaccination with high titre vaccines is no longer recommended,[3] an alternative strategy should be urgently established to protect infants and young children in developing countries.

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V

Windowed FFT – a time-variant spectral analysis: applicability during the head-up tilt test

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The spectral assessment of heart rate variability (HRV) and blood pressure variability (BPV) is a well-established method for the identification of rhythmic fluctuations during stationary conditions, but there is no generally accepted method of describing dynamic changes in such spectral patterns. Our goal was to introduce an alternative means of assessing the dynamics of spectral HRV.

Continuous ECG and non-invasive BP recordings on 29 subjects during head-up tilt testing were subjected to analysis. The total spectral power and the power over the low (LF: 0.04–0.15 Hz) and the high-frequency (HF: 0.15–0.4 Hz) spectral bands were recalculated in an overlapping series with constant time shifting of the initial data-point.

The time course of LFHRV augmentation, with an early peak and subsequent levelling within the first 2 of tilting, was also documented, with parallel changes in LFBPV (LFHRV ms^2/Hz: 290 ± 96 supine, 2707 ± 1557 maximum after tilt, $p < 0.05$ LFBPV $mmHg^2$/Hz: 10 ± 4 supine, 58 ± 26 maximum after tilt, $p < 0.05$). In a subgroup of 7 patients who exhibited syncope upon tilting, a statistically significant early increase HFHRV was also detected, followed by significant decline by the second minute of tilting (HFHRV ms^2/Hz: 143 ± 80 supine, 1054 ± 902 maximum after tilt, 125 ± 84 minimum after tilt). This early HFHRV peak was absent in the group of tilt-negative subjects. Another characteristic feature of the tilt positive group was a second phase of LFBPV and LFHRV elevation preceding the syncopal episode.

Windowed fast Fourier transformation is a suitable method for assessment of dynamic HR and BP spectral changes during upright tilt testing. The method is well applicable for the analysis of tilting-induced autonomic responses. *J Clin Basic Cardiol 1999; 2: 241–4.*

Key words: spectral analysis, heart rate variability head up tilt

Modulation of cardiac interbeat intervals has been subjected to extensive studies. A certain degree of consensus regarding the spectral assessment of the heart rate variability (HRV) has already been reached [1, 2]. When fast Fourier transformation (FFT) is used to assess HRV, a minimum period of 2–3 mins is required [1, 2]. This is somewhat of a paradox: during 2 mins of a manoeuvre in which the autonomic tone can change, 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. A new time-dependent analysis, based on the autoregressive method, was recently reported [3]. Our present goal was to introduce an alternative means of assessing the dynamics of the spectral HRV and blood pressure variability (BPV). As head-up tilt testing (HUT) results in characteristic changes in the cardiovascular autonomic tone, we tested the applicability of our suggested method, windowed FFT, by analysing the records on 29 subjects during HUT. Since the literature data relating to the modification of HRV and BPV during HUT are controversial, a further goal was to describe the HRV and BPV changes during HUT with this novel method, focusing on the dynamics of the changes.

Methods

Patient population

Records on 29 patients during HUT were selected from our data base. All subjects had been referred to the arrhythmia service because of unexplained syncope. During the initial assessment, including history, physical examination, ECG and echocardiography, a cardiac cause of the syncope was definitely excluded in each case. Only virtually noise-free records were considered for further analysis. Records with ectopic beats were also excluded from the study.

Head-up tilt test

The tilt-table 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 utilised, 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.

Data acquisition and analysis

BP was monitored with a photoplethysmograph (Finapres 2300, Ohmeda). ECG signals were recorded by a 5-lead system. 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 analysed 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 mmHg. 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].

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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 [5]; the corresponding equation is:

$$X(f,\tau) = \int_{-\infty}^{\infty} w(t,\tau)x(t)e^{-i2\pi ft}dt$$

where x(t) is the signal to be transformed, w(t,τ) is the window function, τ is the time variable for the time-dependent spectrum X(f,τ).

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 analysed 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.

Statistical analysis

Changes were compared with the baseline by using ANOVA for repeated measures. For parameters exhibiting a non-Gaussian distribution, the Friedman one-way repeated measures ANOVA on ranks was used. The level of statistical significance was set at $p < 0.05$.

Results

Twenty-two subjects exhibited no abnormal reactions during the tilt test (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", characterised by bradycardia and a variable degree of hypotension. Demographic data on the tilt-positive subjects are shown in Table 1. The mean ages of the tilt-positive and negative subjects were similar (22.1 ± 4.4 and 23.5 ± 3.7 years, respectively; p = NS).

Upon tilting, there were immediate fluctuations in HR and systolic BP power spectral components (Figures 1 and 2). A statistically significant increase in the total-frequency systolic BPV (TFBPV) within 2 min from the beginning of the manoeuvre was mainly due to an LFBPV in both groups (Tables 2 and 3). Subsequently, TFBPV and LFBPV decreased in both groups and levelled at lower-than-maximum values for several minutes. Nevertheless, the minimum TFBPV and LFBPV values after tilting in this phase remained significantly higher than the baseline in the tilt-negative group (Table 2). The same tendency was seen in the tilt-positive group, but the difference in minimum TFBPV and LFBPV did not reach statistical significance as compared with the baseline (Table 3). 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. Peaking occurred within the first minute after the completion of tilting. A different response was seen when HFHRV was assessed. 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). The syncopal episode itself in the tilt-positive group was characterised 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. In contrast, the HFBPV component displayed a small and statistically non-significant increase (Table 3).

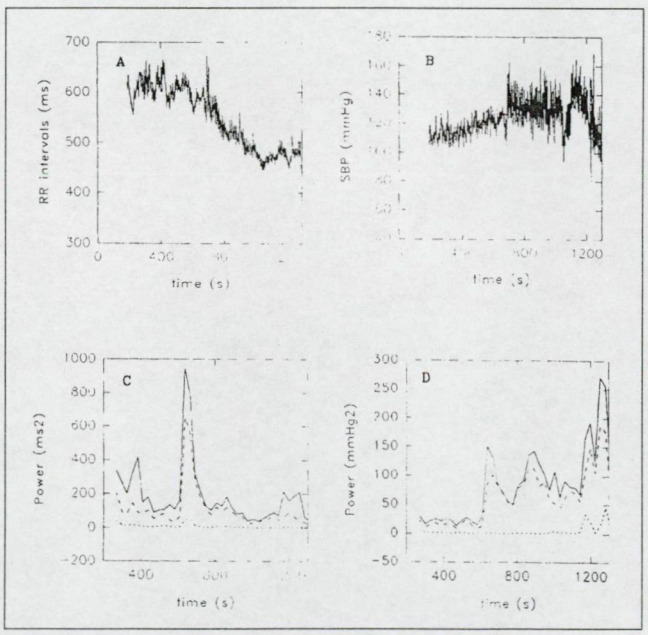

Figure 1. Upright tilt-induced responses of a patient from the tilt-negative group. *Panel A.* Normal heart rate response to upright tilt. The postural change at 600 s of the recording is accompanied by a certain RR interval shortening. *Panel B.* Although the fluctuations in systolic BP are augmented following the upright tilt, no clinically significant hypotension is recorded. *Panels C and D.* Variations in HR (C) and systolic BP (D) powers with time, as assessed by the windowed FFT method. Solid lines indicate total power, dashed lines indicate LF power, and dotted lines indicate HF power. The upright tilt manoeuvre elicits transient elevations in the LFHRV and TFHRV power. The BP power reveal immediate tilt-related increases in the same spectral bands, followed by subsequent fluctuations of lesser magnitude. The terminal increases in the BPV components are related to the motion artefacts at the time of termination of tilting.

Table 1. Demographic and tilt-table test data on patients with positive tilt-table test

Pt. no.	Age (yr)	Gender	Time of syncope from tilting (min)	dSBP (mmHg)	dHR (l/min)
1	22	F	8	135	85
2	30	F	7	50	59
3	18	M	10	72	39
4	22	M	8	95	41
5	24	F	10	47	43
6	23	F	6	62	41
7	16	F	30	68	52

Discussion

The assessment of HRV by spectral analysis is gaining increasing use in clinical cardiology [1, 2]. However, the method of spectral analysis dissects the series of events into discrete sequences, allowing no overlap in the assessment. Only a few publications related to time-dependent spectral assessment [3, 6], but the proposed methods are not yet widely accepted. Thus, spectral analysis currently remains the best possibility for the description of stationary states. Dynamic changes are 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 characterised 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 manoeuvre [7–9], or at a fixed time preceding the syncopal event [10]. 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 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 characterise the tilt-induced responses [11], and presented first documented time-dependent spectral fluctuations. Pagani et al. selected "stationary sections of data both at rest and during tilting" for assessment; the exact timing of their post-tilt data acquisition, however, was not reported [12]. Montano et al. reported on a study in which they applied different extents of angle tilting for periods of 10 min. From the continuous recordings, stationary segments devoid of arrhythmia [200 to 500 RR intervals] were analysed, but the timing of data acquisition in relation to the onset of tilting was not stated [13]. 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 [9]. 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 [7]. 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 [10]. 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 [8]. In general, the investigators tended to select artefact-free stationary segments of the recordings, thereby precluding assessment of the phase of the postural change itself. Most of the cited reports indicated a

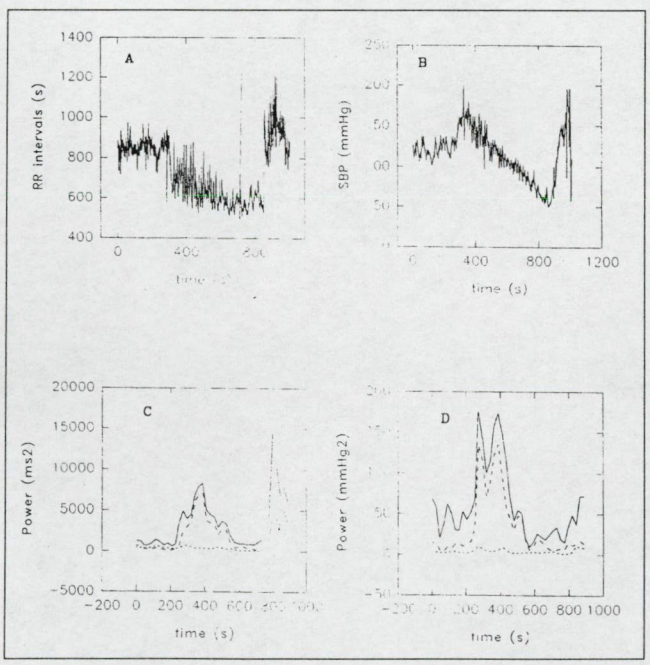

Figure 2. Typical tilt-induced responses of a tilt-positive patient. *Panel A.* The RR interval tachogram. The tilting, indicated by a vertical line is reflected by an immediate HR acceleration. The sudden-onset bradycardia after 800 s of the recording coincides with the syncopal episode. *Panel B.* The systolic BP trend curve reveals transient stabilisation post-tilting, followed by a marked hypotension, eventually leading syncope. *Panels C and D.* Variations in HR (C) and systolic BP (D) with time, as assessed by windowed FFT in the same arrangement as in Figure 1. The upright tilt manoeuvre is paralleled by elevations in the LFHRV and TFHRV powers. The duration of the elevations in these frequency ranges is prolonged, however, relative to the normal, and they are interrupted by further peakings. The subsequent decreases in the spectral components precede the syncopal episode.

Table 2. Changes in spectral components during head-up tilt test in the tilt-negative group (n = 22)

	Supine position	Maximum after tilt	Minimum after tilt
TFHRV (ms^2/Hz)	1255 ± 392	3835 ± 1892*	1618 ± 415*
LFHRV (ms^2/Hz)	514 ± 163	2300 ± 977*	888 ± 223*
HFHRV (ms^2/Hz)	557 ± 234	289 ± 94^{NS}	247 ± 97^{NS}
TFBPV ($mmHg^2$/Hz)	12 ± 3	110 ± 28*	38 ± 10*
LFBPV ($mmHg^2$/Hz)	5 ± 1	54 ± 11*	22 ± 5*
HFBPV ($mmHg^2$/Hz)	1 ± 0	4 ± 1^{NS}	2 ± 0^{NS}

TFHRV: total-frequency heart rate variability, LFHRV: low-frequency heart rate variability, HFHRV: high-frequency heart rate variability, TFBPV: total-frequency blood pressure variability, LFBPV: low-frequency blood pressure variability, HFBPV: high-frequency blood pressure variability; * $p < 0.05$ vs. supine position, ^{NS} non-significant

Table 3. Changes in spectral components during head-up tilt test in the tilt-positive group (n = 7)

	Supine position	Maximum after tilt	Minimum after tilt	During syncope
TFHRV (ms^2/Hz)	673± 202	4379± 2064*	1072± 670*	243± 105*
LFHRV (ms^2/Hz)	290± 96	2707± 1557*	663± 394*	92± 31*
HFHRV (ms^2/HZ)	143± 80	1054± 902*	125± 84^{NS}	38± 28^{NS}
TFBPV ($mmHg^2$/Hz)	24± 9	86± 33*	37± 21^{NS}	17± 6*
LFBPV ($mmHg^2$/Hz)	10± 4	58± 26*	23± 14^{NS}	2± 0*
HFBPV ($mmHg^2$/Hz)	24± 0	2± 0^{NS}	3± 1^{NS}	4± 2^{NS}

TFHRV: total-frequency heart rate variability, LFHRV: low-frequency heart rate variability, HFHRV: high-frequency heart rate variability, TFBPV: total-frequency blood pressure variability, LFBPV: low-frequency blood pressure variability, HFBPV: high-frequency blood pressure variability; * $p < 0.005$ vs. supine position, ^{NS} non significant

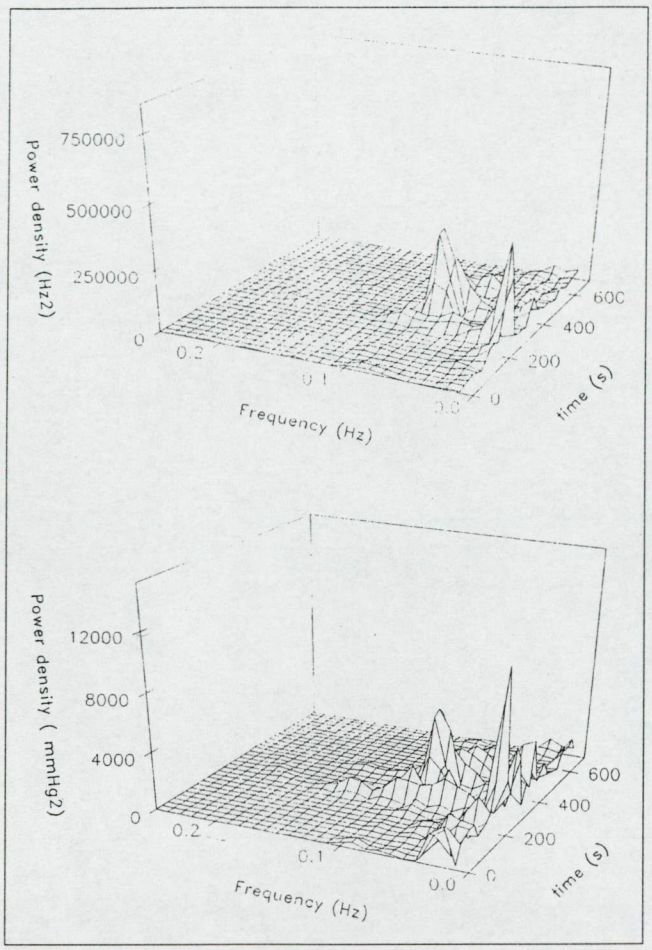

Figure 3. 3D projection of the HRV *(upper panel)* and BPV *(lower panel)* spectral changes following the upright tilt as assessed by windowed FFT in the same syncopal patients as in Fig. 2. The spatial projection allows a thorough assessment of the temporal changes and interrelationship of all spectral components. Thus, it is apparent that the double elevation in the LFBPV response depicted in Fig. 2 is related to changes in different segments of the LF band. Even the two minor elevations in the HFBPV band are quite discernible.

tilt-induced increase in LFHRV, with an increased ratio LFHRV/HFHRV [8–10]. 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 characterised by a predominance of LFHRV markers.

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 an increased HFHRV just prior to an episode of syncope [9, 11], 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, ie, 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 (Figure 1). In contrast, a second phase of LFHRV and HFHRV elevation was seen among tilt-positive subjects prior to syncope (Figures 2 and 3). However, the magnitude of these peaks did not attain the level of the initial increments. Since our program predicted determinations of only one minimum and maximum value for each subject, these presyncopal peaks in the tilt-positive group remained numerically uncharacterized. Nevertheless, similar presyncopal increments in UHRV have been repeatedly reported [11, 14]. The LFHRV component presumably represents sympathetic activation; indeed, studies on presyncopal catecholamine levels [15] and muscle sympathetic nerve activity recordings documented transient pre-syncopal elevations [16, 17]. The mechanism of presyncopal elevation in HFHRV is not clear. The increase may represent the intense vagal stimulation preceding the onset of syncope [9]. However, we have observed a presyncopal increase in HFBPV as well (Figures 2 and 3), 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 hypovolaemia and in increased breathing-related fluctuations in the stroke volume. 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.

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VI

Abnormal cardiovascular autonomic regulation in Parkinson's disease

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Parkinson's disease (PD) is often associated with an autonomic neuropathy. The extent of autonomic involvement, however is poorly defined and unpredictable. In order to assess the autonomic cardiovascular regulation time and frequency domain indices of heart rate and frequency domain indices of blood pressure the variability was determined non-invasively in 20 patients (age: 66 ± 8 years) with PD. The arbitrarily chosen level of spontaneous linear baroreflex sensitivity (BRS) of 3.5 ms/mmHg served to divide PD patients into subgroups. 12 PD patients exhibited a normal (nBRS), and 8 an impaired (iBRS) BRS. The results were compared with those from 18 healthy age-matched volunteers. The group of iBRS PD patients exhibited marked abnormalities in the other indices of cardiovascular autonomic regulation. In contrast, nBRS PD patients displayed only modest deviations. It is concluded that a decreased BRS is a distinctive feature of the impaired cardiovascular autonomic regulation in this heterogeneous population, and may serve as a selection marker for further assessment. *J Clin Basic Cardiol 1999; 2: 245–7.*

Key words: Parkinson's disease, baroreflex sensitivity

It is well documented that Parkinson's disease may be associated with a dysfunction of the autonomic nervous system. In idiopathic Parkinson's disease, this dysfunction can be explained by damage to neurological structures. Histological studies have proven the presence of Lewi's bodies in sympathetic and parasympathetic preganglionary neurons and also in central structures associated with the autonomic regulation [1]. To characterize the cardiovascular autonomic regulation, reflex tests of various complexity, such as the Valsalva maneuver or active orthostasis were traditionally used. These tests were often carried out in panels as a test series [2, 3]. A well-known example is the Ewing Panel [4]. In recent years, several studies have been reported in which Parkinson's disease patients were tested by this method. [5–15]. These tests require an active participation of the patients, and thus the hypokinesis, rigidity and tremor characteristic of Parkinson's disease led to serious limitations. It is obvious that with some patients these tests are infeasible or the results are meaningless. The measurement of autonomic parameters using power spectrum analysis of heart rate (HR) and blood pressure (BP) and a non-invasive determination of baroreflex sensitivity (BRS) does not require the active participation of patients.

One aim of the present study was to determine the markers of heart rate and blood pressure variability (HRV and BPV) in Parkinson's disease. A further goal was to verify our presumption that the pathological BRS level separates the disordered autonomic regulation and autonomically intact subgroups of Parkinson's disease patients.

Methods

Patient population

The study involved 20 patients with Parkinson's disease who were recruited from the Parkinson's Outpatient Clinic of the Neurological Department at Albert Szent-Györgyi Medical University. Patients without history of any cardiovascular disease, and with negative physical examination and normal 12-lead ECG were selected for this study. Neurological characteristics of the patients are shown in Table 1. The average age was 66 ± 7 years. The average age at the time of the diagnosis was 60 ± 8 years. The duration of the Parkinsonian signs was on average 71 ± 62 months. 76 % of the patients were on L-DOPA, 63 % on a MAO inhibitor, 25 % on an anticholinergic drug, 67 % on amantadine and 31 % on bromocryptine. 36 % of the patients took antidepressants and 15 % beta-blockers. All measurements were conducted in the afternoon, 4–5 hours following the last meal. The participants were requested to avoid smoking and to refrain from caffeine-containing beverages for 6 hours prior to the examinations. The subjects were equipped with the peripheral unit of the Penaz system non-invasive BP monitor (Finapres 2300) and ECG electrodes. Continuous BP and HR measurements were started after a 10-min resting period. BP and HR were monitored and recorded in a supine position for 20 mins. Data were analysed off-line. By means of the 3.5 ms/mmHg limit the Parkinson's disease patients were divided into two subgroups: those with a normal BRS (nBRS; 12 patients) and those with an impaired BRS (iBRS; 8 patients). Most of the previous studies utilized pharmacological (phenylephrine) BRS tests. It has been documented that phenylephrine and spontaneous BRS values are closely related but not interchangeable [16]. Therefore we selected our cut off point at 3.5 ms/mmHg.

Table 1. Clinical characteristics and neurodiagnostic test scores of subjects

	Control N = 18	PD nBRS N = 12	PD iBRS N = 8
Age (years)	65 ± 9	64 ± 6	67 ± 7
Sex (M/F)	9/9	6/6	3/5
Age at onset of disease (years)	-	60 ± 7	59 ± 8
MMSE score	29 ± 0.9	27.3 ± 0.7	27.8 ± 0.6
UPDRS	-	26.5 ± 0.3	25.9 ± 0.4
HY	-	2.0 ± 0.1	2.1 ± 0.1

Data are mean ± SD; PD = Parkinson's disease with normal BRS (nBRS) and with impaired BRS (iBRS); M = male, F = female, MMSE = Mini Mental State Examination; UPDRS = Unified Parkinson's Disease Rating Scale; HY = Modified Hoehn and Yahr staging

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Measurements and calculations

The ECG was continuously recorded with a Sirecust 730 (Siemens) monitor. Blood pressure was measured non-invasively with a Finapres 2300 (Ohmeda) device, which reliably reflects blood pressure spectral changes. The Penaz system photoplethysmographic finger blood pressure monitor reconstructs a continuous blood pressure curve that closely follows the invasively recorded blood pressure fluctuations. The R wave of the ECG and the plethysmographic signals were fed through an amplifier, filter and analog-digital converter into an IBM-AT-compatible computer. The computer measured the interval between successive R waves with a precision of 2 ms. The accuracy of ECG signal detection was 40 microvolts and 1 mmHg of the blood pressure measurement. The cardiotachogram and trend-grams of the BP values were continuously recorded on-line. Spectral analysis of the R-R interval and systolic BPV was performed by fast Fourier transformation, using the Hamming window over two frequency bands: low-frequency power (0.04 to 0.14 Hz) and high-frequency power (0.15 to 0.4 Hz). The total frequency power range from 0.01 to 0.4 Hz was considered. The short-term measures of time domain indices as the average normal R-R intervals (mean R-R), SDNN, pNN50, rMSSD were also calculated. BRS was characterized by the spontaneous sequences method as described earlier [17, 18]. 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 blood pressure (delta SBP) were paired with the changes in the subsequent RR intervals (delta RR). This method is referred to as the lag1 technique [17, 18].

Statistical analysis

HRV, BPV and BRS values of patients with Parkinson's disease showed non-gaussian distribution. These data were analysed using the Friedman test. An α level of $p < 0.05$ was considered to be significant.

Results (Table 2)

The average RR interval length was shorter in both Parkinson's disease groups than in the healthy volunteers. The systolic blood pressure, however, was very similar in all three groups. rMSSD was almost the same in the control group and the nBRS group. In the iBRS group, the rMSSD was significantly lower than in the other two groups. There was an even more marked difference in pNN50: the values for the healthy volunteers and the nBRS group were very similar, while that in the iBRS group was 0. There was a similar tendency in the spectral parameters of heart rate variability. TFHRV, LFHRV and HFHRV for the nBRS group were all slightly below those for the healthy volunteers, whereas statistically significant differences were observed between the iBRS group and the healthy volunteer group. The spectral markers of systolic blood pressure variability behaved differently from the other parameters. TFBPV and LFBPV for the iBRS group were significantly higher than those for the control group.

Discussion

Parkinson's disease is a clinical diagnosis. Pathological examinations reveal another syndrome, multiple systemic atrophy (MSA) in one-fourth of patients diagnosed as having Parkinson's disease. The extent and frequency of autonomic involvement differ in the two syndromes [15]. It is even more important that the prognosis and the effect of the applied anti-Parkinson drugs also differ in the two entities, and their differentiation remains an important task for the future [6, 19]. Bordet et al. consider that, because of the difficulties with traditional reflex tests, spectral analysis of heart rate variability may be used even more widely to study the autonomic regulation in Parkinsonian patients in the future [6]. Frontoni et al. have already applied spectral analysis for the same reasons in studies of multiple sclerosis [20]. To the best of our knowledge, this is the first publication relating to a spectral analysis of the HRV and BRS test in patients with Parkinson's disease. The HR spectrum is divided into "high-" and "low"-frequency bands at a commonly agreed value [21]. The high-frequency band is equivalent to breathing arrhythmias and is generated mainly by parasympathetic modulation. The genesis of the low-frequency range is influenced by both sympathetic and parasympathetic nervous mechanisms. While this view is somewhat simplistic, it may be of significance that both frequency ranges were reduced by spectral analysis in the iBRS group, and thus both the sympathetic and the parasympathetic components could be assessed as pathological. Spectral analysis of BPV has not been carried out on Parkinson's disease patients so far. Netten et al. have reported studies on Parkinson's disease patients in which the Finapres blood pressure monitor was used [11], but they targeted the examination of orthostasis tolerance and handgrip-induced vasoconstrictor response. It is of importance that only one of the 23 patients studied by Netten et al. had to be excluded because of hand tremor induced blood pressure artifacts. In our group of 20 patients, we had no such difficulties at all. BRS in Parkinson's disease patients was first examined by Appenzeller and Goss. They used the blood pressure and heart rate response in the overshoot phase of the Valsalva maneuver to characterize BRS, and concluded that these BRS values were pathological in some Parkinson's disease patients [22]. Our study confirms this and demonstrates some more general aspects of this early observation. The time and frequency domain indices of HRV were

Table 2. Comparison of spectral autonomic parameters and BRS values between control, PDn and PDi groups

	Control subjects N = 18	PD nBRS N = 12	PD iBRS N = 8
BRS (ms/mmHg)	9.9 ± 4	9.65 ± 6	2.1 ± 1*
Mean RR (ms)	855 ± 100	689 ± 140	697 ± 9*
Mean SBP (mmHg)	126 ± 21	117 ± 17	117 ± 22
SDNN (ms)	52.4 ± 4.6	49.1 ± 3.1	34.9 ± 2.9*
rMSSD (ms)	27.7 ± 8	27.2 ± 22	11.4 ± 4*
pNN50 (%)	8 ± 7	11 ± 18	0 ± 0*
TFHRV(ms^2/Hz)	847 ± 595	382 ± 662	85 ± 63*
LFHRV (ms^2/Hz)	187 ± 152	63 ± 51	28 ± 23*
HFHRV (ms^2/Hz)	463 ± 480	261 ± 586	28 ± 36*
TFBPV ($mmHg^2$/Hz)	18 ± 16	15 ± 13	34 ± 51*
LFBPV ($mm\ Hg^2$/Hz)	1 ± 2	7 ± 8*	8 ± 10*
HFBPV ($mm\ Hg^2$/Hz)	8 ± 8	2 ± 3*	7 ± 11

Data are mean ± SD; * = $p < 0.05$ compared with control group; PD = Parkinson's disease with normal BRS (nBRS) and with impaired BRS (iBRS); BRS = baroreflex sensitivity; SBP = systolic blood pressure; rMSSD, pNN50, TFHRV, LFHRV, HFHRV, TFBPV, LFBPV, HFBPV: explanation in method section.

also pathological in the group of patients classified as iBRS. A somewhat controversial tendency could be observed in the spectral parameters of systolic BPV. The function of HRV is commonly known to be to buffer changes in BP. Accordingly, it is not surprising that the amplitude of BP surges increases with decreasing HR fluctuation [23]. The significance of the abnormalities in cardiovascular regulation among Parkinson's disease patients is not yet fully known. It is possible that the dysbalance of the sympathetic and the parasympathetic tone is connected with the arrhythmias developing in the ischaemic heart muscles. The pathological BRS that signalled an autonomic dysbalance in the acute phase of myocardial infarction was a sensitive predictor of ventricular arrhythmias and sudden death [18]. The connection between autonomic dysregulation and arrhythmia-related death has recently been considered in other, non-cardiovascular diseases, such as depression [24]. Analogously, we can presume a connection between the autonomic dysbalance and the high mortality among Parkinson's disease patients [25, 26]. The mortality of Parkinson's disease patients is almost twice that for age and sex-matched healthy control groups [25]. The 20-year follow-up study by Ben-Shlomo and Marmot suggested that this increased mortality is connected with an increase in heart ischaemia-related deaths [26]. That study, however, did not distinguish sudden cardiac deaths, and thus the real importance of arrhythmia-related deaths could not be assessed. However, the available data indicate that a wide-ranging prospective examination of autonomic regulation in Parkinson's disease patients would be justified. The examination of spontaneous BRS involves a non-invasive 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 quite suitable for screening autonomic involvement among Parkinson's disease patients. The predictive nature of the pathological BRS in this group necessitates further investigations.

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VII

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Impaired microvascular response to cholinergic stimuli in primary Sjögren's syndrome

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Abstract

***Objective*—Signs of a parasympathetic dysfunction have been revealed in primary Sjögren's syndrome (SS). Its role in the pathogenesis and the clinical picture of the disease is not clear. To investigate the responsiveness of SS patients to a cholinergic agonist, a model was used involving examination of the cutaneous microcirculation. The microvascular response to the administration of carbachol was measured, a muscarinic cholinergic agonist.**

***Methods*—Twenty two SS patients and 12 controls were examined. Carbachol and 0.9% saline solution were administered intracutaneously into the forearm skin at two distinct places. Skin blood flow (SBF) in the injected areas was measured continuously before and for 10 minutes after the injections by means of a laser Doppler perfusion monitor. The increase in SBF in response to carbachol (dSBF), reflecting vasodilatation, was calculated by a formula including the baseline and the maximum SBF values after the injections of carbachol and saline solution.**

***Results*—The vasodilatation was significantly lower in SS patients than in the controls (mean dSBF: 2.1 (range: 1.0–4.5) versus 3.3 (range: 1.7–7.6), p=0.02). With non-responder patients defined as those in whom a smaller response was observed than in any of the controls, 11 of the 22 SS patients proved to be non-responders to carbachol. Comparisons of demographic, clinical and laboratory characteristics and HLA class II genotypes between responder and non-responder SS patients did not show any significant differences.**

***Conclusions*—A diminished or absent response to carbachol indicates a cholinergic dysfunction in SS patients. A disturbance in the neurotransmission at a receptorial or postreceptorial level is hypothesised. Unresponsiveness to cholinergic stimuli may contribute to exocrine insufficiency.**

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Dry mouth and dry eyes, the two fundamental symptoms of primary Sjögren's syndrome (SS), have previously been regarded solely as direct consequences of the loss of functioning glandular tissue caused by an autoimmune inflammatory process. However, this conception is now increasingly questioned.[1,2] The symptoms of dryness and the quantitatively determined saliva and tear production may vary greatly during the course of the disease, even without treatment, and their spontaneous improvement is often observed for varying periods. A marked discrepancy has been reported between the degree of exocrine insufficiency and the histologically verified intensity of the inflammatory infiltration in the salivary glands.[3] In human and animal experiments, several types of abnormalities of the autonomic nervous system have been demonstrated in SS.[4–10]

The question of whether an impairment of parasympathetic innervation may play a part in the elicitation of exocrine insufficiency in SS remains an issue to be examined. Despite previous work, results of human functional studies on the parasympathetic nervous system in SS patients are by no means numerous. If a postulated cholinergic dysfunction does exist, it is not known whether it is caused by a central or a peripheral nervous system dysfunction, or whether it reflects a disorder of the parasympathetic innervation at a receptorial or postreceptorial level. Nor is it clear whether the acetylcholine transmitter system or a non-adrenergic, non-cholinergic transmitter (for example, vasoactive intestinal polypeptide (VIP)) system colocalised in the cholinergic nerve terminals is involved.[11]

For these reasons, we set out to examine the response to the administration of a muscarinic cholinergic agonist, carbachol, in SS patients and to compare it with that in healthy controls. To measure this response, we established a model involving examination of the cutaneous microcirculation. A cholinergic vasodilatory mechanism of the human skin blood vessels has long been verified.[12–14] As this is similar in many respects to the cholinergic innervation of the salivary glands,[13] we decided to use this experimental setting to test the hypothesis that a cholinergic dysfunction exists in SS patients.

Methods

PATIENTS AND CONTROLS

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 criteria[15] 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 (mean (SD)) 50.6 (13.2) years (33–60). The average time since the first symptom had appeared was (mean (SD))11.5 (5.2) years (4–24), while the average time since the establishment of the diagnosis was (mean

(SD)) 9.1 (6.7) years (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 β-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-steroidal anti-inflammatory drugs was suspended at least five days before the examinations. The patients were asked to refrain from smoking or the drinking of coffee on the day of the examination.

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. All gave their informed consent to the procedure and the study design was accepted by the Medical Ethics Board of Albert Szent-Györgyi Medical University.

EXAMINATION OF THE CUTANEOUS VASCULAR RESPONSE

During examinations, the patients and controls were in the supine position. The skin blood flow (SBF) was measured with a laser-Doppler flowmeter (Penflux, Perimed) attached to the skin on the flexor surface of the right forearm, and blood flow values were expressed in arbitrary units.[16] Three ECG electrodes were attached to the chest and the heart rate was monitored continuously, as was the blood pressure by means of a photoplethysmographic blood pressure monitor (Finapres 2300, Ohmeda) attached to a finger of the right hand (beat to beat registration). The subjects were asked to lie at rest with their eyes closed. The room temperature was kept constant (20°C ± 1°C) and all disturbing factors were excluded. The baseline SBF was recorded for five minutes; the blood flow was stable by the end of this period and the SBF value measured at five minutes was defined as SBF basal. 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.

All recorded data (heart rate, systolic and diastolic arterial pressure and SBF) were stored in a computer database and were analysed by means of a self developed software. The change in SBF in response to the injection of carbachol (dSBF) was calculated using the following formula:

$$dSBF = \frac{SBFfinal, carbachol/SBFbasal, carbachol}{SBFfinal, saline/SBFbasal, saline}$$

Thus, dSBF is the ratio of the SBF values measured after and before the injection of carbachol divided by the same ratio for the control solution of physiological saline. This calculation allowed us to eliminate the absolute flow values and also to eliminate possible non-specific microcirculatory effects of the intracutaneous injection. In every subject, the mean values of the RR intervals on the ECG, and the systolic, diastolic and mean arterial pressures were also recorded. The cutaneous vascular resistance (CVR) was calculated by dividing the mean arterial pressure by SBF, while dCVR was calculated with a formula analogous to that for dSBF.

LABORATORY EXAMINATIONS

Laboratory examinations were performed as follows: anti-SSA and anti-SSB antibodies: enzyme linked immunosorbent assay (Epignost, Leonding/Linz, Austria); antinuclear antibodies: indirect immunofluorescence assay, using rat liver as substrate; IgM rheumatoid factor: quantitative measurements by immunoturbidimetry (Boehringer Mannheim, Germany). HLA DRB1, DQA1 and DQB1 genotyping was performed by using methods described in detail by others.[17-20] Schirmer's test and sialometry were performed within one year from the examinations in all SS patients. As sialometric examination, we assessed the unstimulated whole saliva production in 10 minutes. Skin dryness was scored on a scale 0–IV on the basis of the patients' reports of the severity of dry skin complaints and physical examination of the skin, as follows: grade 0: no dry skin; grade I: a transient feeling of skin dryness not requiring treatment, and no objective signs of dry skin; grade II: a recurrent feeling of skin dryness requiring treatment with hydrating lotions, and objective signs of

Table 1 Occurrences of main organ manifestations and laboratory variables in primary Sjögren's syndrome patients (n=22)

Organ manifestations and laboratory variables	*Number of patients (%)*
Articular involvement*	17 (77)
Renal involvement†	4 (18)
Bronchitis sicca	4 (18)
Pulmonary fibrosis	2 (9)
Raynaud's phenomenon	6 (27)
Skin vasculitis	3 (14)
MALT lymphoma in parotid gland	2 (9)
Anaemia‡	6 (27)
Hypergammaglobulinaemia§	15 (68)
Antibody positivity	
Anti-SSA	14 (64)
Anti-SSB	9 (41)
IgM rheumatoid factor	18 (82)
Antinuclear antibody	18 (82)

*Clinically evident arthritis or persistent arthralgia without objective physical signs. †Chronic tubulointerstitial nephritis or renal tubular acidosis. ‡Haemoglobin <100 g/l not attributable to causes other than SS. §γ globulin >16 g/l.

Table 2 Comparison of certain haemodynamic variables in primary Sjögren's syndrome (SS) patients and controls recorded during the blood flow examinations

Group	SBFfinal,carbachol/ SBFbasal,carbachol	SBFfinal,saline/ SBFbasal,saline	dSBF	dCVR	Mean RR (msec)	Mean SBP (mm Hg)	Mean MBP (mm Hg)	Mean DBP (mm Hg)
SS patients	1.89 (1.05)*	0.94 (0.29)	2.07 (1.12)†	0.64 (0.32)‡	827.9 (109.6)	132.4 (22.0)	95.9 (16.5)	76.3 (14.9)
Controls	2.72 (1.27)	0.86 (0.19)	3.30 (1.79)	0.38 (0.17)	797.0 (117.3)	128.6 (14.9)	93.8 (9.8)	74.9 (7.5)

Data shown as mean (SD). SBF: skin blood flow; CVR: cutaneous vascular resistance; RR: RR interval on ECG; SBP: systolic arterial blood pressure; MBP: mean arterial blood pressure; DBP: diastolic arterial blood pressure. SBF and CVR values are in arbitrary units. For the calculation of dSBF and dCVR, see the text (Methods section). *p=0.048, †p=0.019, ‡p=0.013.

decreased sweat production; grade III: a continuous feeling of skin dryness revealed by topical treatment, and severely dry skin with fine scaling; grade IV: a continuous feeling of skin dryness not revealed by topical treatment, and very severely dry skin with excoriations and mild loss of hair.

STATISTICAL ANALYSIS

Differences between means were calculated with an independent sample *t* test, except for the comparison of the means of dSBF values, where Wilcoxon's *t* test was applied because of the non-parametric distribution of the elements of the samples. For the comparisons of frequencies, Fisher's exact test was used, with Bonferroni's correction when the examined variable had more than two possible values (for example, HLA genotype). To investigate correlations with dSBF, Spearman's signed rank test or linear regression analysis was applied. Statistical calculations were performed with SPSS software. Values in the text are expressed as mean (SD). The level of significance was defined as $p<0.05$.

Results

Table 1 gives details relating to the occurrences of the main systemic organ manifestations of SS and the various laboratory parameters in the 22 patients.

Table 2 reports the average values of the most important haemodynamic variables in the two groups recorded during the examinations of the cutaneous microcirculation. We present here the means of the changes in the local blood flow in response to carbachol (SBFfinal,carbachol/SBFbasal,carbachol) and physiological saline solution (SBFfinal,saline/SBFbasal,saline), the dSBF values (which are specific indicators of the cutaneous vasodilatation in response to the cholinergic agonist), and also the corresponding values of the change in CVR. The means of the continuously recorded values of the RR intervals on the ECG and of systemic arterial blood pressure are also included in table 2.

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 3.30 (1.79), which was significantly higher than the average dSBF in SS patients (2.07 (1.12)) (p=0.019) (fig 1). In the controls, the dSBF values reflected a 1.66–7.6-fold increase in microcirculatory blood flow after the injection of carbachol. 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 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

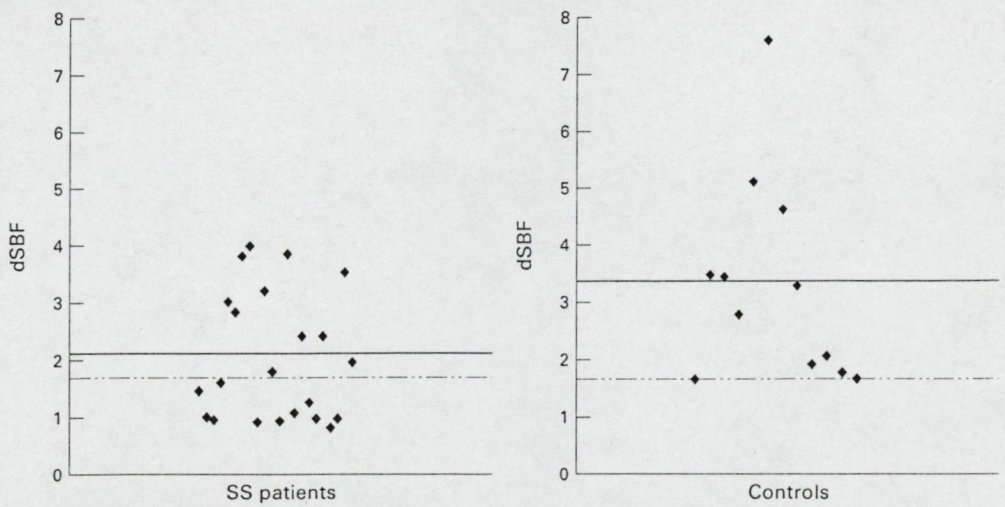

Figure 1 Distribution of dSBF values in primary Sjögren's syndrome (SS) patients and healthy controls. dSBF: change in skin blood flow in response to the injection of carbachol. Continuous lines indicate means. Values above the dashed lines indicate a positive response to carbachol.

Table 3 Demographic characteristics and occurrences of certain organ manifestations of primary Sjögren's syndrome (SS) in carbachol responder and non-responder SS patients

	Responders (n=11)	*Non-responders (n=11)*
Average age (y)	51.0	50.3
Average time since first symptom (y)	11.8	11.2
Articular involvement	9	8
Renal involvement	3	1
Bronchitis sicca	3	1
Pulmonary fibrosis	1	1
Raynaud's phenomenon	5	1
Skin vasculitis	2	1
MALT lymphoma in parotid gland	2	0

For organ manifestations, numbers indicate the number of involved patients. For the precise definitions of articular and renal involvement, see table 1. No significant difference was found between the two subgroups.

Table 4 Numbers of people with various degrees of severity of skin dryness among the primary Sjögren's syndrome patients (n=22)

Degree of skin dryness	0	I	II	III	IV
Number of patients	5	4	6	7	0

For a precise description of the assessment of the severity of skin dryness, see the text (Methods section).

the basis of the pronounced vasodilatation (a more than 1.66-fold increase in SBF).

We examined whether there was any difference in certain demographic characteristics or in the frequencies of certain clinical organ involvements between the SS patients defined as non-responders or as responders to carbachol (table 3). The age of the patients and the time since the appearance of the first symptom of SS was similar in the two groups. Similarly, when tested by linear regression analysis, the above variables did not demonstrate any correlation with dSBF (data not shown). No statistically significant difference was revealed between the two groups in the occurrence of any of the main organ manifestations. However, it is noteworthy that all the examined organ involvements occurred more frequently in the responder patients (with the exception of pulmonary fibrosis, which occurred in one patient in each of the subgroups) (table 3). Dry skin is a common complaint in SS patients. In table 4, we present the proportions of patients with varying degrees of severity of skin dryness. We checked whether there was a correlation between the reactivity of the cutaneous microcirculatory vessels to carbachol (dSBF) and the degree of skin dryness, which is predominantly a consequence of the insufficiency of the exocrine glands of the skin; the answer proved negative. The two subgroups were also comparable as concerns the average values of tear and saliva production (mean tear flow with Schirmer's test: 4.0 (2.12) *v* 4.45 (2.21) mm/5 minutes, unstimulated whole saliva flow: 0.34 (0.28) *v* 0.32 (0.28) ml/10 minutes in the non-responders and in the responders, respectively).

The frequencies of anti-SSA, anti-SSB, IgM RF and ANA positivity, and also the average levels of laboratory markers of a chronic autoimmune inflammatory process, in particular the erythrocyte sedimentation rate, fibrinogen concentration, anaemia and γ globulin concentration, did not differ significantly in the two subgroups of primary SS patients. No HLA DR, DQA1 and DQB1 allele occurred more frequently in one subgroup than in the other, although, in view of the relatively small number of patients and the heterogeneity of the genotypes of the examined persons, a statistically significant difference is hardly to be expected (data not shown).

The drug treatment applied throughout the course of the illness was also surveyed. The proportions of patients treated with non-steroidal anti-inflammatory drugs, hydroxychloroquine or corticosteroids were similar in the two subgroups. Two patients were receiving regular corticosteroid treatment and five hydroxychloroquine treatment at the time of the examination; the use of these drugs did not seem to influence the microcirculatory response to carbachol, as indicated by the similar proportions of users in the responder and non-responder subgroups.

Discussion

The purpose of this study was to determine the proportion of primary SS patients who demonstrate signs of a cholinergic dysfunction, and to evaluate whether there is a relation between the presence of a cholinergic dysfunction and certain clinical, serological and genetic characteristics of the disease. The importance of this question stems from the fact that the salivary glands are richly innervated by cholinergic nerve fibres, and, if the hypothesis that there is a disturbance in the cholinergic innervation proves true; this may provide new aspects as regards the pathogenesis of the sicca symptoms in SS. The issue of abnormalities in the parasympathetic nervous system in SS has arisen in previous human or animal studies in which various stages of the parasympathetic innervation pathway were investigated. Mandl *et al*[6] and Andonopoulos *et al*[7] detected autonomic neuropathy, mainly affecting the parasympathetic nerves. Santavirta *et al*[8] demonstrated that the salivary outputs of VIP (considered a marker of the parasympathetic innervation) and of neuropeptide Y (a marker of sympathetic innervation) were both increased as compared with healthy controls. They suggested that this phenomenon is associated with an altered response to stress in SS patients. In immunohistological examinations, Konttinen *et al*[4] found that VIP containing parasympathetic fibres were absent from regions of the parotid glands where inflammatory cell infiltration and acinar atrophy were severe. Circulating antibodies against rat parotid M3 muscarinic cholinergic receptors were detected by Bacman *et al.*[9] Törnvall *et al*[5] reported that the expression of the β_{II} and α isoforms of protein kinase C, a crucial member of the intracellular second messenger pathway of cholinergic neurotransmission, was deficient on the salivary gland acinar epithelial cells in SS patients as compared with controls. In contrast with healthy controls, the ξ and β_{II} isoforms were undetected on the myoepithelial cells in SS patients. These findings are suggestive of a postreceptorial disturbance of the cholinergic innervation.

In this study, we examined the cutaneous cholinergic vasodilatation in SS patients in response to the local administration of a

muscarinic cholinergic agonist, carbachol. Acetylcholine has been demonstrated to cause vasodilatation in healthy humans.[13] Although the non-adrenergic–non-cholinergic transmitter systems probably play a more important part in the physiological vasoregulation in the skin than do the cholinergic nerves, receptors for acetylcholine can also be found in the cutaneous microvasculature.[21] Carbachol has been demonstrated to induce phospholipase C dependent saliva secretion through stimulation of the muscarinic cholinergic receptors in human salivary acinar cells.[22 23] The same muscarinic receptors have been implicated in the cholinergic vasodilatation in human cutaneous arterioles in response to body heat stress.[13] These findings provide a basis for our choice of an investigation of the cutaneous microcirculation as a model of the parasympathetic innervation of the salivary glands. Nevertheless, firm conclusions can be drawn only after examinations directly involving the salivary glands in SS patients.

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 characterised 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. As our experiments involved the administration of a muscarinic receptor agonist directly to the examined target organ, the detected unresponsiveness favours the hypothesis that a dysfunction may exist at a receptorial or post-receptorial level. To test whether a receptorial dysfunction may result from a structural abnormality of the receptor protein caused by an abnormal genetic coding, we determined the HLA DRB1, DQA1 and DQB1 genotype profiles in our study patients. Certain HLA alleles (most importantly HLA-DR3) are known to occur more frequently in SS patients than in the general population. However, the hypothesis that an acetylcholine receptor pathology may be determined by a genetic predisposition remains an open question, as our relatively small patient population does not allow firm conclusions in this regard. Anti-receptor antibodies, similarly to myasthenia gravis, may be other possible causes of a receptor dysfunction. Indeed, antibodies against rat M_3 muscarinic receptors have been detected in SS patients,[9] though the existence and possible role of anti-receptorial antibodies against human muscarinic receptors have not been verified.

It has been hypothesised that an interaction may occur in the salivary glands between the infiltrating inflammatory cells and certain neuropeptides.[4] In contrast, another hypothesis suggests that the loss of trophic stimuli from the parasympathetic nerves in the salivary glands might be a cause of acinar cell atrophy, and this may contribute to the decreased saliva production.[4] Our results relating to investigation of a target organ distant from the areas of lymphocytic inflammation suggest that a disorder of the cholinergic receptors may not be restricted to the exocrine glands, but may be a more widely occurring phenomenon, and that this defect is possibly independent of the local inflammatory process. Further investigations may yield results of general importance as concerns the interactions between the nervous system and the immune system.

As a considerable proportion of the SS patients displayed a fundamentally insignificant reaction to carbachol, we could divide our population of SS patients into responders and non-responders to the cholinergic agent. The occurrence of unresponsiveness did not correlate with the age of the patients, the disease duration, the presence of a specific organ manifestation (including vascular manifestations of the disease) or of an autoantibody, nor the severity of the functional impairment of the salivary and lachrymal glands. However, all the organ involvements proved to be more common in the responder patients than in the non-responders. Confirmation of this latter finding with statistically significant results is first necessary before an explanation can be suggested.

In summary, we found that half of the examined SS patients proved to be non-responders to the administration of the cholinergic receptor agonist carbachol. Whether this unresponsiveness also relates to the clinically involved exocrine glands, and the precise mechanism of the disorder in the cholinergic innervation, possibly at a receptorial or postreceptorial level, are features that remain to be clarified.

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VIII

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Depressed baroreflex sensitivity in patients with Alzheimer's and Parkinson's disease

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Abstract

Parkinson's disease (PD) and Alzheimer's dementia (AD) are often associated with an autonomic neuropathy. The extent of autonomic involvement, however is poorly defined and unpredic table. In order to assess the autonomic cardiovascular regulation baroreflex sensitivity (BRS) was determined non-invasively in 23 patients (age: 65 ± 9.3 years) with PD and 24 patients with AD (age: 72.3 ± 7.2 years). The results were compared with those on 22 healthy age- and sex-matched volunteers. Patients with PD and AD exhibited marked abnormalities in cardiovascular autonomic reflex regulation showed by markedly depressed BRS. The possible predictive value of centrally based depression of baroreflex sensitivity necessitates further studies.

Keywords: Parkinson's disease; Alzheimer's dementia; Baroreflex sensitivity

1. Introduction

Recently several studies including prospective, randomized, multicenter studies have shown that depressed baroreflex sensitivity (BRS) is an independent predictor for sudden cardiac death and life threatening arrhythmias in patients with cardiac disease [12]. The ATRAMI investigators have shown that measurement of BRS is an appropriate way to identify patients after myocardial infarction who are at high risk of cardiac death or non-fatal cardiac arrest due to ventricular fibrillation [12]. Increased cardiovascular mortality has been reported in patients with Alzheimer's dementia (AD) and Parkinson's disease (PD) [2,3,8]. The aim of the present study was to assess the BRS in patients in whom the baroreflex arch is possibly centrally damaged.

2. Methods

2.1. Patient population

We assessed the BRS 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. 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. Clinical characteristics of the patients are shown in the Table. We excluded patients 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 (C).

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Table 1
Clinical characteristics, neurodiagnostic test scores and comparison of baroreflex sensitivities of probands

	Control	Alzheimer's disease	p values	Parkinson's disease	p values
Age (year	70 ± 6.6	72.3 ± 7.2	>0.05	65 ± 9.3	>0.05
Sex (M/F)	7/15	9/14	—	11/13	—
Age of onset disease (year)	—	66.9 ± 7.4	—	58.9 ± 9	—
MMSE	29 ± 0.9	19 ± 6.5*	<0.05	27.5 ± 2	>0.05
UPDRS	—	—	—	26.7 ± 20.3	—
HY	—	—	—	2.1 ± 0.8	—
dRR (ms)	36.4 ± 7.2	19.6 ± 4.7*	<0.01	21.4 ± 6.7*	<0.01
dBP (mm Hg)	4.4 ± 2.3	6.4 ± 2.1*	<0.05	5.7 ± 2.2*	<0.05
BRS (msec/mm Hg)	8.9 ± 6.9	2.6 ± 1.1*	<0.001	4.3 ± 3.5*	<0.05

Data are mean ± SD; M = male; F = female; MMSE = Modified Mini Mental State Examination; UPDRS = Unified Parkinson's Disease Rating Scale; HY = Modified Hoehn and Yahr staging; BRS = Baroreflex sensitivity; dRR = RR interval oscillation; dBP = systolic arterial blood pressure oscillation; * significant vs. Control, p values are shown vs. Control.

2.2. Measurements and calculations

All measurements were conducted in the afternoon 4–5 h after the last meal. The participants were requested to avoid smoking and to refrain from caffeine-containing beverages 6 h prior to the examinations. The ECG and the breathing were continuously recorded with a Sirecust 730 (Siemens) monitor. Blood pressure (BP) was measured non-invasively with a Finapres 2300 (Ohmeda) device, which reliably reflects BP changes. BP and HR measurements were monitored in a supine rest position for 60 min. The photo-plethysmographic (Penaz system) finger blood pressure monitor reconstructs a continuous BP curve that closely follows the invasively recorded BP fluctuations. The R wave of the ECG and the plethysmographic signals were fed through an amplifier, filter and analog-digital converter into an IBM-AT-compatible computer. The computer measured the interval between successive R waves with a precision of 2 ms. The accuracy of BP signal detection was 1 mm Hg. The cardiotachogram and trend-grams of the BP values were continuously recorded on-line. BRS was characterized by the spontaneous sequences method. 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 [15]. Oscillations in HR (dHR) and BP (dBP) were calculated over the same 60-min period.

2.3. Statistical analysis

One way ANOVA was used for comparison of data showing normal distribution (age, BRS, Age of onset,). *T* tests with Bonferroni correction were used for comparisons between groups. Level of significance was set at $P < 0.05$.

3. Results

The average RR interval length was shorter in both of the PD and AD groups than in the healthy volunteers (C: 855 ± 100 msec, PD: 689 ± 140 msec, AD: 697 ± 110 msec, $P <$ 0.01 vs. C). The systolic blood pressure, however, was very similar in all three groups (C: 126 ± 21 mm Hg, PD: 117 ± 17 mm Hg, AD: 118 ± 22 mm Hg). BRS was markedly reduced in the AD and PD groups compared with C (Table). There was no statistically significant difference between the AD and PD group in BRS values (Table). Patients with AD and PD showed significantly decreased dRR, and increased dBP compared with C (Table). There was no statistical difference between AD and PD patients in dRR and dBP.

4. Discussion

The major observation of our study is that baroreflex regulation is markedly depressed in Alzheimer's dementia and Parkinson's disease, characterized by a significant decrease in BRS.

4.1. 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 [11]. The integrity of the latter was not addressed in our study. 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 [17]. 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 [4,16]. 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 [17].

4.2. 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 [6]. Recently, damage to autonomic-related cortices, which can contribute autonomic imbalance often associated with AD was described in detail [5]. 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 [15] might be related to respiration. 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 [5,6,14]. Decreased BRS may reflect cholinergic deficits in the cardiac autonomic nerves as well as in the central nervous system [14]. 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 [13]. BRS in PD was first examined by Appenzeller and Goss. They concluded that BRS was pathological in some PD patients [1]. Our study confirms this and demonstrates more general aspects of this early observation. 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 [10]. 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 [12].

4.3. Possible clinical implications

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 [12], but the significance of the abnormal baroreflex gain in neurological disorders is unclear. The connection between autonomic dysregulation and arrhythmia-related death has recently been considered in other, non-cardiovascular diseases, such as depression [7]. Analogously, we can presume a connection between the autonomic imbalance and the high mortality among Parkinson's disease patients [2,3]. Increased mortality in Parkinson's disease patients due to ischemic heart disease was reported recently [2,3]. Increased cardiovascular mortality has also been reported in patients with Alzheimer's dementia associated with a tendency to lower blood pressure [9]. The mortality of Parkinson's disease patients is almost twice that for age and sex-matched healthy control groups [2]. 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 [3]. 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.

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