Synchronous and baroceptor-sensitive oscillations in skin microcirculation: evidence for central autonomic control

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1Division of Cardiology, University Hospital Zurich, Switzerland; 2Division of Hypertension, Centre Hospitalier Universitaire Vaudois, Lausanne, and 3Department of Internal Medicine, University of Pavia and Instituto di Ricovero e Cura a Carattere Scientifico S. Matteo, Pavia, Italy

Bernardi, Luciano, Daniel Hayoz, René Wenzel, Claudio Passino, Alessandro Calciali, Roger Weber, and Georg Noll. Synchronous and baroceptor-sensitive oscillations in skin microcirculation: evidence for central autonomic control. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1867–H1878, 1997.—To determine whether skin blood flow is local or takes part in general regulatory mechanisms, we recorded laser-Doppler flowmetry (LDF; left and right index fingers), blood pressure, muscle sympathetic nerve activity (MSNA), R-R interval, and respiration in 10 healthy volunteers and 3 subjects after sympathectomy. We evaluated 1) the synchrony of LDF fluctuations in two index fingers, 2) the relationship with autonomically mediated fluctuations in other signals, and 3) the LDF ability to respond to arterial baroreflex stimulation (by neck suction at frequencies from 0.02 to 0.20 Hz), using spectral analysis (autoregressive uni- and bivariate, time-variant algorithms). Synchronous LDF fluctuations were observed in the index fingers of healthy subjects but not in sympathectomized patients. LDF fluctuations were coherent with those obtained for blood pressure, MSNA, and R-R interval. LDF fluctuations were leading blood pressure in the low-frequency (LF; 0.1 Hz) band and lagging in the respiratory, high-frequency (HF; ~0.25 Hz) band, suggesting passive “downstream” transmission only for HF and “upstream” transmission for LF from the microvessels. LDF fluctuations were responsive to sinusoidal neck suction up to 0.1 Hz, indicating response to sympathetic modulation. Skin blood flow thus reflects modifications determined by autonomic activity, detectable by frequency analysis of spontaneous fluctuations.

autonomic nervous system; skin blood flow; heart rate variability; microcirculation; arterial baroreflexes

THE MICROCIRCULATORY network of the skin, like other body tissues, continuously exhibits rhythmic changes in diameter and flow (7, 30). The fundamental mechanism of these fluctuations is not yet clarified, particularly for the skin microvessels. Some researchers hypothesized that skin blood flow is exclusively under local control (6, 22), whereas others have suggested both general (central) adjustments and baroreflex-induced changes (1, 12, 13, 21, 25). Part of this discrepancy is probably due to the lack of methods to observe and analyze the spontaneous fluctuations in vascular tone and to compare them with other signals. Laser-Doppler flowmetry (LDF) is an accepted and validated method for noninvasive measurement of changes in cutaneous blood flow (3, 14). Spectral analysis techniques can characterize different cardiovascular, neural, and respiratory fluctuations (4, 16, 19, 24). We have previously found in the LDF signal (5) the presence of the so-called low-frequency (LF; ~0.1 Hz) fluctuations, considered a marker of sympathetic activity in the heart and blood pressure (16). These LF fluctuations in the skin circulation were modified by changes in sympathetic tone (posture and local anesthetics; Refs. 4, 5, 19), suggesting that oscillations in microvascular blood flow may therefore be controlled by both central and local mechanisms.

In the present study, we therefore assessed whether spontaneous fluctuations in the skin microcirculation, obtained by LDF, are related to central control mechanisms, by evaluating 1) their synchronism in different body areas (synchronism should be evidence of a central, rather than a local mechanism); 2) their relationship with spontaneous fluctuations observed in signals known to be related to general hemodynamics and/or autonomic activity, such as the blood pressure, R-R interval, respiration, and muscle sympathetic nerve activity (MSNA; absence of synchronization with rhythms of known autonomic origin would be an argument against an autonomic origin of these skin circulation rhythms; 3) their ability to respond to arterial baroreflex stimulations at different frequencies of stimulation, since the autonomic modulation of the cardiovascular system appears to be frequency dependent (2, 18, 23) (for this last purpose, we used a “pure” autonomic stimulus, namely, neck suction, i.e., modifications in cardiovascular system without hemodynamic changes other than reflex; Ref. 2).

METHODS

Subjects

We studied 10 supine healthy volunteers (age 26.3 ± 1.8 yr, mean ± SE) and 3 subjects with bilateral upper limb sympathectomy (3, 10, and 12 mo previously) because of hyperhidrosis. The protocol for the present study was approved by the local ethical committee; all subjects gave their informed consent.

Data Collection

The following signals were directly and continuously recorded on a computer (Macintosh IIfx, Apple) by a 12-bit analog-to-digital converter (NB-Mio-16 board, National Instruments, Austin, TX; sampling rate: 500 samples/s per channel): electrocardiogram (lead II), respiration (by a strain gauge thoracic belt), blood pressure (Finapres, Ohmeda 2300), skin blood flow, and MSNA.

Skin blood flow. Skin arteriolar blood flow was measured by two identical laser-Doppler flowmeters (Perimed model PF2b, Sweden, powered by 1-mW helium-neon laser tubes, supplying a 632.8-nm red light) with identical skin probes, on...
two corresponding volar surfaces of right and left index fingers. Details of the LDF methodology and validation techniques have previously been published (3).

Muscle nerve recording. MSNA was evaluated by conventional microoneurography (24). A tungsten microelectrode (200-µm shaft diam, 1- to 5-µm uninsulated tip; University of Iowa) was inserted in the peroneal nerve and positioned in the sympathetic fibers. An identical reference electrode was inserted subcutaneously 1–2 cm from the recording electrode. Electrodes were connected to a preamplifier (gain 1,000) and amplifier (variable gain 10–50). Neural activity was fed through a band-pass filter (band width 700–2,000 Hz) and then a resistance-capacitance integrating network (time constant 0.1 s) to obtain a mean voltage neurogram. Assessment of the correct position in the fibers supplying the muscle was made by observing typical heart period-related spikes, which increased after simple maneuvers increasing sympathetic activity (mental arithmetic, Valsalva maneuver, and apnea).

Baroreceptor stimulation. Neck suction was applied by means of a molded lead collar connected with a vacuum cleaner whose power was modulated by a second computer (Apple II) equipped with a digital-to-analog board through a phase-control power unit (2). This computer generated a special sinusoidal function, which remained at the frequency of 0.02 Hz for 2 min, then continuously increased up to 0.20 Hz (so-called “chirp” function) over 4 min, and finally remained at 0.20 Hz for 2 more min. The software generated a correction function to compensate for frequency-dependent variations in the vacuum pressure; as a consequence, the pressure within the neck oscillated steadily from 0 to −30 mmHg peak to peak regardless of frequency. The pressure within the neck collar was continuously recorded (Viggo-Spectratone Statham P 23d pressure transducer, Bilthoven, The Netherlands).

Protocol

After at least 20 min were allowed for stabilization, the signals were simultaneously and continuously recorded for 12 min (baseline recording). Respiration was maintained in the range of 0.20–0.26 Hz spontaneously or during neck suction or in subjects with spontaneous slow respiration by active control at 0.25 Hz (to avoid respiratory components in LF region), with care being taken to avoid hyperpnea. Thereafter the recording was repeated during neck suction in the normal subjects but not in the three sympathectomized subjects.

Data Analysis

A “C” language program identified all the QRS complexes in each sequence and then located the peak of each R wave. From these data, the R-R interval time series was obtained; the time series for the respiratory signal was obtained from the value of this signal at the occurrence of each QRS peak, together with systolic and diastolic blood pressure and systolic LDF signal. A time series was also constructed from the MSNA recordings by measuring, during each heart period, the integral within two fixed time limits (specific for each subject) that included the beginning and end of all spikes during all recordings obtained. Linear detrending was applied to the data to remove slow baseline drifts. Premature beats were not observed during this study; hence no interpolation of data was necessary.

Univariate spectral analysis. Power spectral analysis was applied to all signals recorded at baseline, using an autoregressive model, as previously described (19). Spectral components were obtained by a decomposition method (2, 16, 19) to measure the area below each spectral peak and to obtain the distribution of low-, high-, and very low frequency components (LF, HF, and VLF, respectively). The power spectra of all signals except respiration show at least two separate peaks (2, 16, 19): the higher frequency peak is similar in both shape and central frequency to that of respiration. Although, in the R-R interval sequence, this peak seems to reflect mostly the parasympathetic efferent activity, in the circulatory signals, it is interpreted as a mainly mechanical consequence of respiration induced changes in venous return (9, 16, 19). This respiratory component (HF) was identified by its coincidence with the peak of the respiratory spectrum, which served as a reference. The LF component (0.03–0.15 Hz), with usual peak at -0.10 Hz in the R-R interval, has been suggested to reflect changes in sympathetic tone (2, 9, 16), particularly in the blood pressure, but only when slow breaths are absent. Finally, the oscillatory components between 0.01 and 0.03 Hz (designated VLF), whose origin is still unknown, were also measured.

Bivariate spectral analysis (coherence and phase analysis). The fact that two signals could oscillate at the same frequency and that oscillations from such signals undergo similar changes under different experimental settings suggests a common control mechanism. Nevertheless, oscillations may have the same frequency without being linked to each other in a stable way (as would be the case if they were due to the same mechanism). To verify the presence of a stable relationship between the oscillations of pairs of signals, we performed a coherence analysis (by autoregressive algorithms; Ref. 2) between the various signals obtained at baseline. The “squared coherence” function determines whether two signals oscillating at the same frequency maintain a stable phase relationship. This function spans from 0 (i.e., no association) to 1 (i.e., maximal association). If a significant (>0.5) coherence is found, then the two signals have a stable phase relationship for a given frequency of oscillation and hence can be considered as synchronized with each other. In this case, it is then possible to calculate the phase delay (expressed in radians) between pairs of coherent oscillations.

Time-varying spectral analysis. The recordings obtained during chirp sinusoidal neck suction could not be evaluated by the previous spectral methods, since the stimulation and response signals were continuously changing over time. On the other hand, recordings of 2 min, for example, at each neck suction frequency would have been too time consuming considering this complex experimental protocol and not well tolerated by the subject. Unlike a previous study dealing simply with the R-R interval and blood pressure fluctuations (2), whose characteristic predominance in 0.1 Hz and respiratory rhythms is known, it was necessary when dealing with the microcirculation to obtain the widest possible range of stimulation frequencies, particularly since previous reports (20) indicated that the natural resonance frequency of a microvessel is very low, i.e., ~0.03 Hz. To solve this problem, we therefore applied a function capable of performing a stimulation at every frequency; this type of signals can now be analyzed by a new family of spectral methods, allowing computation of the instantaneous spectra. For this purpose, we used the Wigner-Ville transform, which has recently been used with success by different groups (18, 27) in comparable experimental or clinical conditions. The algorithms used have been extensively described and tested (17, 27), and our implementation of the Wigner-Ville transform was written and validated according to previously described methods (27). Finally, to assess the dependence on neck suction oscillations in the various signals and at each frequency, we calculated
The cross-spectrum of each signal vs. the neck suction. This cross-spectrum was obtained by multiplication of each signal spectrum by the spectrum of the neck suction, with the neck suction spectrum maximal peak normalized to a value of 1. As a consequence, the resulting spectra indicated to what extent and at what frequency each signal was dependent on arterial baroreceptor stimulation.

Statistical Analysis

The results are given as means ± SE. Because the power spectrum of the various signals showed a skewed distribution, descriptive statistics were calculated after natural logarithm transformation. Comparisons of the maximal response frequency to neck suction stimulation and of the relative amount of LF or HF at rest in the various signals were obtained by repeated-measures analysis of variance, and if a significant (P < 0.05) overall difference was obtained, the Scheffé test was used to assess differences between various signals. Coherence function was used as a statistical test for each pair of oscillations (11). Linear regression analysis was used to assess the relationship between the oscillations obtained in different body areas and between different signals. A paired t-test was used to assess differences in left vs. right LDF data and to assess differences in LF vs. HF oscillations in the various signals.

RESULTS

Synchronism of Skin Microcirculatory Fluctuations Obtained in Two Index Fingers

Visual inspection of timeseries of signals. The fluctuations observed in two similar skin areas (left vs. right index finger) were consistently synchronous (Fig. 1).

Power spectral analysis, univariate. The spectral analysis (Table 1) identified three kinds of oscillations in the skin microcirculation. The range of 0.1 Hz (LF; 0.081 ± 0.006 and 0.080 ± 0.08 Hz in left and right side, respectively) was invariably present in all subjects and accounted for the greatest percentage of overall variability (61.6% for right side, 57.7% for left side). A consistent fluctuation synchronous with respiration (HF) was also present in all recordings but was only 15.2% (right side)-18.2% (left side) of total variability compared with that observed at lower frequencies. Finally, a third fluctuation at even lower frequency (VLF; ranging from 0.015 to 0.026 Hz) was observed in 5 of 10 subjects, but only in 3 was it detected on both the right and left sides. Figure 2 shows the spectra obtained from the same data as Fig. 1. The spectra obtained from the two LDF signals are very similar, confirming the visual impression of similar waves present in these two signals. Figure 1 shows clear fluctuations in the VLF range, but these did not appear in the LDF spectra, probably due to their variable period.

The average proportion of these fluctuations was similar in the two skin areas examined (Table 1). The power of the fluctuations in the two sides of each signal is similar in the two skin areas examined (Table 1).
subject correlated for the LF and HF (LF: $r = 0.648$, $P < 0.01$; HF: $r = 0.882$, $P < 0.001$) but not for the VLF ($r = 0.103$; not significant, NS).

The percent differences in the power of the 0.1 Hz (LF), respiratory (HF), and VLF fluctuations of left vs. right index finger were 19.7 ± 4.2% for LF, 18.5 ± 3.9% for HF, and 45.7 ± 22.3 for VLF, thus suggesting a more consistently similar pattern for the LF and HF than for the VLF fluctuations. No significant differences were observed in the power of the various fluctuations when left vs. right index fingers (LDF) were compared within the same subject (paired t-test).

Power spectral analysis, bivariate (coherence analysis). The coherences between the pairs of oscillations in these signals were significant in 10 of 10 subjects for the LF oscillations in the two LDF signals (right vs. left index fingers) and in 10 of 10 subjects for the respiratory frequency. The phase delay between left vs. right LDF signals was close to zero for both LF ($0.026 ± 0.106$ rad) and HF ($0.078 ± 0.104$ rad), hence indicating that 1) these two signals were synchronized at these two bands and 2) the corresponding waves occurred with no delay on one side with respect to the other, as if they were generated or propagated simultaneously. The example in Fig. 3 shows the coherence between the left and right LDF signals obtained from the data of Figs. 1 and 2.

Comparison of Microcirculatory Fluctuations with Other Signals

Visual inspection of time series of signals. Not only were the fluctuations in the two similar skin areas (left and right index fingers) similar to each other, but this similarity was also seen in other signals, such as systolic and diastolic blood pressure, MSNA, and R-R interval; in addition, fluctuations synchronous with respiration could be observed in all signals.

Power spectral analysis, univariate. Table 1 reports the results of power spectral analysis of all the signals recorded. The visual similarity of the fluctuations in the various signals is confirmed by the presence of LF and HF waves in all signals, although the relative proportion of LF and HF was different in each signal; VLF components were more evident in the LDF than in any other signal. The LF power in the LDF signals correlated with the LF power in the MSNA ($r = 0.845$, $P < 0.01$) and also with the HF power in the MSNA ($r = 0.808$, $P < 0.01$) but not with the LF power in the...
systolic or diastolic blood pressure and R-R interval. Figure 2 shows the spectra obtained from the signals of Fig. 1. The 0.1-Hz fluctuations are well represented in all signals, including the skin microcirculation and the MSNA. Respiratory fluctuations are also evident in most of the signal spectra. However, their proportions decrease from the R-R interval with respect to the systolic pressure, diastolic pressure, and skin blood flow. These fluctuations, though present, are not apparent in the spectra of Fig. 2 because of their small proportion.

Power spectral analysis, bivariate (LDF vs. other signals). In most subjects, the coherence function was significant in the LF and HF regions when the LDF signal was compared with other signals (Fig. 3).

**LDF VS. SYSTOLIC BLOOD PRESSURE.** Significant coherence was present in 9 of 10 subjects in the LF band, with a positive phase (i.e., LDF leading systolic blood pressure by $1.05 \pm 0.17$ rad), and in 6 of 10 subjects in the HF band, with a negative phase (i.e., LDF lagging behind systolic blood pressure by $-0.13 \pm 0.19$ rad).

**LDF VS. DIASTOLIC BLOOD PRESSURE.** Significant coherence was present in 10 of 10 subjects in the LF band, with a positive phase (i.e., LDF leading diastolic blood pressure by $0.87 \pm 0.20$ rad), and in 6 of 10 subjects in the HF band, with a negative phase (i.e., LDF lagging behind diastolic blood pressure by $-0.46 \pm 0.31$ rad).

**LDF VS. MSNA.** Significant coherence was present in six of eight subjects in the LF band, with a negative phase (i.e., LDF lagging behind MSNA by $2.55 \pm 0.22$ rad), but only in four of eight subjects in the HF band, also with a negative phase and a rather widespread distribution ($-2.77 \pm 0.34$ rad).

**LDF VS. R-R INTERVAL.** Significant coherence was present in 6 of 10 subjects in the LF band, with a positive phase (i.e., LDF leading R-R interval by $1.84 \pm 0.21$ rad), and in 6 of 10 subjects in the HF band, also with a positive phase ($0.44 \pm 0.16$ rad).

**LDF VS. RESPIRATION.** Because all subjects were breathing at a frequency greater than those in the LF band, no LF fluctuations were present in the respiratory spectra obtained and no significant coherences were found in the LF region. Conversely, all subjects showed significant coherences in the HF band, with a negative phase (i.e., LDF lagging behind respiration) corresponding to $-1.91 \pm 0.19$ rad.

The comparative results of the phase analysis are shown schematically in Fig. 4, which indicates that MSNA appears to precede the occurrence of LF waves in all signals and LDF appears to follow MSNA, then diastolic and systolic blood pressure, and finally R-R interval. Figure 4 shows that respiratory (HF) fluctuations in the 0.25-Hz band appear next in the blood pressure signals, then in the LDF, and finally in the R-R interval; the respiratory fluctuations only occur at the end of the respiratory cycle (i.e., end expiration).

**Sympathectomized Subjects**

The subject who underwent bilateral sympathectomy 3 mo earlier showed no evident synchronization of the two microcirculatory signals recorded in the right index finger and no evident synchronization with the LDF fluctuations recorded in the toe (see Fig. 5).
The other two patients who underwent sympathectomy 10 mo and 1 year earlier showed some degree of synchronization in the microvascular fluctuations of both sides and different fingers. However, they also reported signs of partial reinnervation, such as reappearance of sweating in response to psychological stimulation. As a consequence, their results were between those of the first subject and those of the normal subjects.

**Changes in Microcirculatory Fluctuations Induced by Frequency-Dependent Arterial Baroreflex Modulation**

Modulation of arterial baroreceptor activity by chirp sinusoidal neck suction could generate clear fluctuations in the microcirculation in the 0.03- and 0.1-Hz regions, whereas stimulation above the 0.1-Hz region was ineffective. Table 2 lists the frequencies of maximal response in each signal. This behavior was similar to that of systolic and diastolic blood pressure, whereas the R-R interval and, even more, the MSNA were capable of responding also to stimulation at higher frequencies.

Although all signals showed their maximal response in the LF region, the frequency of maximal response was significantly ($P < 0.01$) lower for the LDF (0.058 and 0.055 Hz on left and right sides, respectively) and blood pressures (0.059 and 0.057 Hz for systolic and diastolic, respectively) than for MSNA and R-R intervals (0.077 and 0.088 Hz, respectively). However, the maximal response occurred at a similar frequency in the four “vascular” signals (left and right LDF, systolic and diastolic blood pressures; NS). In addition, a secondary peak of response was found (Table 2) at similar frequencies in the two LDF signals (0.026 and 0.023 Hz on left and right sides, respectively; NS) and at a high frequency in the MSNA signal (0.193 Hz).

As a consequence, with respect to their ability to respond to the arterial baroreceptor modulation, all four vascular signals behaved as a low-pass system, the R-R interval was similar to an all-pass system, and the MSNA was closer to a high-pass system.

Figure 6 shows an example of the data obtained. It is clearly shown that each signal responded with its own characteristics. Although the R-R interval was able to follow the stimulation at almost every frequency, the blood pressure response decreased just above 0.1 Hz and was almost unresponsive in the HF band. Similarly, the microvessels responded well at the lowest frequencies of stimulation but poorly above the LF region. Nevertheless, it is evident from Fig. 6 that all the cardiovascular signals exhibit their maximal response in the 0.1-Hz region. We noticed that the blood

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**Table 2. Frequency of maximal response to neck suction simulation**

<table>
<thead>
<tr>
<th>Signal</th>
<th>Max Response, Hz</th>
<th>Secondary Peak, Hz</th>
<th>Significance vs. R-R interval</th>
<th>Significance vs. MSNA</th>
<th>Significance vs. SBP</th>
<th>Significance vs. DBP</th>
<th>Significance vs. LDF, left</th>
<th>Significance vs. LDF, right</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-R interval</td>
<td>0.088 ± 0.005</td>
<td></td>
<td>NS</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>MSNA</td>
<td>0.077 ± 0.004</td>
<td>0.193 ± 0.005</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP</td>
<td>0.059 ± 0.004</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>DBP</td>
<td>0.057 ± 0.005</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>LDF, left</td>
<td>0.058 ± 0.005</td>
<td>0.026 ± 0.003</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LDF, right</td>
<td>0.053 ± 0.005</td>
<td>0.023 ± 0.002</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tr>
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</table>

SBP and DBP, systolic and diastolic blood pressure; LDF, laser-Doppler flowmetry; NS, not significantly different.

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pressure and, to a greater extent, the microvessels maintained a slow rhythm independent of that imposed by the baroceptor stimulation. This is particularly evident when neck suction was oscillating at frequencies >0.1 Hz, where the only fluctuations left were those at LF or VLF, suggesting that factors independent from the baroceptor stimulation (due to local factors or to a central oscillator or to a combination of both) are also operating at the same time (11).

The cross-spectra of each signal vs. the neck suction showed only the net response of each signal to the arterial baroceptor stimulation. Figure 7 is an example of the cross-spectra obtained from the same data of Fig. 6, showing the low-pass behavior of the LDF and systolic and diastolic blood pressures, the all-pass behavior of the R-R interval, and the high-pass behavior of the MSNA. The pattern of response of the previous figure resulted here more evident.

**DISCUSSION**

Although variations in peripheral blood flow have been well described for over half a century (7), the problem of the origin and significance of these fluctuations remains debated (see introduction). It is now accepted that the LDF signal is proportional (within same subject) to the blood flow in the most superficial (in range of 1 mm) layers of the skin (3). The arterioles and/or the arteriovenous anastomoses, i.e., structures rich in autonomic innervation, are the main determinants of the microvascular fluctuations (30). To assess the influence of central control mechanisms on the fluctuations in skin microvessels, we evaluated the synchronism of LDF fluctuations in different body areas, their relationship with spontaneous fluctuations observed in signals known to be related to general hemodynamics and/or autonomic activity, such as the blood pressure, R-R interval, respiration, and MSNA, and their ability to respond to arterial baroreflex stimulations at varying frequencies, taking into account the possibility that the autonomic modulation of the cardiovascular system could be frequency dependent (2, 18, 23).

Synchronism of LDF Fluctuations in Different Body Areas

The fluctuations in the fingers are similar and synchronized particularly in the LF band and, to a lesser extent, in the HF band.

Nonrespiratory (LF) and respiratory (HF) fluctuations. These fluctuations are highly coherent with zero phase lag; they have similar absolute and relative (i.e., percentage of variance) power, which correlated in each
subject, and similar frequency. This similarity in frequency can easily be understood for the respiratory components (since both left and right LDF HF originate from the activity of respiration) but was quite surprising for the LF, which are nonrespiratory by definition. If these fluctuations were of local origin, there is no reason why they should have the same frequency and amplitude and occur simultaneously in two different body areas.

Although the HF fluctuations in the vasculature are considered to be mostly the result of changes in stroke volume caused by changes in venous return due to respiration, the LF oscillations of blood pressure are considered a marker of sympathetic efferent activity to the vessels (9, 16, 19). Therefore the finding of LF fluctuations in the arteriolar vessels can be considered as a marker of sympathetic modulation of these structures. The presence of these slow fluctuations might be independent of central control and simply be a casual result of local oscillatory phenomenon; however, the finding of synchronous fluctuation in two different limbs is against this hypothesis.

VLF fluctuations. A direct correspondence between two LDF signals is not always evident on visual inspection of the signals, since other rather irregular fluctuations of lower frequencies (0.017–0.028 Hz, VLF) are superimposed on LF and HF fluctuations. We have observed such slower waves in 5 of 10 subjects, but only in 3 of 10 were they present in both right and left LDF signals. The relatively short period of analysis (only few cycles can be observed in 12 min), in addition to likely local influences, might explain the relatively poor synchronization in these fluctuations. Burton (7) suggested that the slowest fluctuations in skin blood flow were due to thermoregulatory influences. If this hypothesis were true, then the VLF should be present particularly in those signals directly related to the thermoregulatory adjustments, such as those in the skin blood flow. Because these fluctuations were in fact present in the LDF more than in any other signal that we have recorded and appeared to be locally mediated, we might speculate that they could indeed be related to thermoregulatory activity (among other factors). However, this statement should be matter of a specific investigation.

Relationship Between Spontaneous Fluctuations in LDF and Other Cardiovascular and Neural Signals

In the present study, we have also compared fluctuations in the LDF signals with those present in heart rhythm, respiration, blood pressure, and MSNA.
Nonrespiratory LF fluctuations. One of the main findings of the present study is that the LF fluctuations in the LDF signals are coherent with those present in most cardiovascular signals and the MSNA, indicating that all such fluctuations are related to each other (Fig. 3). The significant association between these waves allowed us to measure the phase lag between each signal. We found that LF waves first occur in the MSNA, then in the LDF signals, then in the blood pressure, and finally in the R-R interval (Fig. 4). Taken together, even with the limitations due to the lack of skin sympathetic activity signal, it seems that, under normal conditions, these LF originate in the microcirculation, in keeping with the well-known fact that most of the sympathetic modulation of blood vessel diameter occurs at arteriolar level (30). Consideration of the other signals in Fig. 4 also shows that LF blood pressure fluctuations precede those in the R-R interval by ~1 rad. This finding is in close agreement with similar results obtained by de Boer et al. (Ref. 9; they found a delay of 60–90° corresponding to 1–1.5 rad, similar to our finding) and with those of Saul et al. (23) for the same frequency. These findings cannot be due to a spurious delay from arterial blood pressure to the Finapres signal. Theoretical measures suggest a delay of ~0.3 s; preliminary data from our laboratory (unpublished data) indicate a similar delay. In support of the hypothesis of a peripheral origin of the LF, we also found a significant intersubject correlation between the power of the LF fluctuations in the LDF signal vs. those in the MSNA, whereas the correlation with the LF power in the other signals was not significant despite significant coherence. This suggests that there is a close relationship between the skin blood flow fluctuations in the LF band and the sympathetic neural activity directed to the limbs. Although we could record the muscle nerve activity in all subjects, we could never record the skin sympathetic activity for a long enough period to be analyzed. Previous reports (10, 15, 28, 29) on the relationship between skin sympathetic activity/skin blood flow vs. baroreflex or central command stimuli are conflicting. Those studies were based on inspection of short-term recordings, and no mathematical methods were applied to analyze in depth the relationship (if any) between the LF of the various signals. However, the presence of a correlation between the power of LDF LF fluctuations and the power of MSNA LF fluctuations further indicates that the LDF LF fluctuations are not simply of local origin and must have some relationship with the sympathetic efferent activity to the limbs.

Respiratory HF fluctuations. We found that the HF fluctuations (respiratory band) in the LDF signals are coherent with those present in most cardiovascular signals and in the MSNA (Fig. 3), although the presence of significant values was less frequent than for the LF fluctuations. Phase analysis (Fig. 4) revealed a different behavior with respect to the LF. After originating in the respiration, the HF fluctuations appear in the blood pressures, then in the LDF, then in the R-R interval, and finally in the MSNA. This suggests that the HF waves are transmitted "downstream" from the larger arteries to the microvessels (due to respiratory changes in venous return and stroke volume) and are also sensed by the baroceptors and transformed into R-R interval changes and respiratory modulation of MSNA. This hypothesis is also in agreement with previous findings of a transmission of respiratory sinus arrhythmia from blood pressure fluctuations (9, 23). In fact, consideration of the other signals in Fig. 4 shows that blood pressure HF fluctuations precede those in the R-R interval by ~0.5 rad. The R-R interval is nearly in phase opposition with respiration at ~0.25 Hz. As for the LF, this finding is in agreement with similar results obtained by other authors for comparable frequencies (9, 23).

Nevertheless, we have found that the MSNA contains a relevant proportion of respiratory fluctuations in some subjects. In addition, we have found that stimulation of the arterial baroceptors is capable of generating MSNA fluctuations in both the LF and HF regions, with a behavior close to a high-pass system (Table 2, Figs. 6 and 7). This might suggest that sympathetic activity could be responsible for the HF fluctuations, at least in the blood pressure and in the skin blood flow. This hypothesis cannot be ruled out by the present study, but it seems unlikely in view of three other observations. 1) Although the MSNA contains the largest proportion of HF (60% of total variability, Table 1), only a minor fraction of HF can be found in the LDF and blood pressure signals (15–18% of total variability, Table 1). 2) Because the HF waves are clearly initiated by the respiratory activity, if the vascular HF were the result of sympathetic activity, one would have expected the MSNA respiratory fluctuations to occur between respiration and the vascular signal; instead, the MSNA occurred last of all signals, at end expiration (Fig. 4). Our finding is in close agreement with previous observations that MSNA respiratory peak occurs at end expiration (26). 3) Stimulation of the arterial baroceptor at frequencies above the LF region was effective in the LDF and in the blood pressures (see below).

Conversely, the significant correlation between the power in the HF band observed between the LDF and the other signals might suggest that these waves are, at least to a great extent, transmitted from the heart and the larger arteries to the microvessels. This is also supported by the finding that the respiratory fluctuations (HF) of the LDF, unlike the LF, lag behind those of blood pressure signals, as shown in the results and schematically in Fig. 4.

Response of LDF Fluctuations to Frequency-Dependent Arterial Baroreflex Stimulation

We have previously found that maneuvers associated with an increase in sympathetic activity (such as tilting) synchronize the LF in different signals (4, 5). In the present study, we have also clearly demonstrated that the skin microcirculation is sensitive to stimulation of the arterial baroceptors, with a specific frequency-dependent type of response. We have recently proposed
and validated a new simplified model for testing the frequency response of the cardiovascular system to the arterial baroreceptors (2) based on the sinusoidal stimulation of the arterial baroreceptors at two specific frequencies (one in LF region, i.e., 0.10 Hz, and one in the HF region, i.e., 0.20 Hz). In the present experimental study, we have generalized this model by using all frequencies from 0.02 to 0.20 Hz to evaluate the response of the microcirculation with respect to the other signals. We have found that the baroreceptor stimulation is capable of determining clear fluctuations in the LDF signal at a frequency in the LF region similar to the blood pressures. This ability, however, decreases sharply at >0.1 Hz, and at frequencies typical of the respiratory band (i.e., 0.20 Hz), the LDF becomes unresponsive to baroreceptor stimulation, similar to the blood pressure (Figs. 6 and 7). These results demonstrate that skin microvascular fluctuations in the LF region are indeed sensitive to nonthermoregulatory influences. Because this response pattern depends on modulation of arterial baroreflex activity (acting on vessels by efferent sympathetic activity), it can be concluded that the changes observed in the skin microvessels during chirp neck suction are due to modulation of sympathetic activity.

All cardiovascular signals and the MSNA have their maximal sensitivity to the arterial baroreceptor stimulation at a frequency in the LF region. This frequency is not significantly different for the LDF and blood pressures, whereas it is slightly but significantly lower than the frequency of maximal response of R-R interval and MSNA (Table 2). This parallels the result of significant coherence in the LF region obtained in the comparison between the LDF and all other signals (Fig. 3) and indicates that perhaps the whole cardiovascular system has a peculiar attitude to resonate at this specific frequency. The tridimensional spectra relative to the time series of Figs. 6 and 7 and the data of Table 2 clearly show this concept. Despite each signal having its own pattern of response to neck suction, all signals showed their greatest response at a frequency in the LF region.

Additional Findings

Two other findings of the present study are worth mentioning, since they probably are among the first descriptions of supposed ubiquitous phenomena in the cardiovascular system.

Interaction between autonomic modulation and natural resonance frequency of microvessels. There is a maximal response of the LDF signal in the LF region, but, in addition, there is also a second peak in the VLF region at 0.023 and 0.026 Hz (right and left side, respectively; Table 2). This is close to the frequency at which Rosenbaum and Race (20) found that the resistance vessels have a resonant frequency (~0.03 Hz). This result suggests that the LDF LF fluctuations are most likely the result of an external modulation (as one provided by sympathetic neural activity at 0.1 Hz). However, slower stimulation of whatever origin (including neck suction) can easily produce LDF fluctuations in the VLF region at the natural resonance frequency the skin microvessels. This might explain the frequent occurrence of VLF fluctuations in the LDF more than in other signals.

Nonlinear interaction between faster MSNA fluctuations and slower vascular waves. Although the stimulation of the arterial baroreceptors in the HF region was able to increase the MSNA at high frequencies, this did not result in an increase in the LDF HF oscillations, since the behavior of the LDF was clearly similar to a low-pass system. This cannot be attributed to inability of the LDF fluctuations to respond to the baroreceptor stimulation, since this signal, similar to that for MSNA, was highly sensitive to the stimulation in the LF (and also in VLF) region (Table 2, Figs. 6 and 7). Similar considerations also apply to the blood pressure fluctuations, since these signals did not respond to stimulation of the arterial baroreceptors in the HF region but only when the stimulations was limited to the LF region. Hence, for some reason, the faster (i.e., in HF region) components in the sympathetic nerve discharge cannot be "transferred" into oscillations at the same frequency in the microcirculation. One might hypothesize that such fluctuations either are simply ineffective in terms of vascular modulation or they might contribute to the generation of other fluctuations at lower frequencies (i.e., nonlinear interaction).

To date, we are unaware of simple mathematical tools to solve this problem. However, visual inspection of some of the data suggests that faster MSNA fluctuations might be related to the slower fluctuations in the vascular signals (LDF and blood pressures), hence determining a nonlinear interaction. Figure 6 shows quite a clear example of the interaction between LF fluctuations in the MSNA and VLF fluctuations in the LDF and systolic and diastolic blood pressures during chirp neck suction at the lowest frequencies. It is evident from Fig. 6 that the slowest neck suction cycles determine corresponding slow oscillations in the vascular signals. However, in case of a linear relationship, one would expect that these vascular oscillations would result from an identical oscillation in the sympathetic nerve activity. Instead, the MSNA simply responds with a modulation in amplitude and width of its own pulses, which occur consistently at the 0.1-Hz frequency. Hence modulation of a faster wave in the MSNA clearly produces a slow wave in the vascular (blood pressure and LDF) signals. However, as soon as the frequency of the neck suction approaches the spontaneous rhythm present in the MSNA, there seems to be a linear relationship between the oscillations in the various signals. In conclusion, it is very possible that the lack of correspondence between the HF in the MSNA and in the LDF signals can be due to the inability of the vessels to respond to fast autonomic stimulation and further suggests that the observed respiratory (HF) fluctuations are indeed the result, at least to a greater extent, of a passive transmission through the vascular tree.

Limitations of Study

The LDF technique cannot provide measurements of flow in absolute units (3). Direct studies in the microcirculation cannot be performed in vivo. However, the LDF is a useful tool to determine corresponding slow oscillations in the vascular signals.
culation (in which single individual vessels are analyzed; Ref. 8) show that there is a specific distribution in characteristic oscillatory frequencies that is inversely related to vessel diameter. The LDF technique cannot, by definition, observe fluctuations in a single vessel. The volume under measurement (which is thought to be in the range of 1 mm³; Ref. 3) is large enough to include a great number of different microvessels with different sizes and must therefore include fluctuations originating from all types of vascular structures. This must have the effect of enhancing the synchronous fluctuations in the microvasculature and eliminating fluctuations peculiar to a single microvessel, since these, by being strictly local, might occur randomly in each microvessel with respect to the others. Hence the results of this study indicate the presence of a central autonomic modulation of skin blood flow but do not preclude the existence of simultaneous local fluctuations. These could be better seen with methods, such as capillaroscopy, which, on the other hand, are probably less efficient in showing synchronisms in different vessels and skin regions.

In the present study, we have analyzed the LDF signals obtained from structures rich in arteriovenous shunts, such as the pulp of the finger and the ear (30). These areas are known to have the highest spontaneous pulsatility. Although similar results were obtained also from skin areas, such as the forearm (5), which has few arteriovenous shunts (30), the results of the present investigations may not be valid for the entire skin surface. In our subjects, we could only evaluate the muscle and not the skin sympathetic nerve activity; hence we could not have a comparison with the sympathetic activity directly related to the skin microvessels. Nevertheless, our data do provide evidence of a clear relationship between MSNA and LF fluctuations in the LDF signal, thus demonstrating that LF fluctuations in the skin microvessels do have a relationship with the sympathetic neural discharge. Although the plots in Fig. 4 show precedence of signals objectively, the identification of the starting points (for both LF and HF) reflect our personal interpretation. Although this fits with other established physiological facts, other explanations could be possible and are perhaps worth considering in future experimental works.

Conclusions

The data obtained in the present study demonstrate that although local factors may be able to independently influence the microcirculation, oscillations in the 0.1-Hz and respiratory regions are similar and have a stable phase relationship in different parts of the body. Also, in normal subjects, LDF fluctuations are coherent with those of cardiovascular signals and with muscle sympathetic efferent activity to the limbs. Modulation of the arterial baroreceptors determined corresponding oscillations in the skin microvessels up to 0.1 Hz, demonstrating that nonthermoregulatory stimuli have the ability to influence the skin microcirculation. All these findings indicate that the 0.1-Hz fluctuations in the skin microvessels can be considered a marker of sympathetic activity to the circulation. In addition, we provided evidence suggesting that the microvessels are a likely site of origin of the LF fluctuations recorded in the blood pressure and the R-R interval. The 0.1-Hz fluctuations in the skin microvessels thus appear to be a relatively simple technique that can provide information about autonomic regulation at the microvascular level.

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