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Decoding rule from vasoconstrictor skin sympathetic nerve activity to nonglabrous skin blood flow in humans at normothermic rest

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ABSTRACT

Although an importance of vasoconstrictor skin sympathetic nerve activity (SNA) in control of cutaneous circulation is widely recognized, the decoding rule that translate dynamic fluctuations of vasoconstrictor skin SNA into skin blood flow is not fully understood. In 10 male subjects who rested in supine position under normothermic condition, we measured skin blood flow index (by laser-Doppler flowmetry) at the dorsum pedis, and vasoconstrictor skin SNA (by microneurography) that was confirmed to innervate the same region as the flow index. We determined the transfer and coherence functions from the neural activity input to the flow and quantified the contribution and predictability from the input to output by system engineering technique. The results showed that in frequency-domain analysis, the transfer function from vasoconstrictor skin SNA to skin blood flow had low-pass filter characteristics with 3.6 ± 0.1 s of pure time delay. The coherence function was approximately 0.5 between 0.01 and 0.1 Hz and less above 0.1 Hz. In time-domain analysis, the predictability from the SNA to the skin blood flow was approximately 50%. These findings indicate that at normothermic rest, the decoding rule from vasoconstrictor skin SNA to skin blood flow of skin is characterized by low-pass filter with 3–4 s of pure time delay, and that the vasoconstrictor skin SNA contributes to a half of fluctuation of skin blood flow in the condition. The incomplete dependence of skin blood flow on vasoconstrictor skin SNA may confirm nonneural mechanisms to control cutaneous circulation even at normothermic rest.

Human skin sympathetic nerve activity (SNA) has an important role in control of cutaneous circulation [6]. Although skin SNA contains several functional components, vasoconstrictor skin SNA is predominant during normothermic condition and cold stress [6,11,16], whereas sudomotor [6,11,16], and, in some case, vasodilator [14] skin SNA is predominant during heat stress. Although activation of vasoconstrictor skin SNA strongly decreases skin blood flow during cold stress [3], the vasoconstrictor activity also regulates the flow in normothermic condition [4]. However, the decoding rule that translate vasoconstrictor skin SNA into skin blood flow is not fully understood, probably because of ceaseless fluctuations of the nerve activity and the flow. The vascular response to nerve activity includes several time-dependent dynamic processes; release of neurotransmitter (noradrenaline) from presynaptic membrane, binding of neurotransmitter to specific adrenergic receptors in postsynaptic membrane, following cellular responses and resultant changes in microcirculation [12]. Accordingly, we hypothesized that the decoding rule (the transfer function from vasoconstrictor skin SNA to skin blood flow) at normothermic rest has low-pass filter characteristics with a few seconds of pure time delay.

In addition, although vasoconstrictor skin SNA is an important regulator of cutaneous circulation in normothermic condition, nonneural vascular reactions including vascular cutaneous autoregulation (e.g., venoarteriolar response [7,15], myogenic response [4]) also control cutaneous circulation. Although the vascular cutaneous autoregulation was mainly found during postural change [7,15] and forced respiratory maneuver [4], it is possible that these nonneural vascular mechanisms also controls cutaneous circulation even at supine rest. Accordingly, we hypothesized that the contribution of vasoconstrictor skin SNA to a fluctuation of skin blood flow at normothermic rest is limited to be moderate.

To test the two hypotheses, in resting supine posture under normothermic condition, we measured skin blood flow index (by laser-Doppler flowmetry) at the dorsum pedis, and vasoconstrictor skin SNA (by microneurography) that was confirmed to innervate the same region as the flow index. We determined the transfer and coherence functions from the neural activity input to the flow and

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quantified the contribution and predictability from the input to output by system engineering technique.

Subjects were 10 healthy male volunteers with age of 24 ± 4 (mean ± S.D.) year, height of 171 ± 6 cm, and weight of 65 ± 6 kg. We evaluated all subjects as healthy by completing a detailed medical history and by conducting a physical examination, resting electrocardiogram, blood chemistry analyses and psychological testing. None of the subjects smoked or had experience of recreational drugs. All subjects gave informed consent to participate in this study, which was approved by the Ethical Committee of the Research Institute of Environmental Medicine, Nagoya University. The experiments were performed at the Research Institute of Environmental Medicine, Nagoya University, whereas the data were analyzed at the National Cardiovascular Center Research Institute.

Left carpus arterial blood pressure (JENTOW TOM-101, Nippon Colin, Nippon Colin, Japan) was measured continuously. Electrocardiogram (chest lead II, for calculation of R–R interval) and thermistor respirogram were also recorded continuously.

Skin blood flow was measured at the dorsum pedis of the right leg by laser-Doppler flowmetry (ALF 21, Advance, Tokyo). Although the laser-Doppler flow method does not provide absolute flow value, it accurately and continuously reflects changes in skin blood flow [8] that is uninfluenced by muscle blood flow [13]. Sweat rate (mg min^{-1} cm^{-2}) were also measured at the nearly same point (1 cm apart from the point for skin blood flow) by the ventilated capsule method (Kenz-Perspiro 201, Suzuken, Nagoya). The capsule with the cross-sectional area of 0.76 cm^2 was fixed by coupe-face tape. The dry air was drained from the bottle with a temperature (27 °C) as the same as the experimental room. Since the area for measurement was so small, the skin blood flow outside of this capsule was not affected by this air drainage.

Skin SNA were measured from the peroneal nerves of the right leg by microneurography technique [11,16]. Briefly, a tungsten microelectrode (model 26-05-1, Federick Haer and Co., Bowdoinham, ME) was inserted percutaneously into the muscle or skin nerve fascicles of the peroneal nerve at the popliteal fossa without anesthesia. Nerve signals were fed into a preamplifier (Kohno Instruments, Nagoya) with two active band-pass filters set between 500 and 5000 Hz and were subsequently monitored with a loudspeaker. Skin SNA was identified according to the following discharge characteristics [2,11,16]: (1) broad-based neural activity unrelated to the cardiac cycle, (2) afferent activity induced by gentle skin touch, but not in response to tapping of calf muscles, and (3) enhancement during arousal stimuli. Moreover, the skin SNA was confirmed to innervate the dorsum pedis by a response of afferent activity to gentle skin touch of the region and by a reduction of skin blood flow immediately after large bursts of skin SNA. The SNA signals were full-wave rectified, fed through a resistance–capacitance low-pass filter at a time constant of 0.1 s, and then stored on a DAT recorder (PC216Ax, Sony Magnescale, Japan) at a sampling rate of 1000 Hz, together with other variables.

Experiments were performed on 10:00 a.m. We instructed the subjects to refrain from eating for 3 h before the experiments. Subjects were allowed to rest in the supine position in the quiet experimental room. The room temperature was set at 27 °C, a thermoneutral temperature. Microneurography and other measurements were prepared. After 30 min of rest, variables were measured for more than 30 min.

Skin SNA burst was identified and their areas calculated using a computer program custom-built by our laboratory. Since the SNA signal was dependent on electrode position, it was expressed as an arbitrary unit (au). An average burst amplitude during the first 3 min was given 100 au, and other SNA signals during the experimental protocol were normalized to this value. Skin blood flow was quantified by laser-Doppler flowmetry values. The averaged flow value during the first 3 min was given 100 au, and other flow

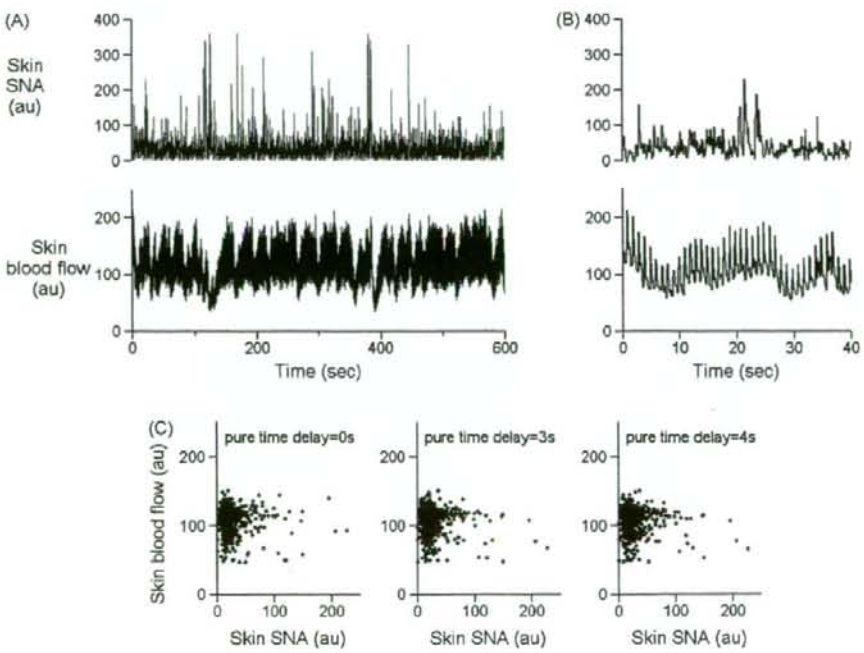

Fig. 1. (A) A typical example in one subjects of time series data of vasoconstrictor skin SNA and skin blood flow during 600 s of normothermic rest. au, arbitrary units. (B) Enlarged the data during the first 40 s of (A). (C) Scatter plotting of the skin SNA and skin blood flow (resampled at 1 Hz) of the same 600 s data as (A), with setting a pure time delay of 0 s (left), 3 s (middle) and 4 s (right).

values during the experimental protocol were normalized to this value.

Using the first 15 min of data, we calculated the transfer (the gain and phase) and coherence functions from skin SNA input to skin blood flow in frequency domain [9,10]. We resampled them at 10 Hz and segmented them into 10 sets of 50% overlapping bins of 2^{10} data point each. The segment length was 102.4 s, which yielded the lowest frequency bound of 0.01 (0.0097) Hz. We subtracted a linear trend and applied a Hanning window for each segment. We then performed fast Fourier transform to obtain frequency spectra of skin SNA and skin blood flow. We ensemble averaged the skin SNA power [$Sxx(f)$], skin blood flow power [$Syy(f)$], and cross power between them [$Syx(f)$] over 10 segments. Thereafter, we calculated the transfer function [$H(f)$] from skin SNA to skin blood flow as follows:

$$H(f) = \frac{Syx(f)}{Sxx(f)}$$

To quantify the linear dependence between skin SNA and skin blood flow in the frequency domain, we calculated the magnitude-squared coherence function [$Coh(f)$] as follows:

$$Coh(f) = \frac{|Syx(f)|^2}{Sxx(f) \cdot Syy(f)}$$

The coherence value ranges from zero to unity. Unity coherence indicates a perfect linear dependence between skin SNA and skin blood flow, whereas zero coherence indicates total independence of these two signals.

To quantify the transfer characteristics in time domain, the step response and predicted response of skin blood flow to changes in skin SNA was calculated by the discrete from convolution integral as follows:

$$Y(t) = \sum_{\tau=1}^{N} h(\tau) \cdot X(t-\tau)$$

where $h(\tau)$ is the impulse response obtained by inverse fast Fourier transform of the transfer function [$H(f)$], N is the total number of data elements, τ is the convolution parameter and t is time in increments of 0.1 s (or 10 Hz). The prediction was performed on the 8 min of data that was randomly selected from the remaining data that was not used for the calculation of transfer function. The predicted skin blood flow was compared with the measured flow while calculating the linear correlation coefficient (r) and the root mean square (RMS) between them when the correlation was judged statistically significant by $p < 0.05$ [5].

Fig. 1A shows a typical example (10 min) of skin SNA and skin blood flow at normothermic rest in one subject. Fig. 1B enlarged the first 40 s of data. Activations of skin SNA (i.e., 110–130 s in Fig. 1A, 20–25 s in Fig. 1B) were followed by a significant reduction of skin blood flow, whereas sweat rate was nearly 0 throughout the experiment, suggesting that the skin SNA was vasoconstrictor activity. However, scatter plotting of 1 Hz skin blood flow over skin SNA shown in Fig. 1A had no significant correlation regardless of pure time delay (Fig. 1C). Similar findings were observed in all subjects ($n = 10$). Skin blood flow had a clear pulse oscillation, whereas skin SNA did not. Mean blood pressure (means ± S.E., 85 ± 3 mmHg), heart rate (66 ± 3 beats/min) and respiratory rate (15 ± 1 breaths/min) were stable during the experimental period.

Fig. 2A shows the autospectra of skin SNA and skin blood flow obtained from all subjects. When the frequency increased from 0.01 to 0.9 Hz, the power of autospectra of skin SNA decreased to approximately 10% of the level at the lowest frequency, whereas that of skin blood flow to approximately 1%. Fig. 2B shows the transfer and coherence functions from skin SNA input to skin blood flow. The gain function decreased from 0.01 to 0.1 Hz and showed steeper decline from 0.1 to 0.3 Hz, indicating the low-pass filter characteristics. The phase function approached to $-\pi$ at the lowest frequency, indicating the negative response of skin blood flow to changes in skin SNA. This was consistent with that the skin SNA observed was vasoconstrictor activity. The coherence function was approximately 0.5 from 0.01 to 0.1 Hz and was below 0.4 above 0.1 Hz.

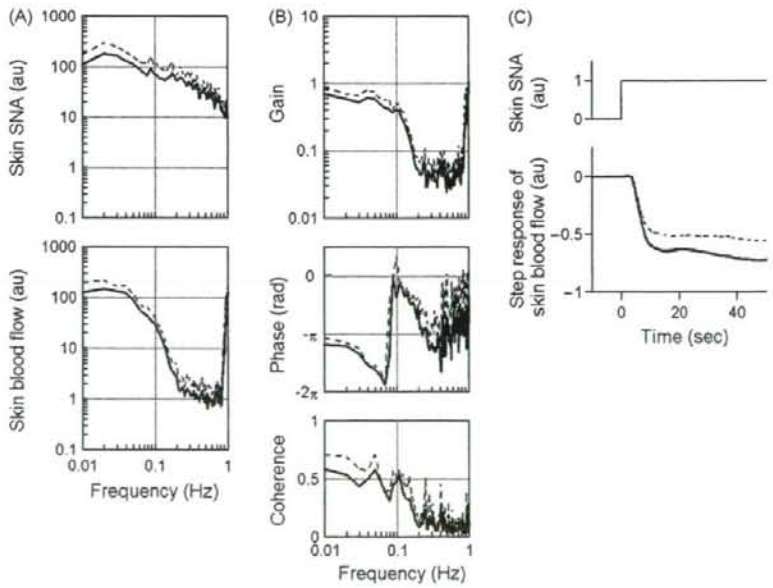

Fig. 2. (A) The autospectra of vasoconstrictor skin SNA and skin blood flow. (B) The gain, phase and coherence of transfer function from skin SNA to skin blood flow. (C) Step response of skin blood flow (to 1 au of step increase in skin SNA at time point 0). Values were shown as mean (solid line) + S.E. (broken line) in all panels ($n = 10$).

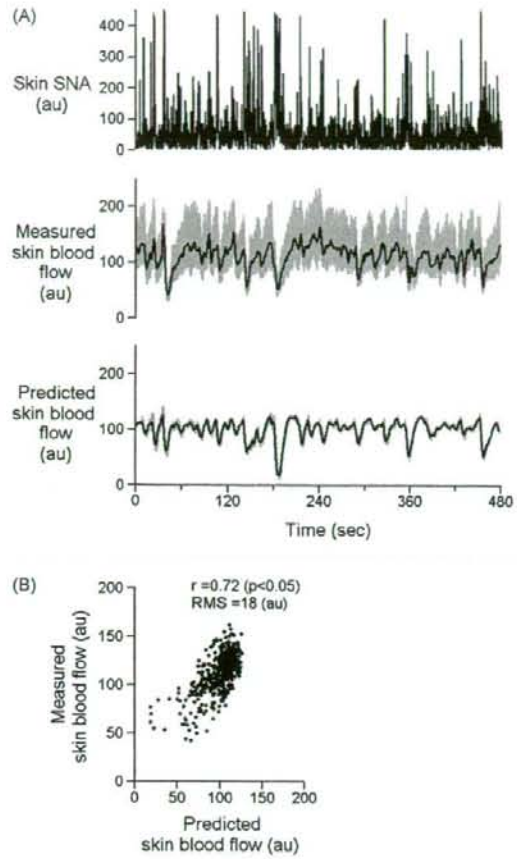

Fig. 3. (A) A typical example (8 min) of the measured vasoconstrictor skin SNA (top panel, 10 Hz) and skin blood flow (middle panel), and the predicted skin blood flow (bottom panel) from the calculated transfer function. The data shown was not used for the calculation of transfer function. In the panels of measured and predicted skin blood flow, gray and black lines show data resampled at 10 and 1 Hz, respectively. (B) Scatter plotting of the predicted and measured skin blood flow (resampled at 1 Hz) of the same data as (A).

Fig. 2C shows step response of skin blood flow (to 1 au of step increase in skin SNA at time 0). The step response had 3.6 ± 0.1 s of a pure time delay and decreased to reach -0.72 ± 0.16 au, that was consistent with the gain function at the lowest frequency. The decreasing response was consistent with the phase function in Fig. 2B.

To quantify the predictability of transfer function from skin SNA input to skin blood flow, skin blood flow responses to 8 min of measured skin SNA (Fig. 3A, top) was predicted (Fig. 3A, bottom). The measured data (skin SNA, skin blood flow) were not used for calculating the transfer function shown in Fig. 2B. The predicted skin blood flow (Fig. 3A, bottom, black line) was moderately similar to the measured skin blood flow (Fig. 3A, middle, black line) while the flows were resampled at 1 Hz. Scatter plotting of these predicted and measured skin blood flows showed significant correlation with r of 0.72 and RMS of 18 au (Fig. 3C). The significant correlations were observed in all subjects with r of 0.7 ± 0.1 and RMS of 18 ± 4 au.

Although an importance of vasoconstrictor skin SNA in control of cutaneous circulation is widely recognized [6,11,16], the decoding rule that translate dynamic fluctuations of vasoconstrictor skin SNA into skin blood flow is not fully understood. The major findings of the present study were that (1) the transfer function from vasoconstrictor skin SNA to skin blood flow in resting normothermic condition had low-pass filter characteristics with 3.6 s of pure time delay (Fig. 2B and C), (2) the coherence function from the SNA to the skin blood flow was approximately 0.5 between 0.01 and 0.1 Hz and less above 0.1 Hz (Fig. 2B) and (3) the predictability from the SNA to the skin blood flow was approximately 50% (Fig. 3). These findings support our two hypotheses that in nonglabrous skin, the decoding rule from vasoconstrictor skin SNA to skin blood flow at normothermic rest has low-pass filter characteristics with 3–4 s of pure time delay, and that the contribution of vasoconstrictor skin SNA to a fluctuation of skin blood flow is limited to be moderate.

The transfer function calculated in this study from vasoconstrictor skin SNA to skin blood flow indicates that the sympathetically mediated skin vascular response has history and frequency dependences. Given the vasoconstrictor function of the skin SNA, instantaneous vasoconstrictor skin SNA could negatively correlate with instantaneous skin blood flow even in normothermic condition. However, no correlation was found in the relation between the nerve activity and the flow (Fig. 1C, left panel). This was not simply because of an inappropriate pure time delay since neither 3 nor 4 s of pure time delay improved the correlation (Fig. 1C, middle and right panels). These time-domain data suggested that skin blood flow at a certain time point is affected by a significant duration of history of vasoconstrictor skin SNA before the time point. In other words, the translation from vasoconstrictor skin SNA to skin blood flow is history dependent, and vary depending on the frequency. This was confirmed by the frequency-domain analysis (Fig. 2B). As the frequency increased, the gain of transfer function, that means a ratio of magnitudes of changes in skin blood flow in response to those in the SNA at the frequency, decreased from 0.01 to 0.3 Hz, indicating the low-pass filter characteristics of the system. The phase of transfer function supported the goodness of the analysis, since it approached to $-\pi$ at the lowest frequency, indicating the negative responsiveness of skin blood flow to the vasoconstrictor skin SNA. Moreover, in time-domain analysis, step response of skin blood flow (in response to 1 au of step increase in the skin SNA) had 3.6 s of pure time delay, initial steep drop and following stable reduction, that was likely characteristics of low-pass filter with pure delay. Accordingly, these findings indicate that sympathetically mediated skin vasoconstrictor response depends on the history and the frequency of skin SNA.

Although the present data determined the decoding rule from vasoconstrictor skin SNA to skin blood flow at normothermic rest, the contribution of the SNA to the flow was limited to be mild to moderate. In frequency-domain analysis, the coherence function was approximately 0.5 between 0.01 and 0.5 Hz, indicating that the SNA determined a half of the variability of skin blood flow in the frequency range. This was consistent with the prediction of skin blood flow from measured skin vasoconstrictor SNA in time domain (Fig. 3). Although the data used for the calculation of transfer function was different from those for the prediction, the predicted skin blood flow (Fig. 3A, bottom panel, black line, 1 Hz resampling) was approximately similar to that measured (Fig. 3A, middle panel, black line, 1 Hz resampling). The prediction had moderate accuracy since it had r of 0.7 and RMS of 18% (Fig. 3B). Since vasoconstrictor skin SNA lacked significant pulse fluctuation (Fig. 1B), the prediction from the nerve activity could not cover the pulse fluctuation measured in skin blood flow (Fig. 3A, middle, gray line, 10 Hz resampling).

The incomplete dependence of skin blood flow on vasoconstrictor skin SNA may confirm that cutaneous microcirculation is

also controlled by nonneural mechanisms even in the supine posture at normothermic rest. In addition to sympathetically mediated vasoconstriction, nonneurally vascular reactions including vascular cutaneous autoregulation (e.g., venoarteriolar response [7,15], myogenic response [4]) is known to regulate skin blood flow. However, the vascular cutaneous autoregulation in normothermic condition has been investigated in postural [7,15] and respiratory vascular responses [4] that were accompanied by a large change in perfusion pressure to the skin. Since in the present study the subjects kept resting in the supine posture without postural or respiratory stress, our results suggest that both sympathetic and nonneural mechanisms control cutaneous circulation even at normothermic rest.

Limitations. First, since we investigated sympathetic cutaneous vasoconstriction at the dorsum pedis (nonglabrous skin), our data cannot simply be applicable to the sympathetic control of cutaneous microcirculation in glabrous skin, that has different (in some case, higher) sympathetic innervation and anastomoses from nonglabrous skin [1]. Second, since we studied only males subjects, it is unclear whether our conclusion holds true with females. Lastly, since the activation of skin SNA in this study was associated with the decrease in skin blood flow and neither increases in skin blood flow nor sweat rate, the SNA would include vasoconstrictor activity as its dominant component. However, we cannot exclude the possibility that the SNA contained some of other nerve types (i.e., vasodilator, sudomotor and pilomotor activity). The possibility can relate with the observed incomplete coherence between the SNA and skin blood flow.

In conclusion, at normothermic rest, the decoding rule from vasoconstrictor skin SNA to skin blood flow in nonglabrous skin has low-pass filter characteristics with 3–4 s of pure time delay. The vasoconstrictor skin SNA contributes to a half of fluctuation of skin blood flow in the condition.

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References

[1] I.M. Braverman, Ultrastructure and organization of the cutaneous microvasculature in normal and pathologic states, J. Invest. Dermatol. 93 (1989) 2S–9S.
[2] C. Cogliati, R. Magatelli, N. Montano, K. Narkiewicz, V.K. Somers, Detection of low- and high-frequency rhythms in the variability of skin sympathetic nerve activity, Am. J. Physiol. Heart Circ. Physiol. 278 (2000) H1256–H1260.
[3] S. Durand, S.L. Davis, J. Cui, C.G. Crandall, Exogenous nitric oxide inhibits sympathetically mediated vasoconstriction in human skin, J. Physiol. 562 (2005) 629–634.
[4] S. Durand, R. Zhang, J. Cui, T.E. Wilson, C.G. Crandall, Evidence of a myogenic response in vasomotor control of forearm and palm cutaneous microcirculations, J. Appl. Physiol. 97 (2004) 535–539.
[5] S.A. Glantz, Primer of Biostatistics, 4th ed., McGraw-Hill, 1997.
[6] K.E. Hagbarth, R.G. Hallin, A. Hongell, H.E. Torebjork, B.G. Wallin, General characteristics of sympathetic activity in human skin nerves, Acta Physiol. Scand. 84 (1972) 164–176.
[7] H. Jepsen, P. Gaehtgens, Postural vascular response in human skin: passive and active reactions to alteration of transmural pressure, Am. J. Physiol. 265 (1993) H949–H958.
[8] J.M. Johnson, W.F. Taylor, A.P. Shepherd, M.K. Park, Laser-Doppler measurement of skin blood flow: comparison with plethysmography, J. Appl. Physiol. 56 (1984) 798–803.
[9] A. Kamiya, T. Kawada, K. Yamamoto, D. Michikami, H. Ariumi, T. Miyamoto, S. Shimizu, K. Uemura, T. Aiba, K. Sunagawa, M. Sugimachi, Dynamic and static baroreflex control of muscle sympathetic nerve activity (SNA) parallels that of renal and cardiac SNA during physiological change in pressure, Am. J. Physiol. Heart Circ. Physiol. 289 (2005) H2641–H2648.
[10] T. Kawada, T. Shishido, M. Inagaki, T. Tatewaki, C. Zheng, Y. Yanagiya, M. Sugimachi, K. Sunagawa, Differential dynamic baroreflex regulation of cardiac and renal sympathetic nerve activities, Am. J. Physiol. Heart Circ. Physiol. 280 (2001) H1581–H1590.
[11] T. Mano, Microneurography as a tool to investigate sympathetic nerve responses to environmental stress, Aviakosmicheskaia i Ekologicheskaia Meditsina 31 (1997) 8–14.
[12] L.B. Rowell, Human Cardiovascular Control, Oxford Univ. Press, 1993, pp. 3–161.
[13] J.L. Saumet, D.L. Kellogg Jr., W.F. Taylor, J.M. Johnson, Cutaneous laser-Doppler flowmetry: influence of underlying muscle blood flow, J. Appl. Physiol. 65 (1988) 478–481.
[14] J. Sugenoya, S. Iwase, T. Mano, Y. Sugiyama, T. Ogawa, T. Nishiyama, N. Nishimura, T. Kimura, Vasodilator component in sympathetic nerve activity destined for the skin of the dorsal foot of mildly heated humans, J. Physiol. 507 (Pt 2) (1998) 603–610.
[15] S.F. Vissing, N.H. Secher, R.G. Victor, Mechanisms of cutaneous vasoconstriction during upright posture, Acta Physiol. Scand. 159 (1997) 131–138.
[16] B.G. Wallin, J. Fagius, Peripheral sympathetic neural activity in conscious humans, Annu. Rev. Physiol. 50 (1988) 565–576.