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RESEARCH ARTICLE

Countdown before voluntary exercise induces muscle vasodilation with baroreflex-mediated decrease in muscle sympathetic nerve activity in humans

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Manabe K, Masuki S, Ogawa Y, Uchida K, Kamijo Y, Kataoka Y, Sumiyoshi E, Takeda Y, Aida T, Nose H. Countdown before voluntary exercise induces muscle vasodilation with baroreflex-mediated decrease in muscle sympathetic nerve activity in humans. *J Appl Physiol* 128: 1196–1206, 2020. First published April 2, 2020; doi:10.1152/japplphysiol.00523.2019.—We examined whether a countdown (CD) before voluntary cycling exercise induced prospective vascular adjustment for the exercise and, if so, whether and how muscle sympathetic nerve activity (MSNA) was involved in the responses. Young men performed voluntary cycling in a semirecumbent position ($n = 14$) while middle cerebral artery blood flow velocity (V_{MCA}; Doppler ultrasonography), heart rate (HR), arterial pressure (AP; finger photoplethysmography), oxygen consumption rate ($\dot{V}O_2$), oxygen saturation in the thigh muscle (St_{O_2}; near-infrared spectrometry), cardiac output (CO; Modelflow method), and total peripheral resistance (TPR) were measured (*experiment 1*). Another group underwent the same exercise protocol but used only the right leg ($n = 10$) while MSNA (microneurography) was measured in the peroneal nerve of the left leg (*experiment 2*). All subjects performed eight trials with a ≥5-min rest between trials. In four trials randomly selected from the eight trials, exercise onset was signaled by a 30-s CD, whereas in the remaining four trials, exercise was started without CD. We found that CD first increased V_{MCA}, HR, CO, and mean AP, and then decreased TPR and increased St_{O_2} and $\dot{V}O_2$ (*experiment 1*; all $P < 0.021$). Furthermore, the CD-induced increase in mean AP decreased total MSNA and burst frequency (*experiment 2*; both $P < 0.048$) through the baroreflex, with decreased TPR and increased St_{O_2} (*experiment 2*; both $P < 0.001$). The vasodilation and increased $\dot{V}O_2$ continued after the start of exercise. Thus CD before starting exercise induced the muscle vasodilatory response with a concomitant reduction in MSNA through the baroreflex to accelerate aerobic energy production after the start of exercise.

NEW & NOTEWORTHY Prospective cardiovascular adjustment occurs before starting voluntary exercise, increasing heart rate and arterial pressure followed by muscle vasodilation; however, the precise mechanisms and significance for this vasodilation remain unknown. We found that during the countdown before starting exercise cerebral blood flow velocity increased, followed by increases in heart rate and arterial pressure, which suppressed MSNA through baroreflex, resulting in thigh muscle vasodilation to increase oxygen consumption rate, which might make it easier to start exercise.

baroreflex; muscle blood flow; muscle sympathetic nerve activity; prospective cardiovascular adjustment; voluntary exercise

INTRODUCTION

It has been suggested that central pressor responses and concomitant muscle vasodilation occur when human subjects intend and/or imagine starting voluntary exercise (10, 14, 24, 28). However, it remains unknown how these mechanisms occur sequentially after potential increase in cerebral activity (15). In addition, there have been few studies experimentally reporting any merits of these responses after starting voluntary exercise by accelerating aerobic energy production in the contracting muscles.

These topics have been studied in human subjects by focusing on the cardiovascular responses to mental stress, e.g., arithmetic calculation (3, 5, 12). This linkage between mental stress and prospective cardiovascular adjustment has been considered one of the "fight or flight" mechanisms engaged when human beings encounter threats or enemies (16). Experimentally, it has been suggested that the central pressor responses of rapid increases in heart rate (HR) and arterial pressure (AP) occur during mental stress (12) and that the responses are accompanied by forearm muscle vasodilation (5, 12, 27); however, the proposed mechanisms for the vasodilation have been controversial. Halliwill et al. (12) suggested that a reduction of muscle sympathetic nerve activity (MSNA) was involved through α-adrenergic receptors in the muscle vasculature, whereas others suggested no reduction in MSNA (5). The first aim of the present study was to assess whether MSNA is involved in muscle vasodilation during mental stress and, if so, whether and how the baroreflex control of MSNA is involved.

Another aim of this study was to assess whether muscle vasodilation occurs in the lower extremities during mental stress. Blair et al. (3) suggested that calf muscle vasodilation occurs during mental stress, whereas others have stated that it does not (5, 27). Regarding the reasons for the inconsistency, we surmise that the mental stress paradigm that they adopted was not as effective in consistently demonstrating the linkage between the stress and the prospective cardiovascular adjustment for voluntary movement. Indeed, Ishii et al. (14) reported that thigh muscle vasodilation occurred when subjects imagined voluntary exercise using the lower extremities without mental stress. These studies suggest that prospective cardiovascular adjustment does not always occur with mental stress. If vasodilation occurred in the lower extremities due to an adequately designed mental stress paradigm, a much higher

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volume of muscles than that in the forearm would be involved in the response so that the oxygen consumption rate ($\dot{V}O_2$) would increase to a sufficiently high level to be detected.

To assess these issues, we continuously measured MSNA in the peroneal nerve, AP, oxygen saturation in the thigh muscle (St_{O_2}) as an index of vasodilation, and $\dot{V}O_2$ in response to a countdown to the start of voluntary exercise in human subjects. The reason for adopting a countdown as an inducer of mental stress was to synchronize the responses among subjects so that we could detect their sequential changes after cerebral activation. Furthermore, we adopted the countdown to a cycling exercise so that subjects could imagine starting the exercise using the lower extremities, which possess a higher circulatory capacity than the upper extremities, to examine any influence of muscle vasodilation on systemic circulation and $\dot{V}O_2$.

We hypothesized that cerebral blood flow would increase first, followed by the pressor response, which would suppress MSNA through the baroreflex so that thigh muscle vasodilation and an increase in $\dot{V}O_2$ would occur. Moreover, the increase in $\dot{V}O_2$ would continue until after the start of exercise to accelerate aerobic energy production in the contracting muscles without delay.

METHODS

Subjects

This study was approved by the Review Board on Human Experiments of the Shinshu University School of Medicine (approval no. 3089), and it conformed to the guidelines of the Declaration of Helsinki. The trial was registered in UMIN (trial registration no. UMIN000016295) on January 21, 2015. After the experimental protocol was fully explained, each subject provided written informed consent before participating in this study. Fourteen and ten healthy young men participated in *experiments 1* and *2*, respectively. Their physical characteristics are summarized in Table 1. All subjects were students at our university who were recreationally active in sports/exercise and were nonsmokers with no histories of illnesses, such as cardiovascular, pulmonary, or intracranial diseases.

Protocol

Experiment 1. We determined the peak oxygen consumption rate ($\dot{V}O_{2peak}$) in all subjects with a graded cycling exercise over at least 3 days before the experiment. The subjects were asked to refrain from consuming caffeine and alcohol and from performing high-intensity exercise for >24 h before the experiment. On the experimental day, the subjects reported to the laboratory at 8:00 AM in a normally hydrated state after fasting for >10 h before the experiment. After emptying their bladders, the subjects were weighed in the nude and then clothed in short pants and shoes. The subjects then entered an artificial climate chamber adjusted to an atmospheric temperature (T_a) of 23 ± 1°C (mean ± range) and a relative humidity (RH) of ~50%. The subjects rested quietly in a semirecumbent position in the contoured chair of a cycle ergometer (Ergomedic 828E; Monark, Vansbro, Sweden) for 60 min while all of the measurement devices were applied.

The subjects then performed a voluntary cycle ergometer exercise at 60 revolutions/min in the semirecumbent position at 50% of their $\dot{V}O_{2peak}$ (~111 W on average), using both legs for 3 min (see protocol, Fig. 1) while middle cerebral artery blood flow velocity (V_{MCA}; Doppler ultrasonography), HR, AP (finger photoplethysmography), $\dot{V}O_2$, and the St_{O_2} in the right thigh muscle (near-infrared spectrometry) were continuously measured. The subjects performed eight trials, intermitted by a ≥5-min rest. In four trials randomly selected from the eight trials, the onset of exercise was signaled by a 30-s countdown (CD+), whereas in the remaining four trials exercise was started with no countdown (CD−). As shown in Fig. 1, in the CD+ condition, the following words were provided before starting exercise: "Thirty sec before starting exercise" at CD 1; "in 15 sec" at CD 2; and "Ten, nine, eight… one. Start exercise" at CD 3. In contrast, in the CD− condition, only the phrase "Start exercise" was provided at the onset of exercise.

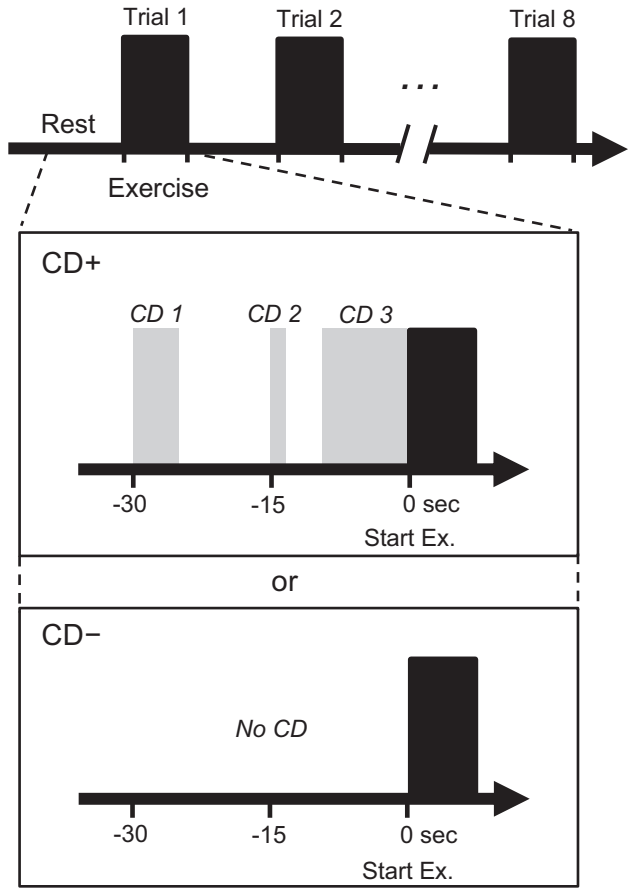

Fig. 1. Experimental protocol. The subjects performed voluntary cycle ergometer exercises using 2 legs (*experiment 1*) or using only the right leg (*experiment 2*) in the semirecumbent position. In all of the experiments, the subjects repeated 8 trials with a ≥5-min rest between trials. In 4 trials randomly selected from the 8 trials, the onset of exercise was signaled by a 30-s countdown (CD+), whereas in the remaining 4 trials, exercise was started without a CD (CD−). In the CD+ condition, the following words were provided before starting the exercise: "30 sec before starting exercise" at CD 1; "in 15 sec" at CD 2; and "10, 9, 8 … 1. Start exercise" at CD 3. In contrast, in the CD− condition the phrase "Start exercise" was provided only at the onset of exercise.

Experiment 2. We determined the $\dot{V}O_{2peak}$ in all subjects except one over at least 3 days before the experiment. The subjects underwent the same exercise protocol as in *experiment 1* but performed the cycling exercise at 60 revolutions/min (30 W) for 1 min in the semirecumbent position using only the right leg while the MSNA in the peroneal nerve of the nonactive (left) leg was continuously measured together with the HR, AP, and the St_{O_2} in the thigh muscle of the active right leg until the start of exercise. We adopted a lower exercise intensity and a shorter duration of exercise in *experiment 2* than in *experiment 1* since in *experiment 2*, we had the subjects not move their left, nonactive leg; as it was the location of the electrode for recording the MSNA, we sought to reduce the risk of the electrode falling off during the exercise.

Measurements

$\dot{V}O_{2peak}$. $\dot{V}O_{2peak}$ was measured with graded exercise using a cycle ergometer in the semirecumbent position at T_a of 23 ± 1°C (mean ± range) and RH of ~50%. After baseline measurements at rest for 3 min, the subjects started pedaling at 60 revolutions/min without loading. The exercise intensity was increased by 60 W every 3 min until it reached 180 W; then it was increased by 30 W every 2 min until it reached 240 W and then by 15 W every 2 min until exhaustion. HR was monitored with an electrocardiogram (ECG). $\dot{V}O_2$ was measured breath by breath from the oxygen and carbon dioxide fractions in the subjects' expired gas and ventilatory volume with a Metamax 3B gas analysis system (Cortex Biophysik, Leipzig, Germany) and then averaged every 15 s and recorded. $\dot{V}O_{2peak}$ was determined by averaging the three largest consecutive values for the 45 s at the end of the exercise.

V_{MCA}. V_{MCA} was measured with a transcranial Doppler ultrasound system (EZ-Dop; Gadelius Medical, Tokyo, Japan). A 2-MHz probe, the head of which was rubbed with an adhesive gel, was placed on the cranial temporal bone window (23). The V_{MCA} was detected according to the standards of practice for probe positioning and orientation (23) while altering the angle between the probe shank and the surface of the temporal bone in order to obtain the maximal Doppler signals from the middle cerebral artery. Then, the angle of the probe was fixed with adjustable headgear to hold the shank during the measurement. Because we could not measure the cerebral artery diameter, the blood flow in the middle cerebral artery was inferred from its velocity, with the assumption that the diameter was constant (1). We used this approach because V_{MCA} measured by transcranial Doppler ultrasound had reportedly been correlated with regional cerebral blood flow measured by single-photon emission computed tomography (22).

HR and AP. HR was monitored as described above. AP was measured beat by beat by finger photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands) and regularly verified by automated sphygmomanometry (STBP model 780B; Colin, Komaki, Japan). We calculated the mean AP (MAP) as diastolic AP (DAP) + [systolic AP (SAP) − DAP]/3. We presented the data from the finger photoplethysmography after correction for a delay time of 1 s as indicated in the system manual.

$\dot{V}O_2$, $\dot{V}CO_2$, and RR. $\dot{V}O_2$, carbon dioxide production rate ($\dot{V}CO_2$), and respiratory rate (RR) were measured with a Metamax 3B gas analysis system (Cortex Biophysik, Leipzig, Germany) as described above.

Near-infrared spectroscopy. We measured oxygenated (Oxy-Hb) and deoxygenated (Deoxy-Hb) hemoglobin of the muscle tissue of the right thigh with a laser tissue oxygenation monitor (BOM-L1 TRW; Omegawave, Tokyo, Japan). We attached the probe to the skin surface 20 cm proximal from the upper end of the kneecap and 5 cm lateral from the midline of the thigh. We measured the oxygenation state at up to 3 cm of depth of the muscle tissue, using the following Beer–Lambert equation:

$$I = \eta I_0 \exp\left[\left(-\alpha V_o - \beta V_d\right)L' - \mu s L\right]$$

where I is the measured photointensity; I_0 the input photointensity; η the coefficient of the system; V_o the Oxy-Hb content per given tissue volume; V_d the Deoxy-Hb content per given tissue volume; α the absorption coefficient of Oxy-Hb; β the absorption coefficient of Deoxy-Hb; L the distance between the laser generator and the photo sensor; L' the distance of the light path; and μs the scattering coefficient of the laser light for a given tissue distance. Since three of the parameters, V_o, V_d, and μs, were not given and the remaining parameters were given by a previous study using model tissue (17), we could determine V_o, V_d, and μs by measuring the three I values corresponding to near-infrared light at three wavelengths: 780, 810, and 830 nm. These values were used to determine St_{O_2} as $V_o/(V_o + V_d)$.

Microneurography. A tungsten microelectrode (microneurography electrodes; FHC, Bowdoin, ME) with an impedance of 4 MΩ at 1 kHz, a 15-to 20-mm length, a <5-μm tip, and a 200- to 250-μm shank diameter was inserted percutaneously into the nerve fascicles of the superficial peroneal nerve at the posterior aspect of the head of the fibula to record multiunit postganglionic MSNA. An Ag-AgCl reference electrode (Vitrode Bs; Nihon Kohden, Tokyo, Japan) was attached on the surface of the skin ~5 cm from the recording electrode. The nerve signal was preamplified 10,000-fold (DAM80; WPI Inc., Sarasota, FL) and passed through a band-pass filter of 700–2,000 Hz. Next, the signal was sent to a loudspeaker and an analog chart recorder and was sent in parallel to a resistance-capacitance circuit to rectify and integrate the signal with a time constant (τ) of 0.1 s. The criteria for identifying an MSNA burst were spontaneous discharges that were *1*) synchronized with the heartbeat, *2*) enhanced by the Valsalva maneuver, and *3*) unchanged in response to cutaneous touch or arousal stimuli (6, 31). We recorded a maximum of eight trials as long as the bursts met these criteria.

Data Acquisition

V_{MCA}, AP waveforms, V_o, V_d, and the rectified and integrated MSNA signals were recorded at a sampling rate of 200 Hz, and an ECG was recorded at 1,000 Hz through an A/D converter using a computerized data acquisition system [AD16-16U (PCI) EH; Contec, Tokyo, Japan].

Analyses

CO and TPR. Cardiac output (CO) was estimated from the AP waveform with the Modelflow method (BeatScope, Amsterdam, The Netherlands), which incorporates age, sex, weight, and height (32). Total peripheral resistance (TPR) was calculated from MAP/CO.

V_{MCA} and MAP response time to CD 1. We calculated the time to peak of V_{MCA} and MAP from CD 1 (ΔT_1 and ΔT_2, respectively) to examine which responded to the CD faster after correcting for the delay time of 1 s from finger photoplethysmography and also for longer pulse wave conduction time from the heart to palmar digital artery by 0.1 s compared with that from the heart to the middle cerebral artery (9, 18, 30).

Baroreflex control of HR. In a previous study using freely moving mice (20), we found that the cerebral blood flow velocity measured by laser-Doppler flowmetry started to increase 2 min before voluntary movement along with increased cerebral activity measured by electroencephalography. These increases were accompanied by the suppression of baroreflex control of HR with increased (less negative) cross-correlation function determined from the HR response to a spontaneous change in MAP. Accordingly, in the present study, we used a cross-correlation function between the HR response (ΔHR) and the corresponding spontaneous change in SAP (ΔSAP) for every 11 sequential cardiac cycles as an index of the current baroreflex control of HR (19, 20). Because changes in SAP and subsequent changes in the duration of the next heartbeat were observed, the ΔSAP and ΔHR were determined from the following formulas:

$$\Delta SAP_n = SAP_n - SAP_{n-1}$$

$$\Delta HR_n = HR_n - HR_{n-1}$$

where n varies from 1 to 11, SAP_n is the SAP occurring during R-R interval$_n$, and HR_n is the HR calculated from R-R interval$_n$. Then, we determined the cross-correlation function at time t as $R(t)$ using 5 pairs of ΔSAP and ΔHR values before and after t, for a total of 11 pairs.

To detect any beat-to-beat changes in the baroreflex control of HR in response to the CD and to assess its relationship with other variables, we used $R(t)$ as an index of the baroreflex control of HR in the present study, whereas the slope of ΔHR/ΔSAP has been generally used in previous studies. We used $R(t)$ because *1*) ΔHR/ΔSAP had been determined only when $R(t)$ was significant, resulting in a ΔHR/

ΔSAP that was not a continuous variable, whereas both R(t) and other variables were determined continuously, and *2*) we previously confirmed that ΔHR/ΔAP was positively correlated with $R(t)$ after it was transformed to $Z_{R(t)}$ in freely moving mice (20). To quantitatively assess the relationship between $R(t)$ and other variables, we transformed $R(t)$ into $Z_{R(t)}$ as follows (8):

$$Z_{R(t)} = \frac{1}{2}\log_e\left[\frac{1+R(t)}{1-R(t)}\right]$$

MSNA. Figure 2 shows typical examples of integrated and total MSNA, together with HR, CO, MAP, $Z_{R(t)}$, TPR, and St_{O_2}, before the start of the cycling exercise with and without a CD during *experiment 2* from one subject. Peaks and leading or trailing edges of each MSNA burst were identified from the trace of the mean voltage neurogram, according to previously published methods (11, 26) after modification (25). The burst amplitude was obtained by subtracting either a leading or trailing edge value, which was less than the other, from the peak value:

$$\text{amplitude} = \text{peak value} - \text{lower edge value}$$

If the amplitude did not exceed the level by twofold more than the baseline fluctuation of a >5-s silent period with no bursts in each trial, they were excluded from the following analyses. For the purpose of quantification, the MSNA was expressed as follows:

$$\text{burst frequency} = \text{burst number/min}\left[\text{bursts/min}\right]$$

$$\text{mean burst amplitude} = \sum_{k=1}^{\text{burst number}} \text{amplitude}(k)/\text{amplitude}(\max)/\text{burst number} \times 100\%\left[\%/\text{burst}\right]$$

$$\text{total MSNA} = \text{mean burst amplitude} \times \text{burst frequency}\left[\%/\text{min}\right]$$

where amplitude (max) is the highest amplitude of the burst at baseline in each trial, assigned a value of 100% to normalize it to the amplitudes of other bursts so that they could be compared between subjects.

Baroreflex control of MSNA. We determined the baroreflex control of MSNA by relating the probability of an MSNA burst occurring to spontaneous changes in DAP according to the standard method (29) after modification for examination during shorter durations. Briefly, we averaged DAP from a period of −120 to −30 s from the start of the exercise to determine the baseline value in individual subjects. Then, we determined the beat-by-beat change in DAP (ΔDAP) from the baseline in every 15-s period: six periods before the CD ($-120 \le t < -105$, $-105 \le t < -90$, $-90 \le t < -75$, $-75 \le t < -60$, $-60 \le t < -45$, $-45 \le t < -30$ s; where t is time) and two periods during the CD ($-30 \le t < -15$, $-15 \le t < 0$ s) in individual subjects. The ΔDAP values for each 15-s period were divided into three bins: ≤0.0, 0.1 to 5.0, and >5.0 mmHg. We calculated the

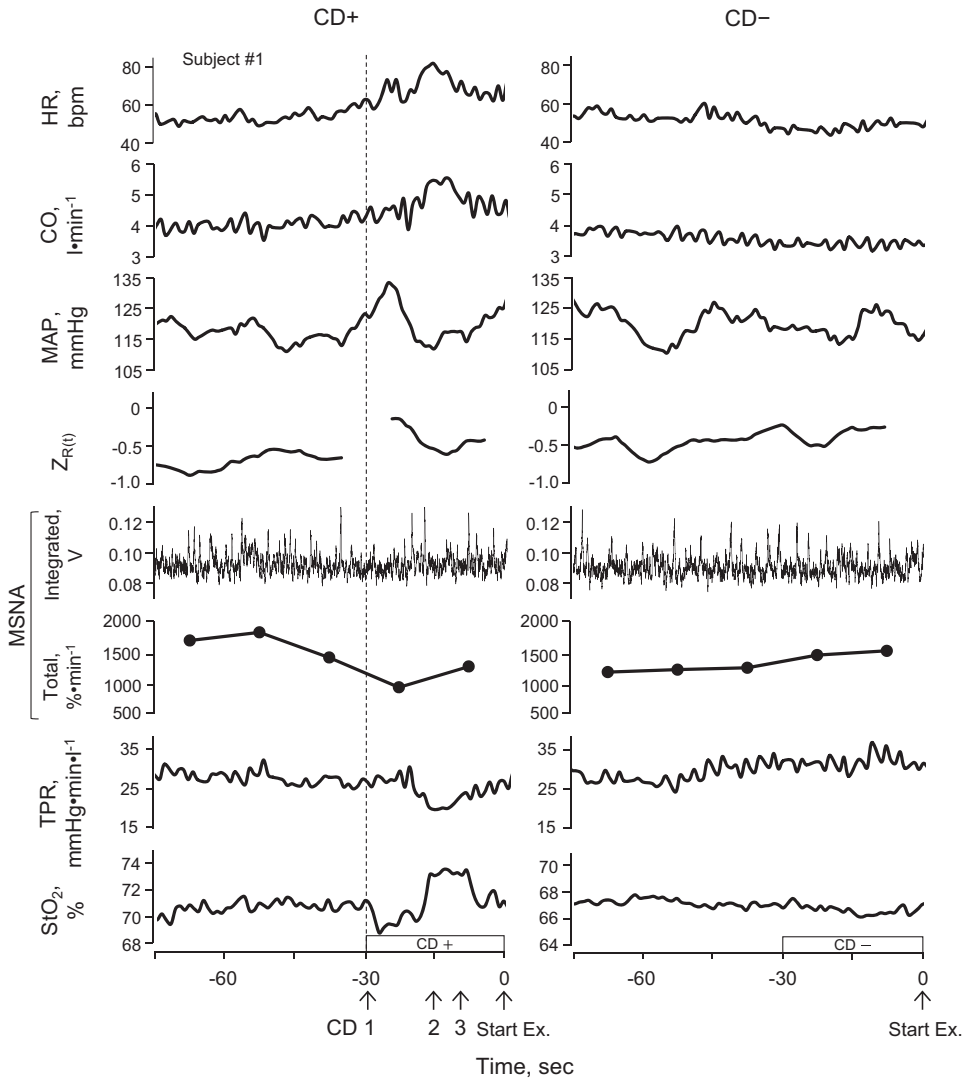

Fig. 2. Typical examples of heart rate [HR, beats per min (bpm)], cardiac output (CO), mean arterial pressure (MAP), transformed cross-correlation function [$R(t)$] between changes in HR and systolic arterial pressure [$Z_{R(t)}$], integrated and total muscle sympathetic nerve activity (MSNA), total peripheral resistance (TPR), and oxygen saturation in the muscle tissue (St_{O_2}) before the start of cycle exercise with (CD+) and without (CD−) a countdown in *experiment 2*. Each subject started the exercise at 0 s. $Z_{R(t)}$ was not determined in the transition phases during the approximately ±5 s from CD 1 and during the approximately ±5 s from exercise onset. The dashed line indicates the start of the CD in the CD+ condition. These variables were averaged as a function of time for the 4 trials in CD− and CD+ in individual subjects and then were presented as the mean and SE values for each experiment in each condition (see Figs. 3 and 4) after confirmation that there were no significant differences in temporal changes in these variables between the first and last trials in each condition.

%MSNA burst occurrence for each bin in every 15-s period from the following equation after considering a 1.2-s delay in an MSNA burst corresponding to a DAP value (29): %MSNA burst occurrence = occurrence of MSNA bursts/number of DAP in each bin × 100. Then, we determined the value before the CD after averaging the values in individual subjects. For the value during the CD, we adopted the values during the first 15-s and latter 15-s periods of the CD separately. The results are summarized in Fig. 5 (see figure legend for more details).

MSNA response to increased MAP. To assess whether a reduction of total MSNA was related to the transient increase in MAP during the first period of CD in the CD+ condition (Fig. 4) as reported in the previous study in which MSNA and MAP responses to mental stress were simultaneously measured (7), we determined the relationship between a peak change in MAP from the baseline (ΔMAP_{peak}, mmHg) and a simultaneous change in total MSNA in individual subjects. The change in total MSNA averaged in the range of ±3.5 s from the time of ΔMAP_{peak} while considering the 1.2-s delayed response of MSNA to the ΔMAP_{peak} (29) and was expressed as a percent change from the baseline (%Δtotal MSNA). Similarly, we determined the relationship between ΔMAP_{peak} and %Δtotal MSNA in the CD− condition.

ΔMSNA vs. ΔTPR and ΔSt_{O_2}. To assess whether a decrease in TPR was related to the reduction of total MSNA after the CD, we determined the relationship between %Δtotal MSNA and %change in TPR from the baseline (%ΔTPR) during the latter period of the CD in the CD+ and CD− conditions in individual subjects. Similarly, to assess whether an increase in St_{O_2} was related to the reduction of total MSNA after the CD, we determined the relationship between %Δtotal MSNA and %change in St_{O_2} from the baseline ($\%\Delta St_{O_2}$) during the latter period of the CD in the CD+ and CD− conditions in individual subjects.

Statistics

One-way analysis of variance (ANOVA) was used to determine any significant differences in physical characteristics of the subjects between *experiments 1* and *2* (Table 1). One-way ANOVA for repeated measures was used to determine any significant differences in the baseline values of V_{MCA}, HR, CO, MAP, $Z_{R(t)}$, MSNA, TPR, St_{O_2}, RR, $\dot{V}O_2$, and $\dot{V}CO_2$ before the CD between the CD+ and CD− conditions (Table 2). The model was also used to examine any significant differences among the baseline values of *trial 1* and those determined by averaging the baseline values for all trials in the CD+ and CD− conditions, for each experiment (Table 2). Furthermore, the model was also used to determine any significant differences in ΔT_1 and ΔT_2 and to examine any significant differences in %MSNA burst occurrence among the baseline periods before the CD and during the CD periods in the CD+ and CD− conditions (Fig. 5). Two-way [CD × time] ANOVA for repeated measures was used to determine any significant differences in V_{MCA}, HR, CO, MAP, $Z_{R(t)}$, TPR, St_{O_2}, RR, $\dot{V}O_2$, and $\dot{V}CO_2$ responses to the CD between the CD+ and CD− conditions in *experiment 1* (Fig. 3) and HR, CO, MAP, $Z_{R(t)}$, MSNA, TPR, and St_{O_2} responses to the CD between the CD+ and CD− conditions in *experiment 2* (Fig. 4), in which subjects and time were treated as repeated factors. Post hoc tests subsequent to ANOVA were performed to determine significant differences in the various pairwise comparisons with the Tukey–Kramer test. A sign test was used to examine any significant effects of ΔMAP_{peak} on %Δtotal MSNA by comparing values for individual subjects in the CD+ condition with those in the CD− conditions. Similarly, the test was also used to examine any effects of %ΔMSNA on %ΔTPR and $\%\Delta St_{O_2}$. P values < 0.05 were considered significant. Values are expressed as means ± standard error (SE) unless otherwise indicated.

RESULTS

As shown in Table 1, there were no significant differences in height, body weight, body mass index, or $\dot{V}O_{2peak}$ between the subjects in *experiments 1* and *2* (all $P > 0.15$).

Table 2 shows the baseline values for the 60 s before the CD of V_{MCA}, HR, CO, MAP, $Z_{R(t)}$, TPR, St_{O_2}, RR, $\dot{V}O_2$, and $\dot{V}CO_2$ for *experiment 1* and those of HR, CO, MAP, $Z_{R(t)}$, MSNA, TPR, and St_{O_2} for *experiment 2*. The values are presented for *trial 1* and for all trials separately. To determine the values for all trials, we averaged the values for the four trials in the CD+ condition and for the four trials in the CD− condition from the eight trials in *experiment 1*. On the other hand, in *experiment 2*, since we completed MSNA measurements in all eight trials for three subjects, in four trials for three subjects, and in two, three, six, and seven trials for one subject each because of the electrode falling off, the number of trials covering the CD+ and CD− conditions varied from one to four depending on the subject. Therefore, in *experiment 2*, we averaged the values for the respective trials in which we successfully determined the MSNA.

As a result, we found that the baseline values of HR, CO, MAP, $Z_{R(t)}$, TPR, and St_{O_2} before the CD in *trial 1* were similar between *experiments 1* and *2*; however, in *experiment 1*, the values of HR, CO, St_{O_2}, RR, $\dot{V}O_2$, and $\dot{V}CO_2$ were increased (all $P < 0.026$) whereas that of TPR was decreased ($P = 0.017$). As a result, we found that the baseline values of HR and CO in the CD+ and CD− conditions and that of St_{O_2} in the CD− condition were higher (all $P < 0.025$), whereas those of TPR in both conditions were lower ($P < 0.030$), in *experiment 1* than in *experiment 2*. However, we found no significant differences in the baseline values between the CD+ and CD− conditions in both experiments (all $P > 0.11$). Additionally, although not shown in Table 2, we found no significant differences in the peak amplitude and burst frequency of the MSNA at baseline between the first trial and the last trial in which we completed MSNA measurement for each subject [0.070 ± 0.009 vs. 0.069 ± 0.002 mV ($P = 0.90$) and 20 ± 0.5 vs. 21 ± 0.4 bursts/min ($P = 0.41$), respectively, for 10 subjects].

Figure 3*A* shows the V_{MCA}, HR, CO, MAP, $Z_{R(t)}$, TPR, and St_{O_2} responses to the CD before and after the start of the cycling exercise in *experiment 1*. The values are expressed as changes (Δ) from the baseline measured from −90 to −30 s from the start of exercise.

Before the start of the exercise, V_{MCA} increased rapidly after CD 1, peaked at −25.7 ± 0.4 s, and then decreased to baseline by −22 s. HR increased sharply by ~4 beats/min compared with the baseline immediately after CD 1 and remained at that level thereafter, the profile of which was similar to that

Table 1. *Physical characteristics of subjects*

	Experiment 1 ($n = 14$)	*Experiment 2* ($n = 10$)
Age, yr	23 ± 1	27 ± 1
Height, cm	174.0 ± 0.9	171.9 ± 2.1
Body weight, kg	64.9 ± 1.8	66.1 ± 2.9
BMI, kg/m^2	21.5 ± 0.5	22.4 ± 0.8
$\dot{V}O_{2peak}$, mL·kg^{-1}·min^{-1}	45.8 ± 2.5	43.8 ± 1.9*

Values are means ± SE for n subjects. BMI, body mass index; $\dot{V}O_{2peak}$, peak oxygen consumption rate. *$n = 9$.

Table 2. *Baseline values before trial 1 and averaged baseline values before all trials*

	Experiment 1 (n = 14)			*Experiment 2* (n = 10)		
	Before *trial 1*	CD+	CD−	Before *trial 1*	CD+	CD−
V_{MCA}, V	0.53 ± 0.03	0.52 ± 0.03	0.52 ± 0.04	NA	NA	NA
HR, beats/min	58 ± 2	67 ± 2*†	69 ± 3*†	58 ± 3	58 ± 3	58 ± 3
CO, L/min	4.6 ± 0.2	5.3 ± 0.2*†	5.4 ± 0.2*†	4.4 ± 0.3	4.4 ± 0.3	4.5 ± 0.3
MAP, mmHg	88 ± 2	90 ± 3	90 ± 3	93 ± 4	97 ± 5	96 ± 4
$Z_{R(t)}$	−0.61 ± 0.15	−0.52 ± 0.12	−0.45 ± 0.14	−0.40 ± 0.14	−0.43 ± 0.15	−0.45 ± 0.16
MSNA						
Burst frequency, bursts/min	NA	NA	NA	20 ± 1	22 ± 2	22 ± 3
Total, %/min	NA	NA	NA	921 ± 24	899 ± 90	965 ± 115
TPR, mmHg·min·L^{-1}	24 ± 1	20 ± 1*†	20 ± 1*†	26 ± 3	27 ± 3	26 ± 3
St_{O_2}, %	67 ± 1	72 ± 1*	73 ± 1*†	68 ± 2	68 ± 2	68 ± 2
RR, breaths/min	17 ± 1	19 ± 1*	19 ± 1	NA	NA	NA
$\dot{V}O_2$, L/min	0.24 ± 0.01	0.28 ± 0.01*	0.28 ± 0.01*	NA	NA	NA
$\dot{V}CO_2$, L/min	0.21 ± 0.01	0.27 ± 0.01*	0.30 ± 0.01*	NA	NA	NA

The baseline values before the countdown (CD) in *trial 1* were calculated by averaging the values from −90 to −30 s in each subject and are presented as the means and SE for n = 14 and 10 subjects in *experiments 1* and *2*, respectively. Similarly, the baseline values before the CD for all trials were calculated by averaging the values from −90 to −30 s in the trial that the subjects completed, and then the mean values were determined for each subject and presented as the means and SE for n = 14 and 10 subjects in *experiments 1* and *2*, respectively. CO, cardiac output; HR, heart rate; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; NA, not applicable; $R(t)$, cross-correlation function between changes in HR and systolic arterial pressure; RR, respiratory rate; St_{O_2}, oxygen saturation in the thigh muscle; $\dot{V}CO_2$, carbon dioxide production rate; $\dot{V}O_2$, oxygen consumption rate; TPR, total peripheral resistance; V_{MCA}, middle cerebral blood flow velocity; $Z_{R(t)}$, transformed $R(t)$. *Significant differences from before *trial 1* at $P < 0.05$. †Significant difference between *experiments 1* and *2* at $P < 0.05$.

of the CO response, although the CO response was slightly delayed. MAP increased immediately after CD 1, peaked at −24.5 ± 0.7 s, and then decreased to baseline by −19 s but increased again gradually at CD 2. Although $Z_{R(t)}$ was not determined in the transition phase for ~11 s, it increased (became less negative) after CD 1, then decreased to baseline by −18 s but increased further at CD 3. TPR decreased gradually after CD 1, reached a minimum at −14 s, and maintained the lower level until the start of exercise. St_{O_2} started to increase gradually from −20 s after CD 1, and the increase became significant at −9 s and remained so thereafter. In contrast, in the CD− condition, these variables remained unchanged from −30 to 0 s. We found that the increases in V_{MCA}, HR, CO, MAP, $Z_{R(t)}$, and St_{O_2} and the decrease in TPR before the start of exercise were greater in the CD+ condition than in the CD− condition, with significant interactive effects of [(CD+ vs. CD−) × time] on these variables (all $P <$ 0.021).

In addition, when we compared the response times of V_{MCA} (ΔT_1) and MAP (ΔT_2) to the CD before the start of exercise, we found that ΔT_1 was faster than ΔT_2 in 9 of 14 subjects, with a significant difference in the mean (P = 0.024).

After the start of exercise, HR and CO increased rapidly, and their significantly higher levels resulting from the CD before the start of exercise remained until 4 and 7 s, respectively, after the start of exercise. Similarly, the decrease in TPR and the increase in St_{O_2} during the CD before the start of exercise remained until ~10 s after the start of exercise. We confirmed that there were significant interactive effects of [(CD+ vs. CD−) × time] on HR, CO, MAP, TPR, and St_{O_2} throughout the periods before and after the start of exercise (all $P <$ 0.0001).

Figure 3*B* shows the RR, $\dot{V}O_2$, and $\dot{V}CO_2$ responses to the CD before the start of exercise in *experiment 1*. The values are expressed as changes (Δ) from the baseline measured from −90 to −30 s from the start of exercise. RR started to increase after CD 1, peaked at −23 s, and then decreased to baseline by −14 s but increased again at CD 3. $\dot{V}O_2$ and $\dot{V}CO_2$ started to increase after CD 1 and then decreased to baseline by −12 s but increased again at CD 3. In contrast, in the CD− condition, these variables remained unchanged from −30 to 0 s. We found that the increases in RR, $\dot{V}O_2$, and $\dot{V}CO_2$ before the start of exercise were greater in the CD+ condition than in the CD− condition (all $P < 0.001$). After the start of exercise, RR, $\dot{V}O_2$, and $\dot{V}CO_2$ increased rapidly, and their significantly higher levels during the CD before the start of exercise remained until ~10, 4, and 4 s, respectively, after the start of exercise. We confirmed that there were significant interactive effects of [(CD+ vs. CD−) × time] on these variables throughout the periods before and after the start of exercise (all $P < 0.01$).

We determined the averaged $\Delta\dot{V}O_2$ in a period from 150 to 180 s, where it reached the plateau demanded by the exercise intensity; we found that it was 1.26 ± 0.05 L/min for the CD+ condition and 1.25 ± 0.05 L/min for the CD− condition, with no significant difference between them (P = 0.27).

Figure 4 shows HR, CO, MAP, $Z_{R(t)}$, MSNA burst frequency, total MSNA, TPR, and St_{O_2} responses to the CD before the start of exercise in *experiment 2*. The values are expressed as changes (Δ) from the baseline measured from −90 to −30 s from the start of exercise, except for MSNA burst frequency and total MSNA. As shown in Fig. 4, these variables, except for the MSNA variables, showed responses similar to those in *experiment 1*. We found that MSNA burst frequency and total MSNA significantly decreased after −30 s in the CD+ condition (P = 0.010 and P = 0.001, respectively), whereas they remained unchanged in the CD− condition (both $P > 0.68$), with significant interactive effects of [(CD+ vs. CD−) × time] on these variables (both $P < 0.048$).

When we analyzed the relationship between ΔMAP_{peak} and %Δtotal MSNA in response to the CD in individual subjects by the sign test, we found that ΔMAP_{peak} was higher for all 10 subjects (P = 0.002) whereas %Δtotal MSNA was lower for 9 of the 10 subjects (P = 0.021) in the CD+ condition than for those in the CD− condition. Moreover, ΔMAP_{peak} was signif-

icantly and inversely correlated with %Δtotal MSNA when all these values were pooled ($r = -0.55$, $P = 0.01$). These results indicate that subjects with the higher ΔMAP_{peak} in the CD+ condition had a greater reduction in total MSNA compared with those in the CD− condition.

In addition, when we analyzed the relationship between %Δtotal MSNA and %ΔTPR or $\%\Delta St_{O_2}$ during the latter period of the CD in individual subjects, we found that in the CD+ condition %Δtotal MSNA was lower for all 10 subjects ($P = 0.002$) and %ΔTPR was lower for 9 of the 10 subjects ($P = 0.021$) whereas $\%\Delta St_{O_2}$ was higher for all 10 subjects ($P = 0.002$) than for those in the CD− condition. Moreover, %Δtotal MSNA was significantly and positively correlated with %ΔTPR ($r = 0.50$, $P = 0.03$) and inversely correlated with $\%\Delta St_{O_2}$ ($r = -0.45$, $P = 0.04$) when all these values were pooled. These results indicate that subjects with larger reductions in MSNA in the CD+ condition had greater decreases in TPR and increases in St_{O_2} compared with those in the CD− condition.

Figure 5 shows the %MSNA burst occurrence in each bin of the ΔDAP during the $-30 \leq t < -15$ s and $-15 \leq t < 0$ s periods of the CD. During the first 15-s period of the CD, we found no significant differences in %MSNA burst occurrence at any bins among the baseline before the CD, CD−, and CD+ conditions (all $P > 0.15$) (Fig. 5*A*), whereas during the latter 15-s period of the CD, we found a significant reduction in %MSNA burst occurrence at the bin of ≤0.0 mmHg in the CD+ condition compared with the baseline ($P = 0.030$) and the CD− condition ($P = 0.006$) (Fig. 5*B*).

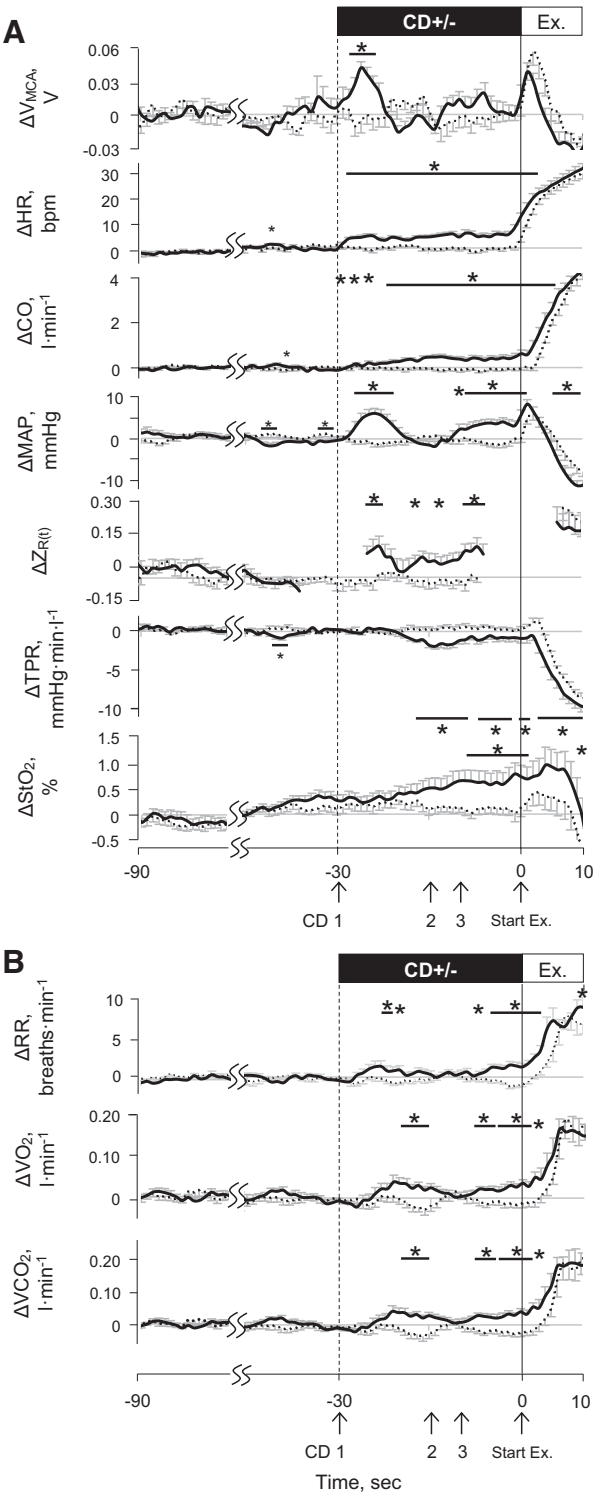

Fig. 3. Cerebral blood flow velocity and cardiovascular and respiratory responses to the CD. *A*: middle cerebral artery blood flow velocity (V_{MCA}), heart rate [HR, beats per min (bpm)], cardiac output (CO), mean arterial pressure (MAP), transformed cross-correlation function [$R(t)$] between changes in HR and systolic arterial pressure [$Z_{R(t)}$], total peripheral resistance (TPR), and oxygen saturation in the muscle tissue (St_{O_2}) before and after the start of the cycle exercise with (CD+) and without (CD−) a countdown in *experiment 1*. Variables are expressed as changes (Δ) from the baseline values determined by averaging the values from −90 to −30 s from the start of exercise. $Z_{R(t)}$ was not determined in the transition phases during the approximately ±5 s from CD 1 and during the approximately ±5 s from exercise onset. The solid line indicates the CD+ condition; the dotted line indicates the CD− condition. *Significant differences from the CD− condition, $P < 0.05$. Mean and SE bars for 14 subjects are presented every 1 s. Significant interactive effects of [(CD+ vs. CD−) × time] were observed in all variables before the start of exercise (all $P < 0.021$) and in HR, CO, MAP, TPR, and St_{O_2} throughout the periods before and after the start of exercise (all $P < 0.0001$). Other symbols are the same as in Fig. 2. *B*: respiratory rate (RR), oxygen consumption rate ($\dot{V}O_2$), and carbon dioxide production rate ($\dot{V}CO_2$) before and after the start of the cycle exercise with (CD+) and without (CD−) a CD in *experiment 1*. Mean and SE bars for 14 subjects are presented every 1 s. Significant interactive effects of [(CD+ vs. CD−) × time] were observed in all variables throughout the periods before and after the start of exercise (all $P < 0.01$). Other symbols are the same as in Fig. 2.

DISCUSSION

This study was unique in continuously measuring MSNA from the peroneal nerve to examine how MSNA was involved in muscle vasodilation for a long period from "before" to "after" starting voluntary cycling exercise initiated by a CD in humans. Regarding the stability and reliability of the MSNA measurements, we confirmed no significant differences in the baseline of the MSNA values between the CD+ and CD− conditions (Table 2) and peak amplitude and burst frequency of the MSNA between the first and the last trial, suggesting that the results obtained from the MSNA analyses were reliable enough. As a result, we found that thigh muscle vasodilation before starting voluntary exercise was caused at least partially by a baroreflex-mediated reduction in MSNA, and these responses were likely induced by an increase in MAP with increased HR (CO) during cerebral activation. In addition, muscle vasodilation was accompanied by an increase in $\dot{V}O_2$.

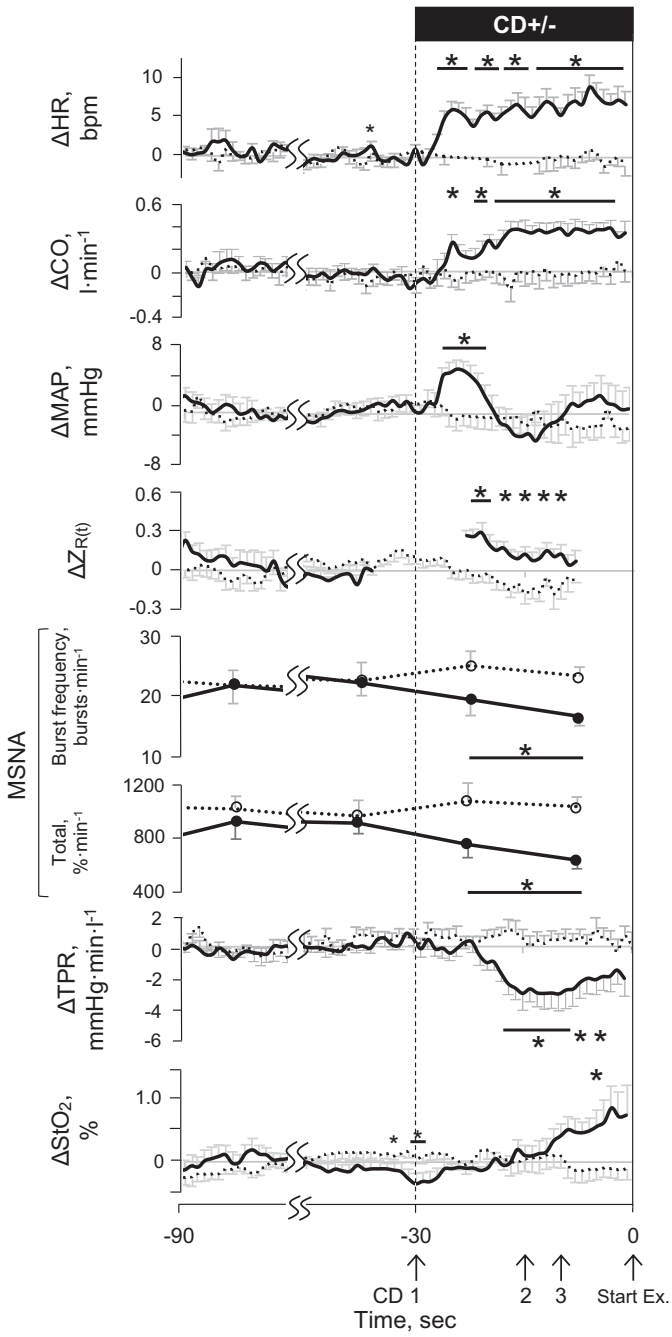

Fig. 4. Heart rate [HR, beats per min (bpm)], cardiac output (CO), mean arterial pressure (MAP), transformed cross-correlation function [$R(t)$] between changes in HR and systolic arterial pressure [$Z_{R(t)}$], muscle sympathetic nerve activity (MSNA) burst frequency and total MSNA, total peripheral resistance (TPR), and oxygen saturation in the muscle tissue (St_{O_2}) before the start of the cycle exercise using only the right leg with (CD+) and without (CD−) a countdown in *experiment 2*. Variables, except for MSNA burst frequency and total MSNA, are presented every 1 s as changes (Δ) from the baseline values determined by averaging the values from −90 to −30 s from the start of exercise. MSNA burst frequency and total MSNA are presented every 15 s. $Z_{R(t)}$ was not determined in the transition phases during the approximately ±5 s from CD 1 and during the approximately ±5 s from exercise onset. The solid line indicates the CD+ condition; the dotted line indicates the CD− condition. *Significant differences from the CD− condition, $P < 0.05$. Mean and SE bars for 10 subjects are presented. Significant interactive effects of [(CD+ vs. CD−) × time] were observed in all variables before the start of exercise (all $P < 0.048$). Other symbols are the same as in Figs. 2 and 3.

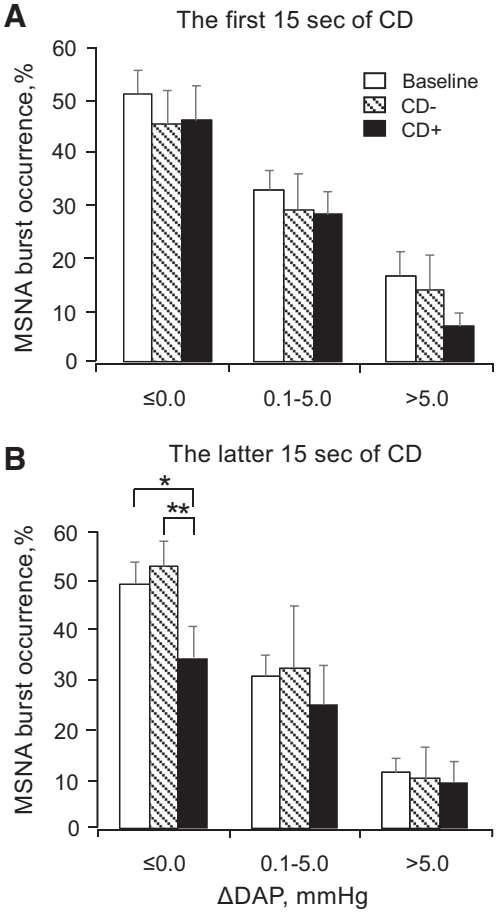

Fig. 5. Percent muscle sympathetic nerve activity (MSNA) burst occurrence in the 3 bins of change in (Δ) diastolic arterial pressure (DAP), ≤0.0, 0.1–5.0, >5.0 mmHg, are shown for the first 15-s period of the countdown (CD) (*A*) and the latter 15-s period of the CD (*B*). The values for 15-s periods before the CD are also shown as baseline values in both *A* and *B* for reference. The white columns indicate the values for the baseline, the hatched columns indicate the values for the condition without a CD (CD−), and the black columns indicate the values for the condition with a CD (CD+). Because the range of DAP varied among individuals, not all of the subjects had DAP values distributed in all of the bins. Therefore, %MSNA burst occurrence was adopted in subjects whose DAP values were available throughout all 3 conditions: the baseline and the CD− and CD+ conditions in each bin. Accordingly, the number of subjects included for the first 15 s of the CD was 10, 10, and 7 and that for the latter 15 s was 9, 8, and 4 in the bins of ≤0.0, 0.1–5.0, and >5.0 mmHg, respectively. The smaller number of subjects in the bin of >5.0 mmHg compared with other bins in the latter period indicates that DAP did not increase by >5.0 mmHg in most subjects after CD 2 (see Fig. 4). Mean and SE bars are presented. *Significant difference between the CD+ and baseline values at $P < 0.05$. **Significant difference between the CD+ and CD− values at $P < 0.01$.

The vasodilation and increased $\dot{V}_{O_2}$ continued until after the start of exercise, leading to accelerated aerobic energy production in the contracting muscle.

V_{MCA} Response to the CD

As shown in Fig. 3*A*, we reconfirmed that V_{MCA} increased at the CD as previously suggested from a voluntary elbow flexion and extension exercise (28). In addition, we found in the present study that the time to obtain the peak values for V_{MCA} was ~1 s faster than that for MAP. This was confirmed after correcting for the delay time from finger photoplethysmogra-

phy and for the longer pulse wave conduction time to the palmar digital artery compared with that to the middle cerebral artery in young people (9, 18, 30). These results suggest that factors other than the increase in MAP, such as cerebral vasodilation with cerebral activation, were involved in the faster increase in V_{MCA} (24). Experimentally, Masuki and Nose (20) measured cerebral activity by electroencephalography and cerebral blood flow velocity by laser-Doppler flowmetry continuously and simultaneously in free-moving mice, and they suggested that the change in the power density ratio of θ- to δ-wave bands, as an index of cerebral activity, was highly synchronized with cerebral blood flow velocity but not with MAP. In the present study, we confirmed this in human subjects.

MAP Response to the CD

As shown in Fig. 3*A*, the increase in MAP before reaching its peak value could be explained by an increase in CO with increased HR. To assess the mechanisms for the increase in HR, we evaluated the baroreflex control of HR using $Z_{R(t)}$ and found that $Z_{R(t)}$ increased (became less negative) significantly due to the CD, although $Z_{R(t)}$ was absent during the transition phase (−35 to −25 s) because of methodological limitations. These results suggest that the baroreflex control of HR was suppressed by the CD. Consistent with these observations, Masuki and Nose (20) reported in free-moving mice that cerebral activity and baroreflex control of HR, determined from $Z_{R(t)}$ (and ΔHR/ΔMAP), were so tightly linked that the baroreflex control of HR was suppressed by cerebral activation to increase HR, which occurred before starting voluntary locomotion. Thus the increase in HR by the CD was likely caused by the suppression of the baroreflex control of HR in response to cerebral activation when human subjects intended to start voluntary exercise.

As shown in Fig. 3*A*, the MAP started to decrease after reaching the peak value at −25 s whereas the elevated levels of HR and CO were maintained. To assess how MSNA was involved in the MAP response during the CD, we analyzed the change in MSNA in relation to changes in the MAP, TPR, and St_{O_2} in another group of subjects in *experiment 2* (Fig. 4). As a result, we found that both MSNA burst frequency and total MSNA, averaged every 15 s, decreased in response to the increase in MAP. Moreover, subjects with the higher ΔMAP_{peak} in the CD+ condition had the greater reduction in total MSNA, suggesting that the reduction in MSNA is mediated by the baroreflex. Similar to our findings, Callister et al. (4) reported that total MSNA from the radial nerve of the arm decreased when arterial pressure increased before starting voluntary leg-cycling exercise, although they did not examine whether cerebral activation and/or muscle vasodilation occurred before starting voluntary exercise. In addition, El Sayed et al. (7) reported that during mental stress ΔMAP_{peak} was greater in subjects who experienced decreases in MSNA burst frequency of the peroneal nerve and suggested that the reduction in MSNA was likely caused by the baroreflex mechanism. These results suggest that the baroreflex accounts for the reduced MSNA in response to the increase in MAP during mental stress or preparation for starting voluntary exercise.

On the other hand, we found that MSNA continued to fall during the latter period of the CD whereas MAP returned to the baseline after reaching the peak value (Fig. 4). To assess this, we evaluated the baroreflex control of MSNA during the CD as done in the previous studies (13, 29). As a result, we found that during the first period of the CD, %MSNA burst occurrence was maintained at the same level as before the CD with a sensitivity of ~-4.5%/mmHg (Fig. 5), similar to those values reported previously in resting subjects (13, 29). On the other hand, during the latter period of the CD, %occurrence of MSNA burst frequency was significantly suppressed in the bin where ΔDAP was lower than or equal to zero, suggesting that the sensitivity of baroreflex control of MSNA was suppressed. These results suggest that the continued decrease in MSNA, despite the return of MAP to the baseline during the latter period of the CD, is partially explained by the suppression of the sensitivity of baroreflex control of MSNA.

The detailed mechanisms for the suppression of baroreflex control of MSNA remain unknown. However, in the present study, we found that the baroreflex control of HR was suppressed immediately after the CD with increased V_{MCA} (Fig. 3*A*). Since it was suggested that the baroreflex control of HR was suppressed during cerebral activation via the cardiovascular center of the medulla (21), it is plausible that this central influence also modulated efferent signals to the peripheral vasculature. Thus the effects of cerebral activation might also be involved in the suppression of baroreflex control of MSNA during the CD. The slightly delayed suppression of baroreflex control of MSNA compared with that of HR might be a defense mechanism against hypotension that would occur when TPR decreases before CO increases.

Taken together, the transient increase in MAP during the first period of the CD was caused by an increase in HR (CO) with increased cerebral activity, resulting in the reduction in MSNA through the baroreflex, the sensitivity of which remained unchanged as that in the baseline. The continued decrease in MSNA in the latter period of the CD despite the return of MAP to the baseline was likely caused by the suppression of the sensitivity of baroreflex control of MSNA. How the reduction in MSNA influenced the peripheral vasculature is discussed in *Effects of the Reduced MSNA on TPR and* St_{O_2}.

Effects of the Reduced MSNA on TPR and St_{O_2}

When we assessed the effects of the reduction in total MSNA on TPR and St_{O_2} during the latter period of the CD in individual subjects, we found that TPR decreased whereas St_{O_2} increased with the reduction in MSNA in 9 and 10 of 10 subjects, respectively, by comparison between the values in the CD+ and CD− conditions. Moreover, subjects with the greater reductions in MSNA in the CD+ condition had the greater decreases in TPR and increases in St_{O_2} when all these values were pooled. These results suggest that the reduction in MSNA during the CD was involved in the vasodilatory responses for each subject. Experimentally, a previous study (14) suggested that the increase in St_{O_2} observed in the thigh muscle paralleled that in femoral blood flow measured by Doppler ultrasound during imagination of the start of voluntary exercise. Indeed, Halliwill et al. (12) suggested that a reduction in arm total MSNA was highly correlated with an increase in forearm vascular conductance, calculated from forearm blood flow measured by plethysmography and MAP, during mental

stress. Taken together, these results suggest that the reduction in MSNA through the baroreflex was likely involved in the vasodilation observed in the present study.

$\dot{V}O_2$ Response to the CD

As shown in Fig. 3, $\dot{V}O_2$ increased as TPR decreased and St_{O_2} increased, suggesting that an increase in oxygen supply to peripheral muscle tissues with increased muscle blood flow increased $\dot{V}O_2$. The major limiting factors for the transfer of oxygen from the blood to the mitochondria in the muscle cells were thought to be the capillary area exposed to the muscle cells and the O_2 pressure gradients from the blood to the mitochondria (2). The finding that the decreased TPR and the increased St_{O_2} were accompanied by an increased $\dot{V}O_2$ suggests that both factors contributed to the increased $\dot{V}O_2$. Importantly, the increased $\dot{V}O_2$ accompanied with an enhanced oxygen transfer mechanism continued for a few seconds after the start of exercise (Fig. 3*B*). On the other hand, there were no significant differences in steady-state $\dot{V}O_2$ after the start of exercise between the CD+ and CD− conditions, suggesting that the energy demanded for a given intensity of exercise was shared more by aerobic energy production for a few seconds after the start of exercise in the CD+ condition than in the CD− condition.

Limitations

As shown in Table 2, the baseline values of the variables before the CD were slightly different between *experiments 1* and *2*. This was due to the difference in the experimental protocols between the experiments. In *experiment 1*, all subjects completed the scheduled eight exercise trials for 3 min with a resting period of ≥5 min between each trial. The baseline values of the measurements before the CD in *experiment 1* were likely influenced by the prior exercise trials. On the other hand, in *experiment 2* not all subjects completed the scheduled eight exercise trials because of the MSNA recording electrode falling off. Moreover, the exercise intensity and duration of the trials in *experiment 2* were lower and shorter than those in *experiment 1*, resulting in a much lesser influence of the trials on the baseline values of the measurements. Experimentally, as shown in Table 2, although the baseline values before the CD in *trial 1* were similar between the experiments, those for all trials in *experiment 1* but not in *experiment 2* were slightly altered by the preceding trials. Despite the limitations, the profiles of the changes in HR, CO, MAP, TPR, and St_{O_2} in response to the CD were similar between the experiments. In addition, regarding potential effects of lower exercise intensity performed in *experiment 2* than *experiment 1* on the reduction in MSNA before stating voluntary exercise, Callister et al. (4) suggested that there were no significant differences in the response among all intensities from 33 to 200 W when subjects intended to start exercise. Therefore, we believed that we could interpret the results by assuming that the physical characteristics of the subjects and the experimental conditions were similar between the experiments.

In conclusion, the presentation of a CD before starting exercise induced a muscle vasodilatory response with a concomitant reduction in MSNA through the baroreflex, which might accelerate aerobic energy production in contracting muscle until after the start of voluntary exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.M., S.M., and H.N. conceived and designed research; K.M., Y.O., K.U., Y.-i.K., Y.K., E.S., Y.T., and T.A. performed experiments; K.M. analyzed data; K.M., S.M., and H.N. interpreted results of experiments; K.M. and S.M. prepared figures; K.M., S.M., and H.N. drafted manuscript; K.M., S.M., and H.N. edited and revised manuscript; K.M., S.M., Y.O., K.U., Y.-i.K., Y.K., E.S., Y.T., T.A., and H.N. approved final version of manuscript.

REFERENCES

1. **Ainslie PN, Hoiland RL.** Transcranial Doppler ultrasound: valid, invalid, or both? *J Appl Physiol (1985)* 117: 1081–1083, 2014. doi:10.1152/japplphysiol.00854.2014.
2. **Bateman RM, Sharpe MD, Ellis CG.** Bench-to-bedside review: microvascular dysfunction in sepsis—hemodynamics, oxygen transport, and nitric oxide. *Crit Care* 7: 359–373, 2003. doi:10.1186/cc2353.
3. **Blair DA, Glover WE, Greenfield AD, Roddie IC.** Excitation of cholinergic vasodilator nerves to human skeletal muscles during emotional stress. *J Physiol* 148: 633–647, 1959. doi:10.1113/jphysiol.1959.sp006312.
4. **Callister R, Ng AV, Seals DR.** Arm muscle sympathetic nerve activity during preparation for and initiation of leg-cycling exercise in humans. *J Appl Physiol (1985)* 77: 1403–1410, 1994. doi:10.1152/jappl.1994.77.3.1403.
5. **Carter JR, Kupiers NT, Ray CA.** Neurovascular responses to mental stress. *J Physiol* 564: 321–327, 2005. doi:10.1113/jphysiol.2004.079665.
6. **Delius W, Hagbarth KE, Hongell A, Wallin BG.** Manoeuvres affecting sympathetic outflow in human muscle nerves. *Acta Physiol Scand* 84: 82–94, 1972. doi:10.1111/j.1748-1716.1972.tb05158.x.
7. **El Sayed K, Macefield VG, Hissen SL, Joyner MJ, Taylor CE.** Blood pressure reactivity at onset of mental stress determines sympathetic vascular response in young adults. *Physiol Rep* 6: e13944, 2018. doi:10.14814/phy2.13944.
8. **Fisher RA.** Frequency distribution of the values of the correlation coefficient in samples from an indefinitely large population. *Biometrika* 10: 507–521, 1915. doi:10.2307/2331838.
9. **Fu X, Huang C, Wong KS, Chen X, Gao Q.** A new method for cerebral arterial stiffness by measuring pulse wave velocity using transcranial Doppler. *J Atheroscler Thromb* 23: 1004–1010, 2016. doi:10.5551/jat.33555.
10. **Gandevia SC, Killian K, McKenzie DK, Crawford M, Allen GM, Gorman RB, Hales JP.** Respiratory sensations, cardiovascular control, kinaesthesia and transcranial stimulation during paralysis in humans. *J Physiol* 470: 85–107, 1993. doi:10.1113/jphysiol.1993.sp019849.
11. **Halliwill JR.** Segregated signal averaging of sympathetic baroreflex responses in humans. *J Appl Physiol (1985)* 88: 767–773, 2000. doi:10.1152/jappl.2000.88.2.767.
12. **Halliwill JR, Lawler LA, Eickhoff TJ, Dietz NM, Nauss LA, Joyner MJ.** Forearm sympathetic withdrawal and vasodilatation during mental stress in humans. *J Physiol* 504: 211–220, 1997. doi:10.1111/j.1469-7793.1997.211bf.x.
13. **Hart EC, Joyner MJ, Wallin BG, Karlsson T, Curry TB, Charkoudian N.** Baroreflex control of muscle sympathetic nerve activity: a non-pharmacological measure of baroreflex sensitivity. *Am J Physiol Heart Circ Physiol* 298: H816–H822, 2010. doi:10.1152/ajpheart.00924.2009.
14. **Ishii K, Liang N, Oue A, Hirasawa A, Sato K, Sadamoto T, Matsukawa K.** Central command contributes to increased blood flow in the noncontracting muscle at the start of one-legged dynamic exercise in humans. *J Appl Physiol (1985)* 112: 1961–1974, 2012. doi:10.1152/japplphysiol.00075.2012.

15. **Jørgensen LG, Perko M, Hanel B, Schroeder TV, Secher NH.** Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia in humans. *J Appl Physiol (1985)* 72: 1123–1132, 1992. doi:10.1152/jappl.1992.72.3.1123.
16. **Joyner MJ, Dietz NM.** Sympathetic vasodilation in human muscle. *Acta Physiol Scand* 177: 329–336, 2003. doi:10.1046/j.1365-201X.2003.01090.x.
17. **Kashima S.** Spectroscopic measurement of blood volume and its oxygenation in a small volume of tissue using red laser lights and differential calculation between two point detections. *Opt Laser Technol* 35: 485–489, 2003. doi:10.1016/S0030-3992(03)00066-5.
18. **Li C, Xiong H, Pirbhulal S, Wu D, Li Z, Huang W, Zhang H, Wu W.** Heart-carotid pulse wave velocity a useful index of atherosclerosis in Chinese hypertensive patients. *Medicine (Baltimore)* 94: e2343, 2015. doi:10.1097/MD.0000000000002343.
19. **Masuki S, Eisenach JH, Dinenno FA, Joyner MJ.** Reduced forearm alpha1-adrenergic vasoconstriction is associated with enhanced heart rate fluctuations in humans. *J Appl Physiol (1985)* 100: 792–799, 2006. doi:10.1152/japplphysiol.00586.2005.
20. **Masuki S, Nose H.** Increased cerebral activity suppresses baroreflex control of heart rate in freely moving mice. *J Physiol* 587: 5783–5794, 2009. doi:10.1113/jphysiol.2009.176164.
21. **Masuki S, Sumiyoshi E, Koshimizu TA, Qian J, Higuchi K, Tsujimoto G, Nose H.** Voluntary locomotion linked with cerebral activation is mediated by vasopressin V1a receptors in free-moving mice. *J Physiol* 591: 3651–3665, 2013. doi:10.1113/jphysiol.2013.251876.
22. **Miyazawa T, Shibata S, Nagai K, Hirasawa A, Kobayashi Y, Koshiba H, Kozaki K.** Relationship between cerebral blood flow estimated by transcranial Doppler ultrasound and single-photon emission computed tomography in elderly people with dementia. *J Appl Physiol (1985)* 125: 1576–1584, 2018. doi:10.1152/japplphysiol.00118.2018.
23. **Nakane M, Nemoto C, Maru H, Murakawa M.** Clinical monitoring of cerebral blood flow velocity with transcranial Doppler sonography. *J Jpn Soc Clin Anesth* 25: 25–32, 2005. doi:10.2199/jjsca.25.25.
24. **Nowak M, Holm S, Biering-Sørensen F, Secher NH, Friberg L.** "Central command" and insular activation during attempted foot lifting in paraplegic humans. *Hum Brain Mapp* 25: 259–265, 2005. doi:10.1002/hbm.20097.
25. **Okada Y, Kamijo Y, Okazaki K, Masuki S, Goto M, Nose H.** Pressor responses to isometric biting are evoked by somatosensory receptors in periodontal tissue in humans. *J Appl Physiol (1985)* 107: 531–539, 2009. doi:10.1152/japplphysiol.91199.2008.
26. **Rudas L, Crossman AA, Morillo CA, Halliwill JR, Tahvanainen KU, Kuusela TA, Eckberg DL.** Human sympathetic and vagal baroreflex responses to sequential nitroprusside and phenylephrine. *Am J Physiol Heart Circ Physiol* 276: H1691–H1698, 1999. doi:10.1152/ajpheart.1999.276.5.H1691.
27. **Rusch NJ, Shepherd JT, Webb RC, Vanhoutte PM.** Different behavior of the resistance vessels of the human calf and forearm during contralateral isometric exercise, mental stress, and abnormal respiratory movements. *Circ Res* 48: I118–I130, 1981.
28. **Sato K, Moriyama M, Sadamoto T.** Influence of central command on cerebral blood flow at the onset of exercise in women. *Exp Physiol* 94: 1139–1146, 2009. doi:10.1113/expphysiol.2009.048587.
29. **Sundlöf G, Wallin BG.** Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *J Physiol* 274: 621–637, 1978. doi:10.1113/jphysiol.1978.sp012170.
30. **The Reference Values for Arterial Stiffness' Collaboration.** Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur Heart J* 31: 2338–2350, 2010. doi:10.1093/eurheartj/ehq165.
31. **Vallbo AB, Hagbarth KE, Torebjörk HE, Wallin BG.** Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev* 59: 919–957, 1979. doi:10.1152/physrev.1979.59.4.919.
32. **Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ.** Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol (1985)* 74: 2566–2573, 1993. doi:10.1152/jappl.1993.74.5.2566.