Central command does not decrease cardiac parasympathetic efferent nerve activity during spontaneous fictive motor activity in decerebrate cats

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Kadowaki A, Matsukawa K, Wakasugi R, Nakamoto T, Liang N. Central command does not decrease cardiac parasympathetic efferent nerve activity during spontaneous fictive motor activity in decerebrate cats. Am J Physiol Heart Circ Physiol 300: H1373–H1385, 2011. First published February 4, 2011; doi:10.1152/ajpheart.01296.2010.—To examine whether withdrawal of cardiac vagal efferent nerve activity (CVNA) predominantly controls the tachycardia at the start of exercise, the responses of CVNA and cardiac sympathetic efferent nerve activity (CSNA) were directly assessed during fictive motor activity that occurred spontaneously in unanesthetized, decerebrate cats. CSNA abruptly increased by 71 ± 12% at the onset of the motor activity, preceding the tachycardia response. The increase in CSNA lasted for 4–5 s and returned to the baseline, even though the motor activity was not ended. The increase of 6 ± 1 beats/min in heart rate appeared with the same time course of the increase in CSNA. In contrast, CVNA never decreased but increased throughout the motor activity, in parallel with a rise in mean arterial blood pressure (MAP). The peak increase in CVNA was 37 ± 9% at 5 s after the motor onset. The rise in MAP gradually developed to 21 ± 2 mmHg and was sustained throughout the spontaneous motor activity. Partial sinoaortic denervation (SAD) blunted the baroreflex sensitivity of the MAP-CSNA and MAP-CVNA relationship to 22–33% of the control. Although partial SAD blunted the initial increase in CSNA to 53% of the control, the increase in CSNA was sustained throughout the motor activity. In contrast, partial SAD almost abolished the increase in CVNA during the motor activity, despite the augmented elevation of 31 ± 1 mmHg in MAP. Because afferent inputs from both muscle receptors and arterial baroreceptors were absent or greatly attenuated in the partial SAD condition, only central command was operating during spontaneous fictive motor activity in decerebrate cats. Therefore, it is likely that central command causes activation of cardiac sympathetic outflow but does not produce withdrawal of cardiac parasympathetic outflow during spontaneous motor activity.

Central command; cardiac vagal and sympathetic efferent nerve activity; tachycardia; exercise; partial sinoaortic denervation

IT REMAINS TO BE SOLVED which of the cardiac autonomic efferent limbs chiefly regulates cardiac rhythm during exercise. Based on indirect estimation by the pharmacological effects of autonomic blockades on the exercise-induced tachycardia and by extrapolation derived from the response of muscle sympathetic nerve activity, it has been proposed that withdrawal of cardiac parasympathetic (vagal) outflow, but not activation of cardiac sympathetic outflow, predominantly contributes to tachycardia at the onset of exercise and throughout a low to moderate intensity of exercise in the absence and presence of an attempted effort with curare (13, 18, 19, 28). However, since the autonomic blockades shift the baseline level of heart rate (HR) and regional sympathetic outflows differentially respond during exercise (21, 29), it is important for better understanding of autonomic regulation of the cardiac function during exercise to directly assess cardiac vagal efferent nerve activity (CVNA) and cardiac sympathetic efferent nerve activity (CSNA). Our laboratory has shown that the increase in CSNA, preceding an increase in HR by 2–5 s, appeared immediately before or simultaneously with the onset of voluntary body movement and during treadmill exercise with a low to moderate intensity in conscious cats (5, 17, 27). These results suggest that cardiac sympathetic outflow plays an important role for the rapid cardiac acceleration at the onset of exercise. On the other hand, the response of CVNA during exercise has never been identified.

With respect to parasympathetic control of HR during exercise, Takahashi et al. (24) have examined the response in HR during voluntary static arm exercise in humans with tetraplegia who lack supraspinal sympathoadrenal control but have intact vagal control. If the conventional assumption for autonomic control of HR during exercise were true, the increase in HR at the onset of static exercise in the tetraplegic subjects with intact vagal efferent limb would be the same as the tachycardia in normal subjects. As a matter of fact, the tachycardia was significantly blunted at the start of static exercise and slowly developed during the later period of exercise in tetraplegic subjects compared with normal subjects (24). In parallel with the slow increase in HR, a high-frequency (HF, at 0.15–0.40 Hz) component of the power spectrum of HR variability, which is known to reflect the size of cardiac parasympathetic outflow, gradually decreased during static exercise (25). The gradual reduction in the HF component of the HR variability power spectrum was almost identical between tetraplegic and normal subjects (25), indicating that cardiac parasympathetic withdrawal during static exercise in tetraplegic subjects was comparable to that in normal subjects. Therefore, we hypothesized that the initial tachycardia at the start of exercise is evoked by the abrupt activation of cardiac sympathetic outflow rather than by cardiac vagal withdrawal. To test the hypothesis, we directly measured the responses of CVNA and CSNA during spontaneous fictive motor activity in unanesthetized, decerebrate cats. We also examined the effects of arterial baroreflex on the cardiac autonomic and cardiovascular responses during spontaneous fictive motor activity by comparing the data between the intact and partially denervated conditions of arterial baroafferents. A part of this study has been preliminarily published (2, 3).

METHODS

The present study was conducted using 10 cats (body wt 2.8 ± 0.4 kg) according to the “Guiding Principles for the Care and Use of Animals in the Fields of Physiological Sciences” approved by the
Physiological Society of Japan. The experimental protocols were approved by the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development, Hiroshima University.

Animal Preparations

We chronically implanted catheters before the experiments in 9 cats; in the remaining one cat, we conducted acute implantation of catheters. They were anesthetized by inhalation of a mixture of N₂O (1.0 l/min) and O₂ (1.0 l/min) with 4% halothane (Fluothane; Takeda Chemical Industries, Osaka, Japan), and then an endotracheal tube was inserted. Subsequently, they inhaled the halothane-N₂O-O₂ mixture through the endotracheal tube during surgery. We continuously monitored electrocardiogram (ECG), HR, rectal temperature, and respiration. HR was derived from the R wave of the ECG with a tachometer (model 1321; GE Marquette Medical Systems, Tokyo, Japan). Rectal temperature was maintained at 37–38°C with a heating pad and an external lamp. To maintain an appropriate level of surgical anesthesia, the concentration of halothane was adjusted in a range of 1.0–2.5% if we observed an increase in HR and/or respiration and/or withdrawal of a limb in response to noxious pinch of the paw and/or a surgical procedure. Polyethylene catheters were inserted in the left external jugular vein for administering drugs and the left carotid artery for measuring arterial blood pressure (AP). The arterial and venous catheters were tunneled subcutaneously and brought to the exterior in the interscapular region. After implantation surgery was completed, antibiotics (benzylpenicillin benzathine, Bicillin tablets, 100,000 U; Banyu Pharmaceutical, Tokyo, Japan) were orally given for successive five to seven postoperative days. After completing a chronic study over 1–2 mo, we started the experiments.

On an experimental day, propofol (Diprivan; AstraZeneka, Osaka, Japan) was intravenously injected with a dose of 11 ± 2 mg/kg as introduction of anesthesia. Subsequently, an endotracheal tube was inserted in the trachea, and the cats inhaled the halothane-N₂O-O₂ mixture through the endotracheal tube during surgery. AP was measured through the carotid artery catheter connected to a pressure transducer (DPTIII; Baxter, Tokyo, Japan). The head of the cat was then mounted on a stereotaxic frame (model SN-2N; Narishige, Tokyo, Japan). Decerebration was performed with an electrocoagulation method at the precculicular-premammillary body level as previously described in detail (6, 14, 20). To do this, a stainless steel electrode, whose insulation was removed 5 mm from the tip, was inserted in the hypothalamus rostral to the mammillary body [from a stereotaxic atlas (23), coordinates from the midpoint of the interaural line were: 13 mm anterior, 6 mm horizontal, and 1–11 mm lateral, with a 14° angle from the perpendicular line]. A negative direct current (1 mA) was passed for 30 s through the electrode. The electrode was withdrawn by 4 mm, and the current was passed again. This procedure was repeated for a total of 42 tracks at 0.5-mm intervals. After the decerebration was completed, the cat was removed from the stereotaxic frame and then placed in the lateral posture. At the end of each experiment, the animal was killed by an overdose of pentobarbital sodium, and the transected area of the brain was examined histologically. From the histological analysis, the transection that started above the dorsal edge of the diencephalon and extended to the optic chiasma through the thalamus and hypothalamus was verified. The cerebral cortex and the rostral parts of the thalamus and hypothalamus (the anterior hypothalamic area, the supraoptic nucleus, and the rostral parts of the lateral and posterior hypothalamic areas) were disconnected from the brain stem as previously reported (20).

Tibial motor nerve activity was measured as a monitor of spontaneous motor drive. The left tibial nerve was exposed in the posterior surface of the thigh and tied peripherally. The tibial nerve was placed on a pair of silver wire electrodes, which was immersed in a liquid paraffin pool surrounded by tissues and skin to prevent the nerve from being dry. Original tibial motor nerve discharge was amplified by a preamplifier (S-0476; Nihon Kohden, Tokyo, Japan) with a band-pass filter of 50–3,000 Hz. The amplified motor nerve activity was rectified and integrated with a resistance-capacitance integrator having a time constant of 20 ms.

Measurements of Cardiac Vagal and Sympathetic Efferent Nerve Activity

The right thoracotomy was performed to remove the first or second to fifth ribs. The right stellate ganglion was carefully exposed with the aid of an operating microscope (OME; Olympus Optical, Tokyo, Japan), and the nerve bundles of the ventral ansa that extend from the stellate ganglion to the thoracic vagus were identified and cut. For recording cardiac sympathetic nerve discharge, a part of the central end of the cut nerve branch or a cardiac sympathetic nerve branch originating from the stellate ganglion to the heart was placed on a pair of Teflon-coated silver wire electrodes (bare diameter, 0.25 mm). Cardiac vagal nerve discharge was measured from a branch of the cardiac vagal nerve that diverged from the right thoracic vagus to the heart; access to the cardiac vagal nerve was previously reported (10, 14). The aygys vein was cut, and the branch of the right cardiac vagus nerve was traced to the right atrium as much as possible. The cardiac vagal branch was cut near the right atrium. For recording cardiac vagal nerve discharge, the central end of the cut vagal nerve branch was placed on a pair of the same electrodes. Cardiac sympathetic and vagal nerves were not glued but they were gently hooked on the recording electrodes, and the nerve-electrode complex was covered with a mixture of Vaseline and liquid paraffin because, in particular, the right cardiac vagal branch was easily damaged by drying and a mechanical manipulation such as pulling and stretching. Cardiac vagal and sympathetic discharges were confirmed by the following criteria: 1) the reduction of CSNA and augmentation of CVNA in response to an increase in AP by intravenously injecting norepinephrine (1–2 μg/kg) and phenylephrine (10–12 μg/kg); 2) the characteristic discharges of CSNA and CVNA synchronized with the AP pulse and respiration-induced fluctuation in AP as previously described in detail (14); and 3) the reciprocal reflex responses in CSNA and CVNA during asphyxia and during mechanical limb stretch. CSNA decreased by 36 ± 20% during asphyxia, whereas CVNA largely increased by 62 ± 16%. In parallel with the reduction in CVNA, HR decreased from 183 ± 10 to 151 ± 12 beats/min during asphyxia; mean arterial blood pressure (MAP) increased from 134 ± 8 to 153 ± 11 mmHg. The reciprocal reflex responses in CSNA and CVNA during mechanical limb stretch were described in RESULTS.

The original multunit discharges of CSNA and CVNA were amplified by a differential preamplifier (S-0476; Nihon Kohden) with a band-pass filter of 50–5,000 Hz. The spikes included in the amplified discharges were converted into standard pulse trains using a digital technique that detected the peaks of the nerve spikes involved in the original signals (16, 31). The pulse trains were integrated continuously with a resistance-capacitance integrator having a time constant of 20 ms. The integrated signals were used as a monitor of CSNA and CVNA. After all surgical and preparatory procedures were completed, inhalation anesthesia was stopped, and pancuronium bromide (1–2 mg; MSD, Tokyo, Japan) was intravenously injected as a muscle relaxant. The lungs were artificially ventilated with a respirator. After allowing a stabilizing period for a few hours, the experiments were started.

Experimental Protocols

Protocol 1: Spontaneous fictive motor activity in the intact condition of arterial baroafferents. The cats decerebrated at the precculicular-premammillary body level were able to induce spontaneous motor activity without any artificial stimulation (6, 20). The cardiac autonomic and cardiovascular responses to spontaneous motor activity...
were measured using four decerebrate cats with the intact arterial baroafferents, which were immobilized with neuromuscular blockade. A total of 89 trials of spontaneous fictive motor activity were obtained, and the average duration of the motor activity was 21 ± 1.5 s. Spontaneous motor activity whose duration was >15 s was selected for analysis, and their average duration of spontaneous motor activity was 29 ± 2.6 s (n = 55 trials).

Protocol 2: Spontaneous fictive motor activity in the partially denervated condition of arterial baroafferents. To examine the effects of arterial baroreflex on the cardiac autonomic responses during spontaneous fictive motor activity, the responses in CSNA and CVNA were measured when arterial baroreceptor input was decreased. Partial sinoaortic denervation (SAD) and left vagotomy were performed by cutting the left cervical vagoaortic nerve complex and crushing bilateral carotid sinus regions. Unfortunately, the right aortic nerve was left intact in the cats because the aortic nerve was usually fused with the cervical vagus nerve and constituted the vagoaortic nerve complex (6, 20). The efficacy of the partial SAD was tested by observing the changes in CSNA and CVNA in response to alterations in AP induced by intravenous injection of norepinephrine (1–2 μg/kg), phenylephrine (10–12 μg/kg), and nitroprusside (3–4 μg/kg). The arterial baroreflex function curves between MAP and CSNA or CVNA were constructed in an individual animal and then were averaged over all animals. The slopes of the average baroreflex function curves were compared between the intact and partially denervated conditions of arterial baroafferents. A total of 164 trials of spontaneous fictive motor activity were obtained from five cats with partial SAD, and the average duration of the motor activity was 30 ± 1.4 s. Spontaneous motor activity, whose duration was >15 s, was selected for analysis, and their average duration of spontaneous motor activity was 33 ± 1.5 s (n = 142 trials).

Protocol 3: Passive stretch of a hindlimb. The right hindlimb was stretched for 60 s by manually extending the hip and knee joints and subsequently by dorsiflexing the ankle joint with careful attention for avoiding any movement of the body trunk. During passive stretch, the hip and knee joints were extended by 17 ± 2.1° and 48 ± 3.6°, respectively, and then the ankle joint was dorsiflexed by 21 ± 9.1° as previously reported (7). The cardiac autonomic and cardiovascular responses to mechanical stretch of the hindlimb were measured in a total of 19 trials from 4 cats with the intact baroafferents.

Data and Statistical Analyses

The data of HR, AP, ECG, CSNA, CVNA, and tibial nerve discharge were simultaneously recorded on an eight-channel pen-writing recorder (8M14; GE Marquette Medical Systems) and also stored in a computer with an analog-to-digital converter (MP 100 or MP150; BIOPACK Systems, Santa Barbara, CA) at a sampling frequency of 1,000 Hz. MAP was calculated from the moving average of the AP data over neighboring 1,000 points sampled. The start of spontaneous motor activity was determined as the time at which the tibial nerve discharge exceeded the baseline fluctuation, and the end of spontaneous motor activity was determined as the time at which the tibial nerve discharge returned within the baseline fluctuation. The average values for 10–30 s before the start of spontaneous motor activity or passive stretch were defined as the baseline levels. The changes in each variable from the baseline level were sequentially calculated every 1 s during spontaneous motor activity and every 3 s during passive stretch. The changes in CSNA and CVNA were expressed as relative percent changes from their baseline levels because the absolute values of CSNA and CVNA varied among the animals. The changes in each variable from the baseline level in an

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**Fig. 1.** The arterial baroreflex responses of cardiac autonomic outflows. Norepinephrine (NEp, 1–2 μg/kg iv) was injected as indicated by arrows. A decrease in cardiac sympathetic efferent nerve activity (CSNA) and an increase in cardiac vagal efferent nerve activity (CVNA) were induced by a pressor response with NEp in the intact condition of arterial baroafferents in A. In contrast, the baroreflex responses of cardiac autonomic outflows were blunted by partial sinoaortic denervation (SAD) in B, although the pressor response with NEp was almost identical between the intact and partial SAD conditions. AP, arterial blood pressure.
individual trial of spontaneous fictive motor activity that lasted for >15 s were aligned at the start or the end of the tibial motor nerve discharge (time = 0 s) and then averaged over all trials.

The time course data of the changes in CSNA, CVNA, HR, and MAP during spontaneous motor activity and during passive stretch were statistically analyzed by a one-way ANOVA. If a significant F-value in the main effect of time was present, a Dunnett’s post hoc test was performed to detect a significant difference in each variable between the baseline level and the value at a given time. The effects of partial SAD on the cardiac autonomic and cardiovascular responses during spontaneous motor activity were analyzed with a two-way ANOVA. Also, the effects of partial SAD on the arterial baroreflex responses in CSNA and CVNA were analyzed with a two-way ANOVA, and the slopes of the baroreflex function curves between MAP and CSNA or CVNA were compared by an unpaired t-test between the intact and partial SAD conditions. The baseline values of CSNA, CVNA, HR, and MAP were also compared by an unpaired t-test between the two conditions. The level of statistical significance was defined as P < 0.05 in all cases. The data are expressed as means ± SE.

RESULTS

Effect of Partial SAD on the Baseline Values and Baroreflex Responses of CSNA and CVNA

The baseline values of CSNA and CVNA in the intact condition of arterial baroafferents were 38 ± 10 and 25 ± 7 impulses/s, respectively. Although partial SAD tended to increase the baseline CSNA to 67 ± 18 impulses/s, the baseline CSNA and CVNA were not significantly affected by partial SAD. Similarly, no significant differences in the baseline HR and MAP were found between the intact and partial SAD conditions (HR, 171 ± 16 vs. 204 ± 5 beats/min; MAP, 139 ± 13 vs. 138 ± 3 mmHg, respectively). However, partial SAD diminished the baroreflex changes of CVNA and CSNA in response to AP increased with norepinephrine (Fig. 1) and blunted the slopes of the baroreflex function curves (Fig. 2). The slope of the MAP-CSNA relationship curve was blunted by partial SAD to −1.0%/mmHg compared with the slope of −3.0%/mmHg in the intact condition; similarly, the slope of the MAP-CVNA relationship curve in the partial SAD condition was 0.4%/mmHg smaller than the slope of 1.8%/mmHg in the intact condition.

The Responses in CSNA and CVNA During Spontaneous Fictive Motor Activity

A typical example of the responses of CSNA, CVNA, HR, AP, and tibial motor nerve discharge during spontaneous fictive motor activity in a decerebrate cat with the intact baroafferents is represented in Fig. 3. There was a rapid increase in CSNA before the onset of spontaneous motor activity, which was followed by rises in HR and AP (Fig. 3A). The increases in CSNA and HR were observed for a short period of 4–5 s before and immediately after the start of the motor activity and were not sustained during the later period of the motor activity. The recovery of CSNA and HR during the motor activity was coincident with the increase in AP. In contrast to the response in CSNA, CVNA slightly increased after the onset of spontaneous motor activity, and the increase in CVNA followed the pressor response and was maintained during the motor activity (Fig. 3B). The rise in AP was maintained throughout the motor activity and returned slowly to the baseline following the cessation of the motor activity.

The time courses of the average responses in CSNA, CVNA, HR, and MAP during spontaneous fictive motor activity are shown in Fig. 4. The increase in CSNA occurred simultaneously with the onset of the motor activity and reached the peak value of 71 ± 12% at 1 s immediately after the motor onset. Thereafter, CSNA returned to the baseline in parallel with the increase in MAP, even though spontaneous motor activity was not ended. Dissimilarly to the CSNA response, CVNA slowly increased by 37 ± 9% at 5 s from the motor onset, and the increase in CVNA was sustained throughout the motor activity. HR increased by 6 ± 1 beats/min at 3 s after the onset of the motor activity, following the increase in CSNA. The rise in MAP was delayed from the onset of the motor activity but sustained throughout the motor activity. The peak rise in MAP was 21 ± 2 mmHg at 10 s after the motor onset. After the cessation of spontaneous fictive motor activity, HR transiently fell 5 ± 1 beats/min below the baseline level. It was of interest that CSNA sometimes decreased immediately after the cessation of spontaneous motor activity, in accordance with the transient reduction in HR, as shown in Fig. 5. MAP gradually returned to the baseline level in 5 s after the end of the motor activity.

The cardiac autonomic and cardiovascular responses during spontaneous fictive motor activity (n = 3 cats) were divided into the three groups according to the duration of motor activity (5–9, 10–19, and 20–29 s), as exemplified in Fig. 5. It was evident that the responses of CSNA and HR were independent of the duration of spontaneous motor activity.
activity, whereas the responses of CVNA and MAP expanded depending on that duration. CSNA and HR increased immediately before and at the start of spontaneous motor activity and thereafter returned near or below the baseline during the later period of the motor activity in association with the rise in MAP. On the contrary, the increases in CVNA and MAP were sustained as long as the spontaneous motor activity continued.

Fig. 3. A typical example of the responses of CSNA, CVNA, heart rate (HR), AP, and tibial motor nerve discharge during spontaneous fictive motor activity in a decerebrate cat with intact baroafferents. In A, CSNA increased before the onset of the motor activity (★), which was followed by rises in HR (★) and AP. In contrast, CVNA slightly increased after the onset of spontaneous motor activity, and the increase in CVNA was maintained during the motor activity in B.
Effect of Partial SAD on the CSNA and CVNA Responses During Spontaneous Fictive Motor Activity

An influence of partial SAD on the responses of CSNA, CVNA, HR, and AP during spontaneous fictive motor activity is exemplified in Fig. 6. CSNA increased immediately before spontaneous fictive motor activity, and this increase in CSNA was sustained during the motor activity (Fig. 6A). On the other hand, partial SAD almost abolished the increase in CVNA observed during the motor activity in the intact condition of arterial baroafferents (Fig. 6B). The increase in HR slowly developed and was sustained during the motor activity, whereas the rise in MAP was enhanced by partial SAD. After the cessation of spontaneous fictive motor activity, CSNA was transiently decreased while CVNA showed a transient increase.

The time courses of the average changes in CSNA, CVNA, HR, and MAP during spontaneous fictive motor activity are shown in Fig. 7. Although partial SAD did not interfere with the occurrence of the rapid increase in CSNA, the peak increase of CSNA became smaller (38 ± 4%) than that in the intact condition. Subsequently, the significant increase in CSNA remained throughout the motor activity. On the other hand, no significant changes in CVNA were found during the motor activity. The initial tachycardia was abolished by partial SAD, although the increase in HR reached the peak of 5 ± 0.4 beats/min at the end of the motor activity. The increase in MAP was delayed but enhanced to 31 ± 1 mmHg by partial SAD. Immediately after the cessation of spontaneous fictive motor activity, CSNA showed a quick drop by 47 ± 4%, whereas CVNA tended to increase although the CVNA increase was not statistically significant. HR and MAP gradually decreased to the baseline control within 10–15 s after the end of the motor activity.

The cardiac autonomic and cardiovascular responses during spontaneous fictive motor activity under the partial SAD (n = 3 cats) were divided into the three groups according to the motor duration (10–19, 20–29, and 30–39 s), as shown in Fig. 8. All of the increases in CSNA, HR, and MAP were
sustained as long as the spontaneous motor activity continued. It was noted that, immediately after the cessation of spontaneous motor activity, the reciprocal responses in CSNA and CVNA were observed in all groups, i.e., a transient decrease in CSNA and a transient increase in CVNA.

**Relationship Between the Changes in MAP and Cardiac Autonomic Outflows**

The relationships between the changes in MAP and CSNA, CVNA, or HR before, during, and after spontaneous fictive motor activity are plotted in scattered diagrams of Fig. 9. A clockwise hysteresis loop was found in the relationship between MAP and CSNA. In the intact baroafferent condition, an upward shift of CSNA during the initial period of the motor activity (at ~2~1 s from the start) was induced, suggesting activation of CSNA by central command. Subsequently, a downward shift of CSNA was recognized in association with a rise in MAP during the mid to late period of the motor activity (at 4 s from the start – the end), suggesting an inhibition of CSNA by the arterial baroreflex. The fundamental characteristics of the hysteresis curve between MAP and CSNA were similar between the intact and partial SAD conditions. Also, there was a clockwise hysteresis in the relationship between MAP and HR in the intact baroafferent condition that was abolished by partial SAD and turned a linear relationship. As a whole, the increase in CVNA during spontaneous motor activity was linearly related to the increase in MAP in the intact baroafferent condition. The regression slope of the MAP-CVNA curve during the motor activity was 1.3%/mmHg, which matched the slope of the resting baroreflex function curve between them (Fig. 2). The linear relationship between MAP and CVNA was greatly attenuated by partial SAD, and the slope of the MAP-CVNA curve became smaller to 0.5%/mmHg, which was comparable to the slope of the resting baroreflex function curve with partial SAD (Fig. 2).

**The Reflex Responses in CSNA and CVNA During Passive Stretch of a Hindlimb**

Passive stretch of a hindlimb transiently increased CSNA by 47 ± 12% at 3 s from the stretch onset, and the increase in...
CSNA was not sustained during the later period of stretch. In contrast, CVNA was gradually decreased during the passive stretch, and the decrease reached a peak value of 40 ± 11% at 36 s from the stretch onset. HR and MAP were increased by 4 ± 1 beats/min and 15 ± 2 mmHg during the stretch, respectively. These cardiac autonomic responses to passive stretch were the same as the previous data reported by our laboratory (14).

**DISCUSSION**

We have studied for the first time the responses of cardiac vagal and sympathetic outflows during spontaneous fictive motor activity using decerebrate cats. The major new findings of the present study are that 1) cardiac sympathetic outflow abruptly increased preceding the tachycardia at the onset of the spontaneous motor activity, and the increase in CSNA lasted only for 4–5 s even though the motor activity was not ended, whereas cardiac vagal outflow increased during spontaneous fictive motor activity, in parallel with a rise in MAP; 2) partial SAD had no significant effect on occurrence of the rapid increase in CSNA at the onset of spontaneous motor activity, even though blunting the increase in CSNA to 53% of the control response; 3) in contrast, partial SAD almost abolished the increase in CVNA during the motor activity; and 4) in the partial SAD condition, the increases in CSNA and HR were sustained throughout the motor activity, whereas the rise in MAP was augmented. Because the cats were immobilized with neuromuscular blockade, afferent input from the skeletal muscle did not contribute to the cardiac autonomic outflow and cardiovascular responses. Therefore, it is postulated that, in the intact baroafferent condition, the cardiac autonomic outflow and cardiovascular responses during spontaneous fictive motor activity were evoked by both central command and arterial baroreflex, whereas, in the partial SAD condition, central command alone evoked them. Taken together, it is likely that central command causes activation of cardiac sympathetic outflow responsible for the cardiac acceleration at the onset of spontaneous motor activity. On the other hand, central command seems not to produce withdrawal of cardiac parasympathetic outflow during spontaneous motor activity, and the increase in CVNA is evoked by the arterial baroreflex instead.

**Neural Mechanisms for the Tachycardia at the Onset of Exercise**

As a neural mechanism responsible for tachycardia at the onset of exercise, it has been considered that withdrawal of cardiac parasympathetic outflow predominantly controls the tachycardia during voluntary exercise that starts immediately before and at the onset of exercise (18, 19). If so, either central command or skeletal muscle mechanoreflex would cause the rapid cardiac vagal withdrawal at the onset of exercise, which may, in turn, produce the rapid tachycardia. With respect to the muscle mechanoreflex, because the previous (14) and present study of our laboratory found that passive stretch of a hindlimb gradually decreased CVNA by 30–40% over a period of 30–36 s, the slow withdrawal of CVNA evoked by stimulation of muscle mechanoreceptors is not sufficient for the rapid acceleration of HR at the onset of exercise. Therefore, central command will be the sole candidate responsible for the rapid vagal withdrawal. When the effect of central command on CVNA during spontaneous fictive motor activity was directly examined in this study, CVNA did not decrease but increased during the motor activity in decerebrate cats with the intact arterial baroafferents, and the increase in CVNA was abolished by partial SAD (Figs. 4 and 7). Thus it is unlikely that central command evokes the rapid cardiac vagal withdrawal in decer-
ebrate cats, and cardiac parasympathetic limb is not responsible for the rapid acceleration of HR at the onset of exercise. This conclusion is supported by previous studies (24, 25), demonstrating that the increase in HR at the onset of static exercise is blunted in tetraplegic subjects with complete cervical spinal cord injury.

In contradiction to the conventional assumption, we consider that cardiac sympathetic outflow plays an important role for the rapid cardiac acceleration at the onset of exercise. In fact, we revealed that CSNA increased immediately before or simultaneously with the onset of spontaneous fictive motor activity in decerebrate cats, in the presence and absence of arterial baroreceptor input (Figs. 4 and 7). As mentioned above, the abrupt increase in CSNA was induced by central command alone. Thus it is suggested that central command may produce a rapid increase in CSNA during voluntary exercise, which may contribute to the rapid tachycardia. In fact, our laboratory has recently reported that CSNA immediately augments at the onset of treadmill exercise in conscious cats (27). The present finding that the response in CSNA is not evoked by exercise pressor reflex but by central command is supported by the recent finding by Tsuchimochi et al. (26) demonstrating that electrical stimulation of the mesencephalic locomotor region rapidly increased CSNA. It is possible that a muscle mechanoreflex during contraction may also produce an increase in HR mediated with a reflex increase in CSNA and a reflex decrease in CVNA because passive stretch of a hindlimb rapidly increased CSNA and slowly decreased CVNA (9, 14, 26).

**Neural Mechanisms for the Changes in HR During the Later Period of and After Exercise**

In the relationships with the change in MAP, downward shifts of CSNA and HR during the mid to late period of spontaneous fictive motor activity may contribute to the rapid tachycardia. In fact, our laboratory has recently reported that CSNA immediately augments at the onset of treadmill exercise in conscious cats (27). The present finding that the response in CSNA is not evoked by exercise pressor reflex but by central command is supported by the recent finding by Tsuchimochi et al. (26) demonstrating that electrical stimulation of the mesencephalic locomotor region rapidly increased CSNA. It is possible that a muscle mechanoreflex during contraction may also produce an increase in HR mediated with a reflex increase in CSNA and a reflex decrease in CVNA because passive stretch of a hindlimb rapidly increased CSNA and slowly decreased CVNA (9, 14, 26).

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ward shifts of CSNA and HR and the upward shift of CVNA were almost abolished by partial SAD. Taken together, it is suggested that the decreases in CSNA and HR and the increase in CVNA during the mid to late period of spontaneous motor activity are chiefly evoked by the arterial baroreflex. It is interesting that the increase in CSNA, which was sustained throughout the motor activity in the partial SAD condition, cannot be explained by both the arterial baroreflex and the exercise pressor reflex, and it must be evoked by central command alone. Taken together, it is evident that a balance between the excitatory input from central command and the opposing inhibitory input from the arterial baroreflex will determine the level of CSNA during spontaneous fictive motor activity. On the other hand, the increase in CVNA during the mid to late period of the motor activity was almost abolished by partial SAD, suggesting that the increase in CVNA during spontaneous fictive motor activity is induced by the arterial baroreflex in association with the rise in MAP.

The transient increase in CSNA at the onset of spontaneous fictive motor activity may explain centrally induced sympathetic regulation of cardiac rhythm during voluntary static exercise in conscious cats because renal sympathetic outflow and HR increase transiently at the beginning of exercise with the same time course as CSNA and HR during the spontaneous motor activity (8). On the other hand, regarding dynamic exercise, our laboratory has reported that both CSNA and HR remained elevated throughout treadmill exercise in conscious cats (27). Central command and/or exercise pressor reflex may contribute to the maintained CSNA and HR during dynamic exercise (9, 26). Furthermore, the sustained tachycardia is determined not only by the augmented CSNA but also by an increase in circulating epinephrine and/or a progressive withdrawal of cardiac parasympathetic nerve activity because adrenal epinephrine contributes to a further increase in HR in ~13 s from the onset of dynamic exercise in rats (30), and HR is able to increase to the same peak level during the later period of exercise in tetraplegic subjects as normal subjects (24, 25).

It has been considered that the HR recovery after exercise is predominantly mediated with restoration of CVNA rather than a concomitant reduction in increased CSNA, based on the data from the pharmacological effects of autonomic blockades on the HR recovery (1, 22). However, because the recovery of HR

Fig. 8. The influence of the exercise duration on the cardiac autonomic and cardiovascular responses. The responses during spontaneous fictive motor activity in a cat with the partial SAD were divided into three groups according to the exercise duration. The increases in CSNA, HR, and MAP were sustained throughout the motor activity depending on the exercise duration. CVNA did not change during the motor activity. After the cessation of spontaneous motor activity, a transient decrease in CSNA and a transient increase in CVNA were observed in all groups. The horizontal dotted lines show the baseline levels of individual variables before the motor activity.
following static exercise was more delayed in tetraplegic subjects compared with normal subjects (25), the contribution of a reduction in CSNA to the HR recovery cannot be neglected. Indeed, we found that the reciprocal responses in CSNA and CVNA, i.e., a transient decrease in CSNA and an increase in CVNA, were evoked as soon as spontaneous fictive motor activity was terminated, particularly in the partial SAD condition (Figs. 6 and 7). This finding suggests that central regulation determined the reciprocal responses in cardiac autonomic outflows for the HR recovery. Taking our previous and present findings into consideration, it is likely that the reduction in CSNA immediately after the cessation of spontaneous motor activity may contribute to the rapid recovery of HR in concert with the augmented CVNA.

Interaction Between Central Command and the Arterial Baroreflex at the Onset of Exercise

Matsukawa et al. (6) and Sadamoto and Matsukawa (20) reported that SAD blunted the centrally induced tachycardia at the onset of spontaneous overground locomotion in decerebrate cats but exaggerated the increases in renal sympathetic outflow and MAP during the locomotion. The findings led to an idea that central command inhibits selectively the gain of the cardiac component of arterial baroreflex at the beginning of exercise, which in turn contributes to the rapid cardiac acceleration. This idea is supported by our laboratory’s further evidence that the baroreflex bradycardia induced by stimulating the aortic depressor nerve (ADN) was temporarily attenuated before or at the onset of voluntary static exercise in conscious cats and at the onset of spontaneous muscle contraction in decerebrate cats, whereas the ADN stimulation-induced depressor response was not affected (4, 15). Neither electrically evoked static contraction nor passive stretch of skeletal muscle in decerebrate cats affected the baroreflex bradycardia (15), indicating that a muscle reflex from contracting muscle does not contribute to the blunted baroreflex bradycardia during exercise. An inhibition by central command of the cardiac component of arterial baroreflex may cause either an increase
in CSNA or a decrease in CVNA to produce the rapid tachycardia at the onset of exercise.

In this study, CSNA substantially increased before and at the onset of spontaneous motor activity while CVNA showed no reduction at that period (Fig. 4). Thus it is conceivable that the central command-induced inhibition of the cardiac component of arterial baroreflex evoked stimulation of cardiac sympathetic outflow but not cardiac vagal withdrawal. If arterial baroafferent input is reduced in the partial SAD condition, the centrally induced disinhibition on the baroreflex circuit will become invalid for full development of the increases in CSNA and HR. Indeed, the peak increase in CSNA at the onset of spontaneous fictive motor activity was decreased by the partial SAD. Therefore, the tachycardia at the onset of exercise is mediated by stimulation of cardiac sympathetic outflow, which is partly explained by the temporal inhibition of the cardiac component of the arterial baroreflex.

Limitations

There are some limitations in the present study. First, the right cardiac vagal branch, with which CVNA was recorded, was amputated to eliminate contamination of cardiac afferent discharge in the measurement of CVNA. Also, the cardiac sympathetic nerves diverging from the right stellate ganglion to the thoracic vagus were cut to avoid contamination of cardiac sympathetic discharge as well. Because the efferent neural signal from the central nervous system to the sinus node via the cardiac vagal branch and sympathetic nerves was partly interrupted, the increase in HR during spontaneous fictive motor activity might be underestimated. Second, afferent input from the skeletal muscle did not contribute to the cardiac autonomic outflow and cardiovascular responses in this study because neuromuscular blockade was used to accomplish stable measurement of CVNA. Based on the previous investigations, it is likely that both central command and exercise pressor reflex are responsible for regulation of cardiac autonomic outflows and cardiovascular function during voluntary exercise in humans and conscious animals (12). The contribution of central command to the responses in cardiac autonomic outflows was estimated with fictive locomotion using unanesthetized, decerebrate cats in this study. However, we have not answered yet to what extent CVNA is influenced by the exercise pressor reflex because of mechanical and metabolic stimulation of muscle thin afferent endings in contracting skeletal muscle, although McMahon and McWilliam (11) suggested that exercise pressor reflex may increase HR at the onset of contraction via rapid vagal withdrawal. More importantly, the response in CVNA during actual voluntary exercise remains to be studied.

In conclusion, it is likely that the rapid increase in CSNA plays an important role in evoking the cardiac acceleration at the beginning of exercise. Central command may increase CSNA via a direct action on the vasomotor centers and via a temporal inhibitory action on the arterial baroreflex circuit, whereas central command does not impose cardiac vagal withdrawal, at least during spontaneous fictive motor activity in unanesthetized, decerebrate cats.

DISCLOSURES

No conflicts of interest are declared by the authors.

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