Augmented renal sympathetic nerve activity by central command during overground locomotion in decerebrate cats

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Matsukawa, Kanji, Jun Murata, and Tetsuya Wada. Augmented renal sympathetic nerve activity by central command during overground locomotion in decerebrate cats. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1115–H1121, 1998.—We examined whether the cerebrum is essential for producing the rapid autonomic adjustment at the onset of spontaneous overground locomotion. Renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP), heart rate (HR), and electromyogram of the forelimb triceps brachialis were measured when freely moving, decerebrate cats spontaneously produced overground locomotion, supporting body weight. Decerebration was performed at the level of the precollicular-premammillary body. RSNA increased 95 ± 14 impulses/s (68 ± 10% of baseline value) at the onset of spontaneous locomotion, which was followed by rises in MAP and HR (7 ± 1 mmHg and 18 ± 2 beats/min, respectively). Concomitantly with the MAP rise, RSNA declined toward control values and then increased again during the subsequent period of locomotion. The same rapid increase in RSNA at the onset of locomotion was observed after sinoaortic denervation and vagotomy. It is concluded that some central site(s), other than the cerebrum and the rostral part of the diencephalon, can generate the centrally induced autonomic adjustment at the onset of spontaneous overground locomotion, which is independent of arterial baroreceptor and vagal afferents.

heart rate; arterial blood pressure; cerebrum; sinoaortic denervation; vagotomy

RENAL SYMPATHETIC NERVE activity (RSNA) and heart rate (HR) increase immediately before or at the onset of voluntary static exercise and dynamic treadmill exercise in conscious animals (10, 15). From these rapid autonomic and cardiovascular adjustments, it has been hypothesized that central descending signals originating from higher brain centers, called central command, increase sympathetic nerve activity, HR, and arterial pressure (AP) at the beginning of voluntary exercise (13). However, the underlying neural mechanisms responsible for generating the descending signals are little known; in particular, the central origin and the neural pathways from the origin to the sympathetic nervous system remain to be resolved. Although the cerebral cortex is believed to have an important role in controlling somatic movements during voluntary exercise, it was unknown whether the cerebrum is also essential for the autonomic and cardiovascular adjustments at the beginning of voluntary exercise. In fact, it has been reported that several sites of the diencephalon and the brain stem outside the cerebrum can produce both somatomotor behaviors and cardiovascular adjustments. Chemical stimulation of neurons in the hypothalamus involving the posterior hypothalamus, the lateral hypothalamus, and a part of the field of Forel, which is called the “locomotor area,” induced both cardiorespiratory changes and locomotor movements (4, 5, 19, 21). Chemical stimulation of neurons in the localized areas of the hypothalamus and the midbrain periaqueductal gray matter, which are called the “defense area,” was capable of producing both cardiovascular changes and defense body movements (1, 2, 8). It is possible that these sites in the diencephalon and the brain stem are responsible for generating central command. We hypothesized that some central site(s) outside the cerebrum can generate a descending signal that produces the rapid autonomic and cardiovascular adjustments associated with spontaneous overground locomotion.

To examine the aforementioned hypothesis, we (16) recently analyzed the time course of the responses in HR and AP at the onset of overground locomotion in freely moving cats that were decerebrated at the precollicular-premammillary body level. These decerebrate cats could induce spontaneous locomotion supporting body weight without any artificial stimulation in the same way as intact, conscious cats. HR began to increase immediately before the onset of electromyogram (EMG) activity of the forelimb triceps brachialis muscle, and AP began to rise almost simultaneously with the EMG onset. This evidence supported our hypothesis that the cerebrum and the rostral part of the diencephalon are not essential for producing the rapid cardiovascular adjustment at the beginning of spontaneous overground locomotion. However, the response in sympathetic effector nerve activity during spontaneous overground locomotion was lacking. To verify our hypothesis further on the basis of sympathetic outflow, it was important to identify the direct response in sympathetic effector nerve discharge. The present study was, therefore, undertaken to determine RSNA during spontaneous overground locomotion in decerebrate cats with and without sinoaortic denervation (SAD) and vagotomy.

METHODS

Preparation. The experiments were performed on seven cats, weighing between 2.0 and 3.5 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. Surgery was conducted for decerebration and implantation of catheters and electrodes. Atropine sulfate (0.5 mg) was given intramuscularly as preanesthetic...
medication to reduce salivation and bronchial secretions. Anesthesia was induced by inhalation of a mixture of halothane (4%), N₂O, and O₂, and then an endotracheal tube was inserted. The cats breathed spontaneously during surgery. Electrocardiogram (ECG), HR, and respiration were continuously monitored. To maintain the level of surgical anesthesia, the concentration of halothane was increased in a range of 1.0–2.5% if an increase in HR and/or respiration and/or withdrawal of the limb in response to noxious pinch of the paw and/or the surgical procedure was observed. Polyvinyl catheters were inserted into the left external jugular vein for administering drugs and into the left carotid artery for measuring AP. A pair of Teflon-coated silver wire electrodes were implanted under the skin of the left chest for monitoring ECG. Rectal temperature was maintained at 37–38.5°C with a heating pad. The electrodes and both arterial and venous catheters were tunnelled subcutaneously and exteriorized at the back of the neck. The head of the cat was then mounted on a stereotaxic frame. Decerebration was performed by electrocoagulation at the precollicular-premammillary body level as described before (16, 20). To do this, a stainless steel electrode with insulation removed 5 mm from the tip was inserted into the hypothalamus rostral to the mammillary bodies (coordinates from the bregma: anterior 13 mm, lateral 6 mm, vertical 11 mm). A negative direct current (1 mA) was passed through the electrode for 30 s. 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. From histological analysis, the cerebrum and the rostral part of the hypothalamus (anterior hypothalamic area, supraoptic nucleus, and rostral part of lateral hypothalamic area) were disconnected from the brain stem and the caudal part of the hypothalamus (posterior hypothalamic area, caudal part of lateral hypothalamic area, and ventromedial nucleus of hypothalamus) was intact (16).

The left kidney was retroperitoneally exposed. With the use of an operating microscope (OME, Olympus), a renal nerve bundle was carefully isolated from the renal plexus and surrounding connective tissue near the renal artery and vein. A pair of silver wire electrodes for recording RSNA were inserted into the renal nerve bundle, and the electrode was connected to the renal artery catheter connected to a pressure transducer (DPT III, Baxter). HR was derived from the arterial pressure pulse by a differential preamplifier (S-0476, Nihon Kohden) with a horizontal 6 mm, lateral 1–11 mm with an angle of 14° from a perpendicular line; from stereotaxic atlas (Ref. 3). A negative direct current (1 mA) was passed through the electrode for 30 s. 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. From histological analysis, the cerebrum and the rostral part of the hypothalamus (anterior hypothalamic area, supraoptic nucleus, and rostral part of lateral hypothalamic area) were disconnected from the brain stem and the caudal part of the hypothalamus (posterior hypothalamic area, caudal part of lateral hypothalamic area, and ventromedial nucleus of hypothalamus) was intact (16).

After all surgery was finished, the cats were housed in their cages and warmed with a heating pad. Antibiotics (penicillin G, 10,000–20,000 U) were given for 2–5 days after the operation. Water and/or 5% glucose (10–30 ml per day) was given orally to the cats every day, and they were able to swallow the fluid.

Recoding of data. AP was measured through the carotid artery catheter connected to a pressure transducer (DPT III, Baxter). HR was derived from the arterial pressure pulse by a tachometer (1321, NEC Sanei). Original RSNA was amplified by a differential preamplifier (S-0476, Nihon Kohden) 13 mm with a band-pass filter of 50–3,000 Hz. The amplified output was converted into standard pulse trains using a digital technique that detected the peaks of the original signal (14). The pulse trains were integrated by a resistance-capacitance integrator with time constant of 20 ms, and the integrated signal was used as a monitor of RSNA. After the implantation surgery, RSNA was continuously recorded over 6–7 days before and after SAD. Once the parameters of the instruments for recording RSNA were determined on the first day, the same parameters were kept until the end of the experiment. RSNA disappeared after administration of a ganglionic blocker (hexamethonium bromide, 3 mg/kg iv), indicating that RSNA originates from sympathetic postganglionic efferent fibers (11). The original EMG was also amplified and integrated with the same procedure as RSNA to obtain the integrated EMG (iEMG). Timings at the start and the end of spontaneous overground locomotion were manually marked with an electric switch. We also defined the onset and offset of iEMG (see Data treatment). Simultaneous RSNA, AP, HR, ECG, EMG, iEMG, and the marking signal for the start and the end of locomotion were continuously recorded on an eight-channel pen-writing recorder (Recti-8K, NEC Sanei) and stored on an FM magnetic tape-recorder (XR-310, TEAC). RSNA, AP, HR, iEMG, and the marking signal were also sampled at 400 Hz in a computer. The beat-to-beat calculated parameters of RSNA, mean arterial pressure (MAP), HR, and iEMG and their corresponding mean values over 1 s were stored on a hard disk using a customized software program (Cordat II, Data Integrated Scientific Systems, Pinkney, MI) for off-line analysis.

Experimental protocol. The locomotion experiments before SAD were performed 2–4 days after the decerebration surgery. At that time the decerebrate cats could walk spontaneously on all four limbs on the floor. To determine the cats were able to walk at the end of surgery and they were able to walk at the end of surgery. A total of 112 trials of locomotion were observed in seven cats.

SAD was performed in three cats 3–4 days after the decerebration surgery. To eliminate baroreceptor input, bilateral carotid sinus nerves and aortic nerves were identified by direct measurement of baroreceptor activity and then disconnected. When it was difficult to separate the aortic nerve from the cervical vagus, the aortic-vagal complex was cut. The right vagal nerve in two cats and bilateral vagal nerves in one cat were also cut. Two to three days after the second surgery for SAD, the locomotion experiments were conducted again according to the same procedure outlined before SAD. A total of 48 trials of locomotion were analyzed in three cats. As control, a total of 55 locomotion trials were analyzed before SAD and vagotomy in the same cats. SAD was tested by observing the responses in RSNA and HR to intravenous bolus injections of phenylephrine (10–15 µg/kg) and nitroprusside (10–15 µg/kg). Although the drug-induced changes in MAP became greater after SAD than before SAD, the responses in RSNA and HR to the alterations in MAP were small and not significant.

Data treatment. The data in each trial of spontaneous locomotion were displayed on a CRT to define the onset and offset of iEMG. The onset of iEMG was visually determined as the time when iEMG exceeded the maximal value of baseline iEMG obtained during the prelocomotion control period. The onset of iEMG was almost identical to the start of locomotion. The offset of iEMG was determined as the time when iEMG returned below the baseline control value before locomotion. The offset of iEMG was delayed from the end of locomotion, because the cats continued body movements for a while after reaching the wall at the end of the walking passage.

Changes in RSNA, HR, and MAP from prelocomotion values (baseline levels) in an individual trial were aligned at the onset of iEMG and then averaged. Data for RSNA, HR,
and MAP obtained for 20 s before the onset of iEMG were defined as the baseline levels.

Statistical analysis. Changes in RSNA, HR, and MAP during overground spontaneous locomotion were statistically analyzed by using a one-way ANOVA. When a significant F-value in the main effect of time was present, a Dunnett post hoc test was performed to detect a significant difference between the baseline control level and the value at a given time. The effects of SAD on the changes in RSNA, HR, and MAP were analyzed using a two-way ANOVA. The duration of locomotion, the baseline values of RSNA, HR, and MAP, and their peak changes during locomotion observed before and after SAD were compared by an unpaired t-test. The level of statistical significance was defined as P < 0.05. The data are expressed as means ± SE.

RESULTS

Locomotor movements and RSNA response. A typical example of HR, AP, RSNA, and iEMG of the triceps brachialis muscle of the forelimb during spontaneous overground locomotion in a decerebrate cat is shown in Fig. 1. Spontaneous locomotion occurred while the cat was sitting or squatting. When iEMG rapidly increased, the cat stood up and began to step on the floor. The increase in iEMG, which was almost identical to the start of spontaneous locomotion, was followed by rhythmic bursts of iEMG, indicating coordinated overground locomotion. The animal could maintain appropriate posture and body equilibrium during locomotion, supporting its body weight on all four limbs. After the end of locomotion, the elevated iEMG lasted for 10 ± 1.4 s because of continued body movements. This pattern of spontaneous coordinated locomotion was similarly observed before and after SAD and vagotomy. The average duration of locomotion was 14 ± 0.7 s before and 16 ± 2.7 s after SAD and vagotomy.

Augmented RSNA during spontaneous overground locomotion is shown in Fig. 1. This increase in RSNA was induced at the onset of iEMG, which was followed by increases in AP and HR. After the initial increase, RSNA decreased toward the control level and then increased again during the subsequent period of locomotion. It was noted that the decline in RSNA appeared in parallel with the rise in AP. When the cats continued body movements after reaching the wall at the end of the walking passage, RSNA, AP, and HR were further increased. After the cessation of body movements RSNA showed an abrupt drop, whereas AP gradually returned to the baseline level.

RSNA was characterized by synchronized grouped bursts during locomotion as well as during resting, whose interburst interval ranged from ~100 ms to cardiac cycles (250–500 ms) and respiratory cycles (several seconds) (Fig. 1). The increase in RSNA during spontaneous overground locomotion was caused by an increase in the frequency and magnitude of synchronized grouped bursts (Fig. 1). If AP was highly elevated by norepinephrine during resting, RSNA was almost completely inhibited by the arterial baroreflexes (Fig. 2). Subsequently, when spontaneous locomotion was obtained during the period with elevated AP, the response in RSNA to spontaneous locomotion was markedly attenuated.

Time course of RSNA, HR, and MAP during locomotion. The overall baseline values of RSNA, MAP, and HR were 132 ± 25 impulses/s, 101 ± 4 mmHg, and 142 ± 13 beats/min, respectively. RSNA, MAP, and HR were significantly increased during spontaneous locomotion. The time course of the responses in RSNA, MAP, and HR before, during, and after spontaneous locomotion is shown in Fig. 3. At the beginning of spontaneous locomotion, a significant increase in RSNA appeared at the onset of iEMG and reached the maximum value of 95 ± 14 impulses/s at 2 s from the iEMG onset, which corresponded to 68 ± 10% of the baseline control value before locomotion. The rise in MAP started 3 s after the onset of iEMG and reached an early peak of 7 ± 1 mmHg at 4 s. In parallel with the rise in MAP, a decrease in RSNA by 60 impulses/s toward the control value was observed. The increase in HR occurred 1 s after the iEMG onset and reached the peak of 18 ± 2 beats/min at 4 s. RSNA, HR, and MAP remained elevated above the control value during the subsequent period of spontaneous locomotion; after the end of locomotion, the elevated iEMG lasted for 10 ± 1.4 s because of continued body movements.
locomotion, they were further increased because of continued body movements.

Effect of SAD and vagotomy. RSNA, MAP, and HR were continuously measured in the same animals before and 2–3 days after SAD with unilateral or bilateral vagotomy. Baseline RSNA was increased by SAD from the control value of 131 ± 28 impulses/s and remained at the augmented level of 303 ± 112 impulses/s after SAD. Baseline HR was also increased by SAD in this study (151 ± 15 beats/min before SAD vs. 210 ± 15 beats/min after SAD). In contrast, there was no significant difference in the baseline value of MAP before and after SAD (95 ± 7 vs. 87 ± 7 mmHg), although MAP was transiently increased by SAD.

The effect of SAD and vagotomy on the time course of the responses of RSNA, MAP, and HR during spontaneous locomotion is shown in Fig. 4. At the beginning of spontaneous locomotion with SAD and vagotomy, the increase in RSNA occurred at the onset of iEMG and reached the maximum value of 129 ± 14 impulses/s at 1 s from the iEMG onset, which corresponded to 40 ± 5% of the baseline control value before locomotion. The time course and magnitude of the increase in RSNA at the beginning of locomotion were identical with those of the increase in RSNA before SAD and vagotomy (136 ± 25 impulses/s at 2 s from iEMG onset). MAP also started to increase at the beginning of locomotion with the same time course as the rise in MAP before SAD and vagotomy, whereas the early increase in HR was attenuated by the intervention.

In the subsequent period of spontaneous locomotion, SAD and vagotomy had a significant effect on the time course and magnitude of the increases in RSNA, MAP, and HR. It was of interest that RSNA did not show a sharp decrease in parallel with the rise in MAP (Fig. 4). Furthermore, SAD and vagotomy augmented the peak increase in MAP (5 ± 1 mmHg before vs. 12 ± 1 mmHg after SAD and vagotomy) but attenuated the peak increase in HR (26 ± 3 beats/min before vs. 8 ± 1 beats/min after SAD and vagotomy), as demonstrated in Fig. 4. The contrasting effects of SAD and vagotomy on the peak increases in MAP and HR were similarly observed in individual cats.

DISCUSSION

The direct response of sympathetic nerve activity to the kidney during spontaneous overground locomotion was studied before and after SAD and vagotomy using freely moving, decerebrate cats. Our major new finding
These results imply that contamination of bioelectrical noise and/or artificial artifacts, if any, was small in the recording of RSNA during locomotion in this study.

Early studies demonstrated that renal sympathetic discharge, HR, MAP, and respiration increased during spontaneous or electrically induced locomotor activity in nonanesthetized, decerebrate cats (4, 5, 7). Hajduczok et al. (7) reported that the increase in renal sympathetic outflow occurred during locomotion after SAD and vagotomy or during fictive locomotion, suggesting that central command can increase sympathetic drive in the absence of feedback from contracting muscle and arterial and cardiopulmonary baroreceptors. However, the locomotor movements observed in the early studies were substantially different from natural locomotion in conscious cats, because the animals were suspended on a treadmill or table by a stereotaxic device and spinal clamps. This difference in locomotor movement may affect the cardiorespiratory and autonomic responses to locomotion. Moreover, the time course of the cardiovascular and sympathetic nerve responses at the beginning of locomotion was not investigated quantitatively. To examine this further, we analyzed the time course of the response in RSNA during overground locomotion in freely moving, decerebrate cats, which were able to start spontaneous locomotion on the floor, supporting body weight without any artificial stimulation or support. We found that the increase in RSNA occurred at the onset of EMG activity of the forelimb triceps brachialis muscle and peaked by 68% at 2 s from the EMG onset. This abrupt increase in RSNA suggests that the autonomic adjustment at the onset of spontaneous overground locomotion is caused by central descending signals from higher brain centers, i.e., central command, but not by a feedback signal from contracting muscles when a number of limb muscles are recruited to support and propel the body trunk, although it cannot be ignored that a reflex originating from receptors in contracting muscles may modify RSNA after the start of locomotion (9, 12). Because SAD and vagotomy did not affect the time course and magnitude of the increase in RSNA at the beginning of overground locomotion, it is unlikely that the initial increase in RSNA is caused by feedback signals from arterial and cardiopulmonary baroreceptors in agreement with a previous study (7).

The rapid response in RSNA that decerebrate cats produced during spontaneous overground locomotion seems identical to the rapid autonomic adjustment observed during static exercise, eating, and grooming in conscious, intact cats (10, 11). We suppose, therefore, that the cerebrum and the rostral part of the diencephalon are not essential for generating central command responsible for the rapid autonomic adjustment during locomotion in awake animals. On the other hand, chemical stimulation of neurons in the hypothalamic locomotor area induced both cardiorespiratory changes and locomotor movements (4, 5, 19, 21). Neurons in the localized areas of the hypothalamus and midbrain periaqueductal gray matter were capable of producing both cardiovascular changes and defensive body move-
ments (1, 2, 8). These sites of the hypothalamus and the brain stem may be responsible for generating central command to activate renal sympathetic outflow during spontaneous locomotion. Regarding the neural pathway from the central command origin to the sympathetic nervous system, central command may interact with input from arterial baroreceptors on the descending way, because the increase in RSNA during locomotion was inhibited by the arterial baroreflex. It is known that neurons in the rostroventral lateral medulla (RVLM) that project to the spinal cord are inhibited by input from arterial baroreceptors and play a role in the arterial baroreflex arc. Furthermore, neurons in the RVLM receive input from the hypothalamic and midbrain periaqueductal gray defense areas (2, 8). The central command, which is assumed to originate from some sites rostral to RVLM such as the hypothalamus and midbrain periaqueductal gray, may have a synaptic relay in the ventrolateral medulla.

It has been reported that MAP is transiently elevated by SAD but returns to the control level within several days after SAD (17). With the use of long-term recording of RSNA in the same animals, we have shown for the first time that RSNA remained elevated to 231% 2–3 days after SAD and vagotomy, whereas MAP returned to the control level. The recovery of MAP after SAD is in agreement with a previous study (17). This dissociation between the changes in baseline RSNA and MAP after SAD indicates that the recovery of MAP is not caused by a decrease in sympathetic efferent nerve activity due to a central adaptation mechanism. As another explanation of the recovery of MAP, it is conceivable that the vascular response to sympathetic nerve discharge is weakened after SAD because of a peripheral adaptation mechanism. However, this possibility is also unlikely because the response in MAP during locomotion was augmented by SAD and vagotomy. As a third possibility, increasing MAP may induce pressure natriuresis and pressure diuresis in combination with the change in the renin-angiotensin system, which in turn decreases blood volume and cardiac output (6). If so, the recovery of MAP will be accompanied with a gradual decrease in cardiac output. However, the time course of the changes in cardiac output and total peripheral resistance after SAD remains to be resolved.

We think that the arterial baroreceptor-RSNA reflex is operating during spontaneous overground locomotion, because RSNA declined toward the control level when MAP increased during locomotion. The arterial baroreflex may decrease sympathetic discharge and counteract the pressor response. When MAP was raised by norepinephrine and arterial baroreceptors were stimulated, the increase in RSNA during spontaneous overground locomotion was inhibited, suggesting that the increase in RSNA during locomotion is overcome by inhibition from exaggerated input of arterial baroreceptors. On the other hand, if the inhibition of the baroreflex is eliminated, the increase in RSNA during locomotion will be sustained and thereby the pressor response will be enhanced. Indeed, the decline in RSNA that occurred in parallel with the rise in MAP was abolished by SAD and vagotomy. Furthermore, the pressor response during locomotion was augmented after SAD and vagotomy, in agreement with the previous findings during spontaneous overground locomotion in decerebrate cats (16) and during dynamic exercise in conscious dogs (18, 22). Taken together, activation of central command mechanisms resulting in an overall stimulation of the sympathetic nervous system is likely to increase MAP in the initial period of spontaneous locomotion, which in turn may elicit the arterial baroreflexes and counteract the pressor response by inhibiting sympathetic nerve activity in the succeeding period of locomotion.

In conclusion, it is likely that the increase in RSNA obtained at the onset of spontaneous overground locomotion in decerebrate cats is caused by direct descending signals that couple with locomotor activity and not by a reflex arising from the contracting muscle and from arterial and cardiopulmonary baroreceptors. Some central sites(s), other than the cerebrum or the rostral part of the diencephalon, can generate a central descending signal that can activate renal sympathetic efferent nerve activity at the onset of locomotion.

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