Cardiovascular responses during spontaneous overground locomotion in freely moving decerebrate cats

TOMOKO SADAMOTO AND KANJI MATSUKAWA
Department of Cardiac Physiology, National Cardiovascular Center Research Institute, Suita, Osaka 565, Japan

Sadamoto, Tomoko, and Kanji Matsukawa. Cardiovascular responses during spontaneous overground locomotion in freely moving decerebrate cats. J. Appl. Physiol. 83(5): 1454–1460, 1997.—To examine whether the cerebrum is essential for producing the rapid cardiovascular adjustment at the beginning of overground locomotion, we examined heart rate (HR), mean arterial blood pressure (MAP), and integrated electromyogram (iEMG) of the forelimb triceps brachialis muscle in freely moving decerebrate cats during locomotion. Two to four days after decerebration surgery performed at the level of the precollicular-premammillary body, the animals spontaneously produced coordinated overground locomotion, supporting body weight. HR began to increase immediately before the onset of iEMG, and MAP began to rise almost simultaneously with the iEMG onset. Their increases in HR and MAP (24 ± 3 beats/min and 22 ± 4 mmHg) were sustained during locomotion. Sinoaortic denervation (SAD) did not affect the abrupt changes in HR and MAP at the beginning of locomotion (0–4 s from the onset of iEMG), whereas SAD had a contrasting effect during the later period of spontaneous locomotion. Sinoaortic denervation (SAD) on the responses of HR and ABP during spontaneous overground locomotion. It has been hypothesized that areas of the brain stem outside the cerebrum can produce the rapid cardiovascular adjustment associated with spontaneous overground locomotion.

To verify the aforementioned hypothesis, we examined the time course of the responses in HR and ABP at the onset of overground locomotion in freely moving cats, which were decerebrated at the level of the precollicular-premammillary body. These kinds of decerebrate cats could induce spontaneous locomotion without any artificial stimulation in the same way as intact awake cats performed voluntary locomotion. In addition, to identify whether the cardiovascular adjustment at the beginning of spontaneous locomotion in decerebrate cats was independent of the arterial baroreflexes, we examined the effect of sinoaortic denervation (SAD) on the responses of HR and ABP during spontaneous overground locomotion. It has been hypothesized that tonic inhibitory effects of the arterial baroreflexes are suppressed (9) and/or the operating point of the baroreflexes is shifted during dynamic exercise (4, 15), which, in turn, may contribute to the increases in HR and ABP at the onset of voluntary exercise. If so, SAD may diminish the cardiovascular responses at the onset of spontaneous overground locomotion, as found during treadmill exercise in conscious dogs (15) and rabbits (4, 9).

METHODS
Preparation. The experiments were performed in six cats, weighing between 2.1 and 3.8 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 intramuscularly given as a preanesthetic medication to reduce salivation and bronchial secretions. The anesthesia was induced by inhalation of a mixture of halothane (4%), N₂O, and O₂, and an endotracheal tube was then inserted. The cats breathed spontaneously during surgery, and an electrocardiogram (ECG), HR, and respiration were monitored. Two to four days after decerebration surgery performed at the level of the precollicular-premammillary body, the animals spontaneously produced coordinated overground locomotion, supporting body weight. HR began to increase immediately before the onset of iEMG, and MAP began to rise almost simultaneously with the iEMG onset. Their increases in HR and MAP (24 ± 3 beats/min and 22 ± 4 mmHg) were sustained during locomotion. Sinoaortic denervation (SAD) did not affect the abrupt changes in HR and MAP at the beginning of locomotion (0–4 s from the onset of iEMG), whereas SAD had a contrasting effect during the later period of spontaneous locomotion.
continuously monitored. To maintain the level of surgical anesthesia, the concentration of halothane was increased in a range of 1.5 to 2.0% if an increase in HR and or respiration and/or withdrawal of the limb in response to noxious pinch of the paw and/or 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 ABP. A pair of stainless steel wire electrodes were implanted under the skin of the left chest for ECG monitoring. Rectal temperature was maintained at 37–38.5°C with a heating pad. The electrodes and both arterial and venous catheters were tunneled subcutaneously and exteriorized at the back of the neck. Then, the head of the cat was mounted on a stereotaxic frame. Decerebration was performed by electrococulation at the level of the precollicular-premammillary body as described previously (22). To accomplish this, a stainless steel electrode, the insulation of which was exposed along a region 5 mm from the tip, was inserted into the hypothalamus rostral to the mammillary bodies (coordinates from the midpoint of the interaural line: 13 mm anterior, 6 mm horizontal, 1–11 mm lateral with an angle of 24° from the perpendicular line with reference to a stereotaxic atlas (3)). A negative direct current (1 mA) was passed through the electrode. Then, the electrode was withdrawn by 4 mm, and the current was passed again. This procedure was repeated for the total of 42 tracks at 0.5-mm intervals. The animals were killed with an overdose of pentobarbital sodium at the end of experiments, and the transected area of the brain was examined histologically.

After decerebration surgery, the cats were housed in their cages and warmed with a heating pad. The cats recovered from anesthesia 1 day after surgery. Antibiotics (penicillin G, 10,000–20,000 U) were given for 2–5 days after the operation. Water and/or 5% glucose (volume 10–30 ml/day) was given orally to the cats every day, and they were able to swallow the fluid. Recording of data. ABP was measured through the carotid artery catheter connected to a pressure transducer (Baxter, DPT II). Mean arterial blood pressure (MAP) was obtained by integrating the ABP signal with a time constant of 1 s (NEC Sanei, Recti-8K) and stored on an FM magnetic tape recorder (TEAC, XR-310). ABP, ECG, iEMG, and the marking signals were also sampled at 400 Hz in a computer, and their beat-to-beat calculated parameters and corresponding mean values over 1 s were stored in a hard disk by using a customized software program (Cordat II, DISS) 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 the floor. The cats were placed in a sitting or squatting posture at one end of a walking passage (3 × 0.2 × 0.35 m) enclosed by a plastic wall. Without any artificial stimulation, the cats could initiate spontaneous locomotion at variable intervals and move all four limbs in a coordinated manner during overground locomotion, supporting their own body weight. The cats walked toward the other end of the walking passage. In most cases, the cats were unable to stop body movement spontaneously when they reached the end of the walking passage. Each animal produced 13–22 trials of coordinated locomotion. From these data, a total of 65 trials of locomotion were randomly selected from 5 cats (13 trials/cat), and the cardiovascular responses to overground locomotion were analyzed.

SAD was performed in four of five cats 3–4 days after the decerebration surgery. To eliminate baroreceptor input, the bilateral carotid sinus nerves and aortic nerves were identified by direct measurement of baroreceptor activity and then dissected. When it was difficult to isolate the aortic nerve from the cervical vagus, the aortic-vagal complex was cut as shown in Table 1 (the right vagal nerve in 2 cats and bilateral vagal nerves in 1 cat were additionally cut). In 2–3 days after the second surgery for SAD, the locomotion experiments were conducted again according to the same procedure outlined before SAD. A total of 52 trials of spontaneous locomotion (13 trials/cat) obtained from 4 cats were analyzed. Also, by using one decerebrate cat with chronic SAD (the SAD surgery was done 34 mo before the experiment), the cardiovascular responses to locomotion were examined. SAD was tested by observing the HR responses to intravenous bolus injections of phenylephrine (9–15 µg/kg) and nitroprusside (10–15 µg/kg). Before SAD, HR was decreased by 49 ± 13 beats/min in response to phenylephrine-induced pressor response (25 ± 5 mmHg in MAP) and was increased by 44 ± 13 beats/min in

Table 1. Duration of spontaneous overground locomotion, baseline values of HR and MAP, and peak changes during overground locomotion in cats before and after SAD

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Duration of Locomotion, s</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>Peak change</td>
<td>Baseline</td>
</tr>
<tr>
<td>1</td>
<td>17 ± 1/ —</td>
<td>176 ± 2/ —</td>
<td>36 ± 3/ —</td>
<td>122 ± 1/ —</td>
</tr>
<tr>
<td>2</td>
<td>32 ± 4/28 ± 1</td>
<td>168 ± 1/141 ± 1*</td>
<td>17 ± 1/11 ± 1*</td>
<td>108 ± 1/111 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>27 ± 3/29 ± 3</td>
<td>151 ± 2/172 ± 3*</td>
<td>26 ± 5/17 ± 3*</td>
<td>117 ± 1/107 ± 2*</td>
</tr>
<tr>
<td>4</td>
<td>10 ± 1/10 ± 1</td>
<td>148 ± 2/192 ± 1*</td>
<td>15 ± 4/13 ± 1*</td>
<td>135 ± 2/135 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>21 ± 1/19 ± 3</td>
<td>193 ± 2/181 ± 1*</td>
<td>34 ± 4/14 ± 3*</td>
<td>107 ± 1/118 ± 2*</td>
</tr>
<tr>
<td>6</td>
<td>— /23 ± 1/22</td>
<td>— /182 ± 1 — /11 ± 2</td>
<td>— /11 ± 2</td>
<td>— /27 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE for 13 trials/cat in 6 cats. HR, heart rate; MAP, mean arterial pressure; SAD, sinoaortic denervation. *Significant difference before and after SAD, P < 0.05.
response to nitroprusside-induced depressor response (37 ± 3 mmHg). Although the drug-induced changes in MAP (an increase of 41 ± 10 mmHg and a decrease of 46 ± 4 mmHg) became greater after than before SAD, the responses in HR (2 to 3 ± 1 beats/min) to the alterations in MAP were small and not significant.

Data treatment. The data in each trial of spontaneous locomotion were displayed on a cathode-ray tube 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 (ciEMG) 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 ciEMG. The offset of iEMG was delayed from the end of locomotion because the cats continued body movements for a few seconds after reaching the wall at the end of the walking passage.

The changes in HR and MAP from prelocomotion values (baseline levels) in an individual trial were aligned at the onset of iEMG or at the offset of iEMG and then averaged over 65 trials before SAD and over 52 trials after SAD. The data of HR and MAP obtained for 20 s before the onset of iEMG were defined as the baseline levels.

Statistical analysis. The changes in HR and MAP during overground spontaneous locomotion were statistically analyzed by using a one-way analysis of variance with repeated measures. When a significant F-value in the main effect of time was present, Tukey's post hoc test was performed to see a significant difference between the baseline level and the value at a given time. The effects of SAD on the changes in HR and MAP were analyzed by using a two-way analysis of variance with repeated measures. When a significant F-value in the main effect of SAD was present, the mean values before and after SAD obtained at an individual time were compared by Tukey's post hoc test. The duration of locomotion and the baseline values of HR and MAP and their peak changes in the main effect of SAD were analyzed by using a one-way analysis of variance with repeated measures. When a significant F-value in the main effect of SAD was present, the mean values were aligned and compared by a paired t-test. The level of statistical significance was defined as P < 0.05. The data are expressed as means ± SE.

RESULTS

Locomotor movements. A typical change in 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 with negligible tonic iEMG. When iEMG rapidly increased, the cat stood up and began to step on the floor. The onset of iEMG (Fig. 1, up arrow) was almost identical to the start of spontaneous locomotion. The increase in iEMG was followed by rhythmic bursts, 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 6 ± 2 s because of continued body movements. This pattern of coordinated locomotion was similarly observed before and after SAD.

The duration of locomotion varied among animals before and after SAD (range 10-32 s before and 10-29 s after SAD) as shown in Table 1. The average duration of locomotion was 21 ± 4 s (n = 5 cats) before SAD, which corresponded to 14 ± 3 cm/s in the speed of locomotion. The average duration and speed after SAD were 22 ± 3 s and 14 ± 3 cm/s, respectively. In a given animal, the duration of locomotion seemed quite constant because there was no significant difference in the locomotor duration obtained before and after SAD (Table 1), and the coefficient of variation of the duration was low (23 ± 6% before and 24 ± 6% after SAD).

Time course of changes in HR and MAP during locomotion. A typical example of HR and ABP in response to spontaneous overground locomotion is shown in Fig. 1. HR began to increase before the onset of iEMG and continuously increased, reaching a peak in the later period of spontaneous locomotion. ABP increased concurrently with the onset of iEMG and often showed two peaks during spontaneous locomotion, as shown in Fig. 1. Immediately after the end of overground locomotion, HR showed an abrupt drop and ABP gradually returned to the baseline value.

The early and late peaks of the pressor response to overground locomotion were observed in 52 (80%) of 65 locomotor trials performed by 5 cats before SAD. The early peak of the pressor response was 13 ± 2 mmHg.
and appeared at 3.8 ± 0.2 s from the onset of iEMG, and the late peak of the pressor response was 26 ± 3 mmHg at 15.5 ± 1.1 s from the iEMG onset. In the following analysis of the average time course of the cardiovascular responses, all data in 65 trials were collected and averaged.

The baseline values of HR and MAP and their maximum changes during spontaneous locomotion in individual cats are summarized in Table 1. The overall baseline values of HR and MAP were 168 ± 7 beats/min and 118 ± 4 mmHg (n = 5 cats), respectively. HR increased by 15–36 beats/min and MAP increased by 5–60 mmHg during spontaneous overground locomotion produced by decerebrate cats (Table 1). The average time course of the increases in HR and MAP at the beginning of spontaneous locomotion was analyzed as shown in Fig. 2A. A significant increase in HR occurred 1 s before the onset of iEMG and reached the maximum value of 24 ± 3 beats/min at 13 s from the onset of iEMG. Also, a significant increase in MAP occurred 1 s after the onset of iEMG and reached the maximum of 22 ± 4 mmHg at 15 s.

The time course of changes in HR and MAP after the cessation of spontaneous locomotion is shown in Fig. 2B. The changes in HR and MAP began to decrease as soon as locomotion ended, despite the presence of increased iEMG. HR showed a quick drop of 14 beats/min within 5 s, which was followed by a gradual decline toward the baseline level in the subsequent 80-s recovery period. In contrast, MAP decreased by 18 mmHg within 10 s and remained at this level.

Effect of SAD. The effects of SAD on the baseline values of HR and MAP varied among cats (Table 1), and the overall changes in these baseline values were not significant before and after SAD. During spontaneous locomotion after SAD (Fig. 3), there were significant increases in HR and MAP. In the initial period of locomotion (0–4 s from the onset of iEMG), the average time course and magnitude of the increases in HR and MAP were identical before and after SAD (Fig. 3). However, in the subsequent period of locomotion, SAD had a significant effect on the magnitude of increases in HR and MAP. SAD attenuated the peak increase of HR (21 ± 2 beats/min observed at 15 s before SAD vs. 9 ± 1 beats/min observed at 12 s after SAD) but augmented the peak increase in MAP (13 ± 2 mmHg observed at 16 s before SAD vs. 30 ± 3 mmHg observed at 16 s after SAD). These contrasting effects of SAD on the peak increases in HR and MAP were similarly observed in individual cats (Table 1). Conversely, the time in which HR and MAP peaked in the average time course analysis was not different before and after SAD.

Indeed, in 33 (63%) of 52 trials performed by 4 cats after SAD, the early and late peaks of the pressor response were discriminated. In the trials the early peak of the pressor response was 11 ± 1 mmHg and appeared at 3.6 ± 0.2 s from the iEMG onset, indicating that there was no significant difference in the amplitude and time of the early peak of MAP. In contrast, the late peak amplitude of the pressor response was significantly greater after SAD (33 ± 3 mmHg at 14.9 ± 1.3 s from the iEMG onset) than before SAD (26 ± 3 mmHg at 15.5 ± 1.1 s from the iEMG onset).

In the 80-s recovery period (Fig. 3B), the time courses of the changes in HR observed before and after SAD were almost identical, but the HR level after SAD was shifted to a lower value than that before SAD. The time course of the changes in MAP in the recovery period was also similar before and after SAD, except for a brief period immediately after the end of locomotion.
From the histological analysis (n = 6 cats), it was observed that the transection occurred in the middle of the hypothalamus in the sagittal plane, as shown in Fig. 4. In all cats, it was found that the cerebrum and the rostral part of the hypothalamus (the anterior hypothalamic area, supraoptic nucleus, and rostral part of the lateral hypothalamic area) were disconnected from the brain stem. However, the caudal part of the hypothalamus (the posterior hypothalamic area and the caudal parts of the lateral hypothalamic area, and the ventromedial nucleus of the hypothalamus) were intact.

DISCUSSION

The time course of the cardiovascular responses during spontaneous overground locomotion was studied before and after SAD in freely moving decerebrate cats. Our major new finding is that HR began to increase immediately before the onset of iEMG and MAP began to rise almost at the onset of iEMG. The time course and magnitude of the increases in HR and MAP observed in the initial period of locomotion were identical before and after SAD. These results suggest that the rapid adjustment of HR and MAP occurs in parallel with locomotor movement in animals without the cerebrum and the rostral part of the diencephalon and that the neural mechanism responsible for this adjustment is independent of the arterial baroreflexes.

The present finding that the increase in HR preceded the onset of EMG activity of the forelimb triceps brachialis muscle and the rise in ABP occurred almost simultaneously with the iEMG onset suggests that this rapid cardiovascular adjustment is directly initiated by descending signals from higher brain centers, although it cannot be excluded that a reflex originating from receptors in the exercising muscle may contribute further to the cardiovascular adjustment after the start of locomotion (8, 13). Furthermore, SAD did not affect the increases in HR and MAP at the initial period of spontaneous overground locomotion (before and within 4 s after the iEMG onset). Taken together, it is likely that central descending signals were capable of generating the rapid cardiovascular adjustment at the onset of
spontaneous locomotion in decerebrate cats without feedback signals from the contracting muscles and arterial baroreceptors. Our idea is supported by previous findings using unanesthetized decerebrate cats showing that responses in HR, MAP, and RSNA occurred during spontaneous fictive locomotion (5–7).

In intact awake animals, such rapid cardiovascular adjustment has been observed during static and dynamic exercises and other voluntary behaviors. RSNA and HR began to increase immediately before or at the onset of voluntary static exercise in cats (12) and at the first step of treadmill exercise in rabbits (18). The instantaneous adjustment of RSNA and HR was also observed during eating (12), grooming (12), and defense reactions (1) in conscious cats. Because the rapid cardiovascular adjustment that decerebrate cats produced during spontaneous overground locomotion seems identical to the results observed in intact awake animals, the cerebrum and the rostral part of the diencephalon may not be essential for generating direct descending signals responsible for the rapid cardiovascular adjustment during locomotion in awake animals.

Possible sites in the brain stem producing both cardiovascular adjustment and somatomotor behavioral changes have been reported. Chemical stimulation of neurons in the hypothalamus involving the posterior hypothalamus, the lateral hypothalamus, and a part of the field of Forel induced both cardiorespiratory changes and locomotor movements (5–7, 20, 23). Neurons in the localized areas of the hypothalamus and the midbrain periaqueductal gray matter were capable of producing both cardiovascular changes and body defense movements (2, 10, 21). These sites of the brain stem may be responsible for generating direct descending signals for the rapid cardiovascular adjustment during spontaneous locomotion evoked by decerebrate cats.

Because stimulation of arterial baroreceptors induced by injection of norepinephrine inhibited the increase in RSNA during voluntary static exercise in awake cats (11), it is assumed that when MAP increases considerably during locomotion, the arterial baroreflexes decrease sympathetic efferent nerve activity and thereby counteract the pressor response. If the inhibition of the baroreflexes is eliminated, the pressor response during locomotion will be enhanced. Indeed, we found that SAD augmented the response in MAP observed in the late period of locomotion, whereas the denervation did not affect the rapid increases in HR and MAP in the initial 4-s period of locomotion. Stimulation of the sympathetic nervous system is likely to increase MAP in the initial period of spontaneous locomotion, which in turn may inhibit sympathetic nerve activity and counteract the pressor response. However, a time lag from a decrease in sympathetic nerve activity to a change in MAP mediated via a relaxation of vascular smooth muscles should be taken into account because the time lag was ~6 s when an increase in RSNA during static exercise in the conscious cat was followed by a rise in MAP (11). On the other hand, the augmenting effect of SAD on the late increase in MAP suggests that the arterial baroreflexes can counteract the increase in MAP in the late period of spontaneous locomotion in decerebrate cats. This effect is in agreement with the previous findings made during dynamic exercises in conscious dogs (19, 24). In addition, the augmented response in MAP is presumably caused by a marked increase in sympathetic vasomotor activity to peripheral vascular beds because the HR response was decreased after SAD.

In contrast, the opposite effect on the HR response of SAD was found. SAD reduced the increase in HR in the late period of spontaneous locomotion. This attenuated HR response might be caused by the loss of vagal efferents to the heart because we cut the vagal nerve in some cats during the denervation surgery. However, this is unlikely because the cats with and without vagotomy showed the same reduction of the HR response after SAD (Table 1). Alternatively, to explain the contrasting effect of SAD on the responses in HR and MAP, we considered that the two (cardiac and vasomotor) components of the arterial baroreflexes are controlled differently during spontaneous locomotion. The cardiac component of the arterial baroreflexes may be inhibited during spontaneous locomotion, whereas the vasomotor component of the arterial baroreflexes is operating. If the inhibition of the cardiac component of the arterial baroreflexes contributes to cardiac acceleration during locomotion, the lack of arterial baroreceptors with SAD will result in a decreased HR response. In support of this explanation, previous studies showed that the cardiac component of the arterial baroreflexes was inhibited by electrical stimulation of muscle afferent fibers (14, 17) or by stimulation of the central nervous system (16). Thus, the attenuating effect on the HR response of SAD suggests that the cardiac component of the arterial baroreflexes is inhibited during locomotion, which in turn contributes to an increase in HR observed in the late period of spontaneous locomotion.

In conclusion, it is likely that the increase in HR obtained immediately before the onset of spontaneous 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 baroreceptors. It is concluded that some central site, other than the cerebrum and the rostral part of the diencephalon, generates a central descending signal that can increase HR before spontaneous locomotion.

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and by research grants from the Japan Heart Foundation and from the Japan Cardiovascular Research Foundation.

Address for reprint requests: T. Sadamoto, Dept. of Sport Science, Faculty of Letters, Nara Women's Univ., Kitaouyamishi-machi, Nara 630, Japan

Received 20 December 1996; accepted in final form 18 July 1997.

REFERENCES
1. Baccelli, G., R. Albertini, A. Del Bo, G. Manicia, and A. Zanchetti. Role of sinoaortic reflexes in hemodynamic pat-

Downloaded from http://jap.physiology.org/ by 10.220.33.1 on October 30, 2017