Central command, but not muscle reflex, stimulates cutaneous sympathetic efferents of cats

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Hill, Janeen M., and Marc P. Kaufman. Central command, but not muscle reflex, stimulates cutaneous sympathetic efferents of cats. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1552-H1559, 1998.—We determined the effects of stimulation of the mesencephalic locomotor region (MLR) and the muscle reflex, each evoked separately, on the discharge of cutaneous sympathetic fibers innervating the hairy skin of decerebrate cats. Electrical stimulation of the MLR was performed while the cats were paralyzed with vecuronium bromide. The muscle reflex was evoked while the cats were not paralyzed by electrical stimulation of the tibial nerve at current densities that did not activate directly group III and IV muscle afferents. MLR stimulation increased, on average, the discharge of the 23 cutaneous sympathetic fibers tested (P < 0.05). The muscle reflex, in contrast, had no overall effect on the discharge of 21 sympathetic fibers tested (P > 0.05). Both maneuvers markedly increased mean arterial pressure and heart rate (P < 0.05).

Prevention of the baroreceptor reflex with the anesthetic halothane (5%) and O2-N2O (4:1). This conclusion was based on two findings. First, exercise-induced increases in cutaneous sympathetic outflow (19, 25). In these studies, activation of central command was always accompanied by muscular contraction, raising the possibility that a muscle reflex contributed at least in part to the exercise-induced increases in cutaneous sympathetic discharge. In addition, activation of central command may have been accompanied by arousal, which also activates skin sympathetic discharge (3).

The role played by each mechanism in the control of cutaneous sympathetic discharge is not clear. The available evidence, which has been obtained solely in humans, suggests that central command plays a larger role than the muscle reflex in evoking the exercise-induced increase in cutaneous sympathetic outflow (19, 24, 25). This conclusion was based on two findings. First, exercise-induced increases in cutaneous sympathetic discharge immediately preceded the onset of muscle tension development (24, 25), and, second, that postexercise ischemia, which evokes the muscle chemoreflex (17), did not maintain the exercise-induced increase in cutaneous sympathetic discharge (19, 25). In these studies, activation of central command was always accompanied by muscular contraction, raising the possibility that a muscle reflex contributed at least in part to the exercise-induced increases in cutaneous sympathetic discharge. In addition, activation of central command may have been accompanied by arousal, which also activates skin sympathetic discharge (3).

EXERCISE IS WELL KNOWN to increase sympathetic outflows to muscle, skin, and the viscera. These increases are believed to be caused by two neural mechanisms, central command and the muscle reflex. Central command, a feed-forward mechanism, is defined as the parallel activation of central neural circuits controlling locomotion and sympathetic discharge (4, 11). The neuroanatomical substrate for central command involves primarily supramedullary structures, which include the motor cortex, the hypothalamic locomotor region, which is found in or near the H fields of Forel, the mesencephalic locomotor region (MLR), which is found in the cuneiform nucleus, and the amygdala (26). The muscle reflex, a feedback mechanism, is defined as the contraction-induced activation of sympathetic discharge, the afferent arm of which is composed of group III and IV muscle afferents (2, 15).

The role played by each mechanism in the control of cutaneous sympathetic discharge is not clear. The available evidence, which has been obtained solely in humans, suggests that central command plays a larger role than the muscle reflex in evoking the exercise-induced increase in cutaneous sympathetic outflow (19, 24, 25). This conclusion was based on two findings. First, exercise-induced increases in cutaneous sympathetic discharge immediately preceded the onset of muscle tension development (24, 25), and, second, that postexercise ischemia, which evokes the muscle chemoreflex (17), did not maintain the exercise-induced increase in cutaneous sympathetic discharge (19, 25). In these studies, activation of central command was always accompanied by muscular contraction, raising the possibility that a muscle reflex contributed at least in part to the exercise-induced increases in cutaneous sympathetic discharge. In addition, activation of central command may have been accompanied by arousal, which also activates skin sympathetic discharge (3).

These uncertainties prompted us to clarify the roles played by central command and the muscle reflex in evoking exercise-induced increases in cutaneous sympathetic discharge. Using decerebrate unanesthetized cats, we recorded the discharge of sympathetic postganglionic fibers that were presumed to innervate blood vessels of the hindlimb hairy skin. We evoked the muscle reflex by electrically stimulating the tibial nerve, a maneuver that caused the triceps surae muscles to contract statically. We evoked a part of the central command mechanism by electrically stimulating the MLR (4) while the cats were paralyzed. Both maneuvers allowed us to manipulate separately each of the two mechanisms. We tested the hypothesis that central command, but not the muscle reflex, increased the discharge of sympathetic postganglionic fibers that innervate the vasculature of skin.

METHODS

Experiments were performed on 26 cats of either sex with weights ranging from 1.9 to 2.5 kg. The cats were anesthetized with halothane (5%) and O2-N2O (4:1). The cervical trachea was cannulated while the cats breathed the anesthetic gas mixture through a nose cone. The tracheal cannula was then attached to a respirator, and the lungs were ventilated with the anesthetic-O2 mixture. The right common carotid artery and external jugular vein were cannulated for the measurement of arterial blood pressure and for the introduction of fluids and drugs, respectively.

Each cat was suspended over a treadmill, and the head was fixed in place with a Kopf stereotaxic unit. A decerebration was performed according to the method of Shik et al. (21). The brain stem was sectioned transversely at a 30° angle starting ~1 mm rostral to the superior colliculi. All tissue rostral to the section including the cortex was removed, and bleeding was controlled. The inhalant anesthetic was discontinued, and the cat's lungs were ventilated with room air and supplemental O2.

Each cat was placed in a Kopf spinal unit secured to the treadmill. The chest was opened. A side port of the tracheal cannula was then attached to a pressure transducer (Statham P23XL) that measured airway pressure. The left calcaneal tendon was severed at its junction with the calcaneal bone and attached to a force displacement transducer (Grass FT-10). The left tibial nerve was isolated, and a hook stimulating electrode was placed around it. The nerve and electrode...
were covered with a mixture of petroleum jelly and mineral oil to prevent drying.

Skin flaps from the right hindlimb formed a pool, which contained warm mineral oil (37–38°C). The right sural nerve was isolated, and the central cut end was placed on a small platform and desheathed. Small filaments were teased from the sural nerve and placed on a platinum hook recording electrode until the discharge of one or two postganglionic sympathetic fibers was recorded. Arterial PO₂, PCO₂, and pH were measured periodically (ABL-3; Radiometer) and were maintained within normal limits by supplementing inspired air with O₂, adjusting ventilatory rate, and administering sodium bicarbonate (9% iv). Temperatures of the cats were monitored intraoesophageally and were maintained between 36.5 and 38°C.

A part of the central command mechanism was evoked by electrical stimulation of the MLR (4). A monopolar stainless steel electrode (model SNEX-300; Rhodes) was positioned 4 mm lateral to the midline and 1 mm caudal to the anterior margin of the inferior colliculus. The electrode was lowered to a depth of 2 mm below the surface of one inferior colliculus, and then current was passed through the electrode while advancing it in 0.5-mm increments from 2 to 10 mm below the collicular surface. A central command site was identified when electrical stimulation (20–30 Hz; 0.7–1.0 ms; 60–100 µA) increased arterial blood pressure and heart rate (HR) as well as causing the cat to walk with all four limbs on the treadmill.

The muscle reflex was evoked by static contraction of the left triceps surae muscles. The left tibial nerve was stimulated electrically at current intensities (20 Hz; 0.025 ms; ≈2× motor threshold) that do not activate directly groups III and IV afferents (18). The tension developed by the contracting triceps surae muscles was measured by a force displacement transducer (Grass FT10) attached to the central cut end of the calcaneal tendon.

Protocol. We recorded from sural nerve filaments the discharge of postganglionic sympathetic fibers that presumably innervated the vasculature of skin. The sural nerve only supplies the hairy skin of the hindlimb (12). No pilomotor axons have been identified in the sural nerve, and sudomotor axons are rare (5). Additionally, postganglionic vasodilator neurons supplying skin have not been identified in cats (8), leading us to assume that the discharge of sympathetic postganglionic fibers recorded in our experiments served a vasoconstrictor function.

In our experiments, a fiber was classified as a sympathetic postganglionic efferent only if its spontaneous discharge was abolished by intravenous injection of hexamethonium bromide (20 mg/kg). In addition, the fiber’s responses to an increase and to a decrease in arterial blood pressure were sometimes examined. Blood pressure was increased by a bolus injection of phenylephrine (5–40 µg iv) and was decreased by a bolus injection of nitroprusside (2.5 µg/kg iv). These maneuvers, which altered blood pressure, were performed to assess the effect of the baroreflex on the discharge of cutaneous sympathetic efferents. In addition, the dose of phenylephrine given was adjusted to increase arterial blood pressure by the same amount as that increased by central command (see RESULTS). Finally, conduction velocities of some fibers were calculated. Conduction time was obtained by electrically stimulating the right sciatic nerve and recording on a storage oscilloscope the time between the stimulus onset and the evoked action potential. Conduction distance was obtained by measuring the length of a thread placed between the stimulating and recording electrodes.

For the most part, the fibers were tested for their responses to either stimulation of the MLR or the muscle reflex, but not both. This assignment was done randomly and was not based either on their responses to changes in baroreceptor discharge or their spontaneous discharge pattern (see below). We recorded the response of each fiber to at least one of the following protocols: muscle reflex, stimulation of the MLR, muscle reflex before and after phentolamine (1 mg/kg iv), and stimulation of the MLR before and after phentolamine (1 mg/kg iv). We gave phentolamine, an α-adrenergic antagonist, to attenuate the pressor response to either MLR stimulation or the muscle reflex. This attenuation, in turn, would minimize any baroreflex buffering of the responses of cutaneous sympathetic fibers to either mechanism. The cat was paralyzed with vecuronium bromide (0.1 mg/kg iv) before a fiber’s response to MLR stimulation was determined. This dose abolished all limb and respiratory muscle movement.

Data analysis. The discharge of each sympathetic postganglionic fiber was recorded for an equivalent time period before, during, and after a maneuver. This period was usually 30 s. Baseline discharge of a fiber represents the average impulse activity from a fiber for the time period before a maneuver. Response discharge of a fiber represents the average discharge of a fiber during the time period of a maneuver. Recovery discharge of a fiber represents the average discharge of a fiber during the time period after a maneuver (see Figs. 4 and 5). When either the MLR was stimulated or the muscle reflex was evoked, the discharge was averaged in 10-s intervals throughout the duration of the maneuver. Onset latencies were determined by identifying the time at which a maneuver (central command or the muscle reflex) evoked an increase in the fiber’s discharge when compared with its discharge pattern during baseline.

Some sural nerve filaments, even when finely split, contained the discharge of two postganglionic sympathetic fibers. These fibers were included in the data only if the waveform and amplitude of each impulse were clearly distinguishable. Otherwise, only single-unit recordings were analyzed.

The criterion for an individual fiber to be stimulated by either the muscle reflex or central command was a 25% increase in impulse activity from baseline. Values for all reported data are means ± SE. Statistical significance was determined using a paired t-test or, when appropriate, a repeated-measures analysis of variance followed by a Newman-Keuls post hoc test. The criterion for significance was P < 0.05.

RESULTS

The impulse activity of 41 spontaneously active sympathetic postganglionic fibers with axons traveling in the sural nerve was recorded. At the end of the study, the spontaneous activity of each fiber was abolished by intravenous injection of hexamethonium bromide, an effect that confirmed that they were sympathetic postganglionic efferents (Fig. 1).

General discharge properties. The spontaneous discharge of 23 of the 41 (56%) fibers was synchronous with lung deflation (Fig. 2A). In contrast, the spontaneous discharge of the remaining 18 fibers was not related to either inflation or deflation of the lungs (Fig. 2B). The discharge of 26 of 41 fibers was recorded when arterial blood pressure was increased by phenylephrine injection. On average, baseline discharge was attenuated from 0.8 ± 0.2 to 0.5 ± 0.2 impulses/s (P < 0.05) when mean arterial pressure (MAP) was increased
from 111 ± 6 to 170 ± 6 mmHg (P < 0.05). The discharge of 18 of 41 fibers was recorded when arterial blood pressure was decreased by nitroprusside injection. On average, baseline discharge was increased from 0.4 ± 0.1 to 0.6 ± 0.1 impulses/s (P < 0.05) when MAP was decreased from 124 ± 4 to 72 ± 4 mmHg (P < 0.05). Finally, the conduction velocities of eight fibers averaged 0.8 ± 0.2 m/s.

MLR stimulation. We recorded the responses to stimulation of the MLR of 23 of the 41 cutaneous sympathetic postganglionic fibers. MLR stimulation increased the discharge of 16 of 23 fibers tested. In addition, MLR stimulation significantly increased (P < 0.05) the average discharge of the 23 fibers tested (onset latency: 2.8 ± 1.2 s; Table 1). Moreover, MLR stimulation significantly increased over baseline levels during the first, middle, and final 10 s of stimulation (Figs. 3 and 4). In addition, MLR stimulation significantly increased MAP and HR (Table 1). The discharge of 12 of the 23 fibers was recorded when MAP was increased by phenylephrine injection to the same levels (i.e., 65 mmHg) as those increased by MLR stimulation (i.e., 65 mmHg). The discharge of these fibers was decreased by the pressor response to phenylephrine injection (0.8 ± 0.2 to 0.4 ± 0.2 impulses/s; P < 0.05).

We assessed the responses of five fibers to MLR stimulation before and after α-adrenergic blockade with phentolamine. Before blockade, MLR stimulation changed the discharge of these fibers from 0.9 ± 0.3 to 1.1 ± 0.3 impulses/s (P > 0.05), whereas, afterward, MLR stimulation increased the discharge of the five fibers from 1.7 ± 0.6 to 3.0 ± 0.9 impulses/s (P < 0.05). Before blockade, MLR stimulation increased MAP from 120 ± 14 to 175 ± 5 mmHg (P < 0.05); after blockade, MLR stimulation increased MAP from 67 ± 5 to 81 ± 6 mmHg (P < 0.05). Additionally, when the discharge of these fibers was compared during the first, middle, and final 10 s of MLR stimulation, the discharge of these fibers was stimulated (P < 0.05) more after blockade than before (Fig. 4).

Muscle reflex. We recorded the responses to static contraction of the triceps surae muscles of 21 of the 41 fibers. On average, contraction did not increase (P > 0.05) their discharge (from 0.7 ± 0.1 to 0.8 ± 0.2 impulses/s, n = 21). Likewise, the muscle reflex did not increase the discharge of these 21 fibers over their baseline levels during the first, middle, and final 10 s of contraction (Fig. 5). Nevertheless, contraction increased the discharge of seven of the 21 fibers tested (P < 0.05; Table 2). The onset latency of the seven fibers stimulated by contraction averaged 3.7 ± 0.7 s (range 2–6 s). Activation of the muscle reflex significantly increased (P < 0.05) MAP and HR (Table 2).

We assessed the responses of five fibers to static contraction of the triceps surae muscles before and after α-adrenergic blockade with phentolamine. Before blockade, contraction had no effect on the discharge of
DISCUSSION

Before any conclusions can be drawn from our findings, the identity and function of the fibers whose discharge we recorded must be ascertained. Several lines of evidence support the hypothesis that these fibers were sympathetic postganglionic efferents causing vasoconstriction in hairy skin. First, and most important, the spontaneous activity of every fiber was silenced by hexamethonium, an agent that blocks synaptic transmission between pre- and postsynaptic ganglionic neurons. Second, each of the fibers was spontaneously active, a discharge property characteristic of sympathetic vasoconstrictor efferents. Third, the discharge of each of the fibers was recorded from the sural nerve, which innervates the hairy skin of the hindlimb. Moreover, the sural nerve does not innervate the footpad, a site that contains the only sweat glands of the hindlimb (8). Fourth, in a limited number of instances, the conduction velocities of the fibers were measured and were found to be consistent with those reported (8) for sympathetic vasoconstrictor efferents. Likewise, the modest attenuation of the fiber’s spontaneous discharge by the baroreflex was similar to that reported for these efferents (9).

We found that a part of the central command mechanism, evoked by stimulation of the MLR, significantly increased the overall discharge (i.e., n = 23) of sympathetic postganglionic fibers innervating the hairy skin. In contrast, the muscle reflex, evoked by static contraction of the triceps surae, had no overall significant effect on this sympathetic discharge (i.e., n = 21). α-Adrenergic blockade with phentolamine, which attenuated the pressor response to both central command and the muscle reflex, did not unmask a stimulatory effect on cutaneous sympathetic efferents by the latter mechanism. Consequently, the arterial baroreflex did not increase the overall discharge (i.e., n = 23) of sympathetic postganglionic fibers innervating the hairy skin.

Table 1. Responses to central command

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<tr>
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<th>Impulses/s</th>
<th>Peak MAP, mmHg</th>
<th>Peak HR, beats/min</th>
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<tr>
<td><strong>Central Command</strong></td>
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<tr>
<td><strong>Baseline</strong></td>
<td>0.4 ± 0.1</td>
<td>110 ± 5</td>
<td>187 ± 13</td>
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<tr>
<td><strong>Stimulation</strong></td>
<td>0.9 ± 0.2†</td>
<td>178 ± 8†</td>
<td>198 ± 14†</td>
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<tr>
<td><strong>Recovery</strong></td>
<td>0.4 ± 0.1</td>
<td>136 ± 11*</td>
<td>185 ± 13</td>
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<tr>
<td><strong>n = 16</strong></td>
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Data are means ± SE; n, no. of fibers. Effects of central command on the discharge of 23 sympathetic postganglionic fibers that innervate skin. Central command increased (P < 0.05) both the discharge of 16 of 23 fibers studied and increased (P < 0.05) peak mean arterial pressure (MAP) and peak heart rate (HR). Central command did not increase (P > 0.05) the discharge of 7 of 23 fibers studied but did increase (P < 0.05) peak MAP and peak HR. *Significant difference from baseline; †significant difference from recovery (P < 0.05).
not buffer the stimulatory effect of the muscle reflex on cutaneous sympathetic outflow.

In humans, the exercise-induced increase in cutaneous sympathetic outflow preceded the development of muscle tension by 2 s (24, 25). This finding excluded the muscle reflex as a cause for the increase in sympathetic discharge occurring before exercise started. Alternatively, the increase might have been caused by either arousal, conditioning (3, 13), or central command. Our use of decerebrate cats allowed more control over these mechanisms than is possible in human experiments. For example, conditioning was unlikely to occur in our experiments because the cats had no cerebral cortex or thalamus. Hence, the pairing of an external stimulus to the electrical stimuli applied to the MLR was highly improbable. The only way that arousal could be evoked in our experiments was if it was part of the central command pathway arising from the MLR. Therefore, the most likely mechanism responsible for the increase in cutaneous sympathetic discharge evoked by MLR stimulation in our experiments was central command.

Both respiratory and thermal stimuli exert large effects on cutaneous sympathetic discharge (3, 9). We attempted to minimize these stimuli by controlling ventilation as well as by contracting a small muscle mass for a brief period of time. Consequently, vagal feedback from the lungs was constant when both central command and the muscle reflex were activated in our experiments. Likewise, skin temperature was unlikely to change much in our experiments when the triceps surae muscles were contracted statically for 30 s. Last, arterial PCO₂ and pH were unlikely to change much during activation of central command because the cats were paralyzed. If these variables did change during static contraction, they had no effect on cutaneous sympathetic discharge.

Cutaneous venomotor tone in humans increases in response to static handgrip (20, 25). Specifically, this increase is transient, occurring during the first 20 s of handgrip, and then returns to baseline even though the maneuver is continued (25). Central command probably is responsible for this transient increase in venomo-
tor tone. Our findings in cats as well as those in humans (25) indicate that, even though the end organ response is transient, the cutaneous sympathetic activation causing this increase in venomotor tone is sustained. One possible explanation for this apparent discrepancy might be that the sustained sympathetic activation is overridden by a neurohumoral vasodilation arising from sweat glands (1, 16).

Any conclusions drawn from our findings must be limited to the sympathetic innervation of blood vessels in skin. Our findings, therefore, cannot be compared with those of Saito et al. (19) who found that electrically

Fig. 4. Summary data (mean ± SE) for effects of stimulating the MLR, i.e., central command, on both the discharge of cutaneous sympathetic fibers and MAP. A: effects of 30 s of MLR stimulation on the discharge of the 23 sympathetic fibers tested. B: effects of MLR stimulation on MAP while the discharge of the 23 fibers was recorded. C: effects of 30 s of MLR stimulation on the discharge of 5 sympathetic fibers before (*) and after (○) α-adrenergic blockade with phentolamine. D: effects of 30 s of MLR stimulation on MAP while the discharge of the 5 fibers was recorded. ●, MAP before α-adrenergic blockade with phentolamine; ○, MAP after blockade; B, baseline activity or MAP (60 s); R, recovery. *Significantly different (P < 0.05) value from baseline (B); ○, significantly different (P < 0.05) value from its corresponding value in time after α-adrenergic blockade with phentolamine.

Fig. 5. Summary data (mean ± SE) for effects of stimulating the MLR, i.e., central command, on both the discharge of cutaneous sympathetic fibers and MAP. A: effects of static contraction of the triceps surae muscles on the discharge of the 21 cutaneous sympathetic fibers tested. B: effect of static contraction on MAP while the discharge of the 21 sympathetic fibers was recorded. C: effects of static contraction on the discharge of the 5 sympathetic fibers before (*) and after (○) α-adrenergic blockade with phentolamine. D: effects of static contraction on MAP while the discharge of the 5 fibers in C was recorded. ●, MAP before blockade; ○, MAP after blockade. B, baseline activity (60 s); R, recovery (60 s). * Significantly different (P < 0.05) from baseline (B); ○, significantly different value (P < 0.05) from its corresponding value in time after α-adrenergic blockade with phentolamine.
induced static contraction increased sudomotor discharge in humans. These investigators raised the possibility that the muscle reflex, as well as central command, increased volar sudomotor discharge to the foot.

Two limitations must be kept in mind when interpreting our data. First, the central command evoked by stimulation of the MLR was representative of dynamic exercise, whereas the reflex evoked by stimulation of the tibial nerve was representative of static exercise. Second, the recruitment order and discharge pattern of the α-motoneurons activated by the electrical pulses applied to the tibial nerve were opposite to that evoked by static exercise performed voluntarily (6). Nevertheless, the level of static contraction used in our experiments has been shown to be a potent stimulus to both group III afferents, most of which are mechanosensitive, and group IV afferents, most of which are metaboliically sensitive (10). In humans, moreover, percutaneous electrically evoked static contraction, a maneuver similar to that used in our experiments on cats, is the accepted method of evoking the muscle reflex without the confounding effects of central command (7, 19, 22).

In humans, the neural mechanisms that control the sympathetic outflow to the vasculature of the skin and skeletal muscles during mild to moderate levels of exercise appear to differ (14, 19, 23, 25). Specifically, central command appears to control the sympathetic outflow to the skin vasculature, whereas the reflex appears to control the sympathetic outflow to the skeletal muscle vasculature. Nevertheless, whenever central command has been elicited in these experiments on humans, it has always been accompanied by muscular contraction (14, 19, 23–25), raising the possibility that the muscle reflex played some role in causing the observed effects. In contrast, our findings in decerebrate paralyzed cats have provided evidence that activation of central command increases sympathetic outflow to the vasculature of the skin, whereas the muscle reflex appears to have no effect.

REFERENCES


