MLR stimulation and exercise pressor reflex activate different renal sympathetic fibers in decerebrate cats

SHAWN G. HAYES AND MARC P. KAUFMAN
Division of Cardiovascular Medicine, Departments of Internal Medicine and Human Physiology, University of California, Davis, California 95616

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Hayes, Shawn G., and Marc P. Kaufman. MLR stimulation and exercise pressor reflex activate different renal sympathetic fibers in decerebrate cats. J Appl Physiol 92: 1628–1634, 2002; 10.1152/japplphysiol.00905.2001.—Although mesencephalic locomotor region (MLR) stimulation and the exercise pressor reflex have been shown to increase whole nerve renal sympathetic activity, it is not known whether these mechanisms converge onto the same population of renal sympathetic postganglionic efferents. In decerebrate cats, we examined the responses of single renal sympathetic postganglionic efferents to stimulation of the MLR and the exercise pressor reflex (i.e., static contraction of the triceps surae muscles). We found that, in most instances (24 of 28 fibers), either MLR stimulation or the muscle reflex, but not both, increased the discharge of renal postganglionic sympathetic efferents. In addition, we found that renal sympathetic efferents that responded to static contraction while the muscles were freely perfused responded more vigorously to static contraction during circulatory arrest. Moreover, stretch of the calcaneal (Achilles) tendon stimulated the same renal sympathetic efferents as did static contraction. These findings suggest that MLR stimulation and the exercise pressor reflex do not converge onto the same renal sympathetic postganglionic efferents.

Central command; static contraction; tendon stretch; autonomic nervous system; exercise; mesencephalic locomotor region

EXERCISE-INDUCED INCREASES in blood pressure, heart rate, and cardiac contractility are caused, at least in part, by increases in sympathetic nervous system output. Two neural mechanisms have been proposed to cause these increases, namely, the exercise pressor reflex (11, 21) and central command (38). The first mechanism proposes that the responses are due to a reflex originating in the contracting muscles. The second mechanism proposes that these responses are due to a direct action of central motor areas on the medullary and spinal neuronal pools controlling cardiovascular and respiratory function.

Previous studies have shown that both central command [i.e., mesencephalic locomotor region (MLR) stimulation] and the exercise pressor reflex increase renal sympathetic nerve activity (RSNA) in cats (7, 16, 34). Renal nerve-induced increases in vascular resistance (17) are thought to be important in maintaining blood pressure in the face of metabolically induced vasodilation in exercising muscles. Because these previous studies (7, 16, 34) recorded whole nerve activity, it is not known whether the two mechanisms converge onto the same postganglionic sympathetic fibers. Consequently, we were prompted to investigate whether stimulation of the MLR and the exercise pressor reflex activated separate populations of renal postganglionic sympathetic efferents.

Using decerebrate unanesthetized cats, we recorded the responses of single renal sympathetic efferents to four stimuli, namely, MLR stimulation, static contraction of the triceps surae muscles while they were freely perfused, static contraction of these muscles while their circulation was occluded, and calcaneal tendon stretch. The first stimulus, which was evoked while the cats were paralyzed, used only central command to stimulate RSNA (6). The second stimulus used mechanical and metabolic stimuli to stimulate RSNA (34). The third stimulus, contraction while the circulation was occluded, used stronger metabolic stimuli than did contraction while the muscles were freely perfused to stimulate RSNA (18, 31). The fourth stimulus, tendon stretch, used a pure mechanical stimulus to evoke RSNA (30). We tested the hypothesis that MLR stimulation and the exercise pressor reflex activated separate populations of renal postganglionic sympathetic efferents.

METHODS

General. Adult cats (n = 14) were anesthetized with a mixture of 5% halothane and oxygen. Catheters were placed in the right jugular vein and common carotid artery for delivery of drugs and measurement of arterial blood pressure, respectively. The carotid artery catheter was connected to a Statham pressure transducer (model P23 XL) to measure arterial pressure. Heart rate was calculated beat-to-beat from the arterial pressure pulse by a Gould Biobak ampliﬁer. The trachea was cannulated, and the lungs were ventilated mechanically (Harvard Apparatus). The cat was placed in a Kopf stereotactic and spinal unit, after which the brain stem was transected through the middle of the superior
colliculus. The plane of section was perpendicular to the brain stem. All neural tissue rostral to the section was removed. Hemostasis was achieved, and the cranial vault was filled with agar (37°C). After the decerebration procedure was completed, the lungs were ventilated with a mixture of room air and oxygen. In preparations that were not paralyzed, airflow was measured with a pneumotachograph (Fleisch) that was attached in series with the tracheal cannula. The measurement of airflow enabled us to determine whether renal sympathetic postganglionic fibers discharged synchronously with a particular phase of the ventilatory cycle. All variables were registered on a chart recorder (model ES1000, Gould) as well as into an analog-to-digital converter (model 4000, Vetter) and video cassette recorder (JVC).

Renal nerve recording. The renal nerve was exposed using a retroperitoneal approach while the cat remained in the Kopf stereotaxic frame and spinal unit. Space for a pool was formed around the renal nerve and filled with warm (37°C) mineral oil. The nerve was placed over a black plastic platform and sectioned, and its central end was divided into filaments, which were draped over the end of a bipolar hook electrode. Impulse activity from the filaments was fed into a high-impedance probe (model HIP 511, Grass), amplified (model P511, Grass), and displayed on a storage oscilloscope (Hewlett-Packard) as well as a scrolling monitor (model V 1000, Gould).

While viewing the renal nerve with a dissecting microscope, we split the filaments until the spontaneous discharge of no more than three individual fibers could be distinguished (Fig. 1). In addition, we injected sodium nitroprusside (30 μg/kg iv) to determine whether activity was increased by the fall in arterial blood pressure that was induced by this vasodilator agent. For most filaments, and especially for silent filaments, the dose of nitroprusside was adjusted so that it decreased diastolic blood pressure to 20 mmHg. This severe decrease in arterial pressure, which removed baroreflex inhibition of sympathetic discharge, helped us determine the number of potentially active fibers in a filament. If this number exceeded three, the filament was split or discarded.

Protocols. The exercise pressor reflex, the muscle mechanoreceptor reflex, and central command were evoked in each cat in which satisfactory renal sympathetic nerve recordings were obtained. The exercise pressor reflex was evoked by electrically stimulating (15–25 Hz, 0.025 ms, <2 times motor threshold) the tibial nerve near its junction with the triceps surae muscles. The common peroneal nerve was sectioned. The calcaneal bone was severed, and its tendon was attached to a force transducer (model FT 10, Grass), which in turn was attached to a rach-and-pinion. The knee was clamped in place. The calcaneal tendon was stretched so that baseline tension was 1.0 kg. The duration of the contraction was 60 s. In some experiments, we also examined the effect of circulatory arrest on the responses of renal sympathetic efferents to static contraction of the triceps surae muscles. Circulatory arrest was achieved by occluding the external iliac artery and vein ipsilateral to the triceps surae muscles being contracted. Occlusion was initiated 5 min before the onset of contraction and remained for 1 min after the end of contraction. In all, occlusion was maintained for 7 min. A muscle mechanoreceptor reflex (30) was evoked by stretching the calcaneal (Achilles) tendon by manually turning the rach-and-pinion, which was placed in series with the force transducer. Baseline tension was set at 1.0 kg. Tendon stretch and muscle contraction were performed on the same cats. Central command was evoked by electrically stimulating (15–20 Hz, 0.5 ms, 40–100 μA) the MLR for 60 s with an FHC monopolar stainless steel electrode. When successful, MLR stimulation evoked locomotion in the unparalyzed cat. After finding a site in the MLR that evoked locomotion, we paralyzed the cat by injecting vecuronium bromide (0.1 mg/kg iv). The duration of this effect on the neuromuscular junction was 45–60 min.

Data analysis. Values for mean arterial pressure, heart rate, tension development by the triceps surae muscles, and discharge rates for renal sympathetic postganglionic fibers are means ± SE. Baseline values for mean arterial pressure, heart rate, and tension development were taken immediately before a maneuver; peak values represent the highest levels reached during a maneuver. Impulse activity arising from single renal sympathetic efferent fibers was counted over 1-min periods and then divided by 60 so that these values could be expressed as impulses per second. Fisher’s exact test was used to determine whether the proportions of fibers responding to the different stimuli were significant. Paired t-tests were used to compare the discharge properties of the fibers. The criterion for statistical significance was P < 0.05.

RESULTS

We recorded the discharge of 28 sympathetic fibers traveling in the renal nerves of decerebrate cats. Each of the 28 fibers responded to a decrease in arterial pressure that was induced by intravenous injection of sodium nitroprusside (30 μg/kg iv; Fig. 2). Each of the 28 fibers discharged in synchrony with the arterial pressure pulse; in addition, they discharged more during lung deflation than during lung inflation. Most importantly, the spontaneous discharge, as well as all responses to MLR stimulation, tendon stretch, muscular contraction, and nitroprusside-induced hypotension, was abolished by injection of hexamethonium bromide (20 mg/kg iv). Consequently, each of the 28 fibers from which we recorded discharge was classified as a sympathetic postganglionic renal efferent. Of the 28 fibers, 4 came from filaments in which only 1 fiber was active, 12 came from filaments in which 2 fibers...
were active, and 12 came from filaments in which 3 fibers were active.

**MLR stimulation.** MLR stimulation increased the discharge of 11 of the 28 renal sympathetic fibers tested (Fig. 3). When averaged across the group of 28, MLR stimulation increased activity from $0.05 \pm 0.01$ to $0.33 \pm 0.09$ impulses/s ($P < 0.05$). The onset latency of the renal sympathetic efferents responsive to MLR stimulation averaged $0.7 \pm 0.2$ s ($n = 11$). There was a significant tendency for renal sympathetic efferents responsive to MLR stimulation to be nonresponsive to static contraction while the triceps surae muscles were freely perfused (Table 1; $P < 0.01$). MLR stimulation significantly increased mean arterial pressure (from $112 \pm 3$ to $139 \pm 4$ mmHg, $P < 0.05$) and heart rate (from $193 \pm 9$ to $221 \pm 10$ beats/min, $P < 0.05$).

**Static contraction.** Static contraction while the triceps surae muscles were freely perfused increased the discharge of 19 of the 28 renal sympathetic efferents tested (Fig. 4). When averaged across the group of 28

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**Fig. 2.** Depressor response induced by injection of sodium nitroprusside (NP, 30 μg/kg iv; horizontal bar) was used to identify renal sympathetic postganglionic efferents.

**Fig. 3.** A single renal sympathetic postganglionic efferent that is responsive to stimulation of the mesencephalic locomotor region (MLR) but not to static contraction or stretch of the calcaneal tendon. Histograms show discharge of fiber before, during (horizontal bar, 60 s), and after stimulation of the MLR (A), static contraction of the triceps surae muscles (B), and stretch of the calcaneal tendon (C). Arrows on histograms correspond to point in neurogram (a–c). Right: arterial blood pressure (mmHg), renal nerve activity, and airflow (inspiration downward) during stimulation of the MLR (a), static contraction (b), and tendon stretch (c). *Action potential; vertical lines in a are artifacts caused by MLR stimulation.
Table 1. Responses of renal sympathetic efferents to MLR stimulation, freely perfused contraction, and tendon stretch

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Values represent number of renal sympathetic efferents out of a total of 28. MLR, mesencephalic locomotor region. Probability of obtaining distributions was <0.01 (by Fisher’s exact test).

efferents, activity increased from 0.06 ± 0.02 to 0.38 ± 0.06 impulses/s (P < 0.05). The onset latency of renal sympathetic efferents responsive to contraction averaged 1.9 ± 0.3 s. There was a significant tendency for renal sympathetic efferents responsive to static contraction while the muscles were freely perfused to be nonresponsive to MLR stimulation (Table 1; P < 0.01).

We compared responses of 17 of the 28 renal efferents to contraction while the muscles were freely perfused with their responses to contraction while the circulation was occluded. We found that contraction while the muscles were freely perfused increased the activity of these 17 efferents from 0.07 ± 0.03 to 0.42 ± 0.09 impulses/s (P < 0.05), whereas contraction while the circulation was occluded increased the activity of these 17 efferents from 0.07 ± 0.02 to 0.59 ± 0.13 impulses/s (P < 0.05). Most importantly, the increase in activity during contraction with the circulation occluded was significantly greater than the increase in activity with the circulation not occluded (P < 0.01). Likewise, the increase in activity after contraction but during circulatory occlusion was significantly greater than that after contraction while the triceps surae muscles were freely perfused (0.19 ± 0.05 vs. 0.06 ± 0.02 impulses/s, P < 0.02).

Static contraction while the triceps surae muscles were freely perfused significantly increased mean arterial pressure and heart rate (from 111 ± 4 to 135 ± 4 mmHg and from 178 ± 8 to 188 ± 8 beats/min, both P < 0.05). Peak developed tension averaged 3.1 ± 0.2 kg. Static contraction of the triceps surae muscles during circulatory occlusion also significantly increased mean arterial pressure and heart rate (from 117 ± 5 to 142 ± 5 mmHg and from 187 ± 9 to 193 ± 10 beats/min, both P < 0.05). Peak developed tension averaged 2.8 ± 0.3 kg. Arterial pressure remained slightly but not significantly higher (P > 0.05) during circulatory occlusion after the completion of contraction than after the completion of contraction when the triceps surae muscles were freely perfused. Specifically, mean arterial pressure averaged 124 ± 4 mmHg during circulatory occlusion and 120 ± 6 mmHg during the time in which the muscles were freely perfused (n = 8). The two arterial pressures were compared at the same time after the completion of contraction (i.e., 35 s).

Tendon stretch. Stretching the calcaneal (Achilles) tendon increased the discharge of 12 of the 28 renal sympathetic efferents tested. When averaged across the group of 28 efferents, tendon stretch increased activity from 0.05 ± 0.02 to 0.20 ± 0.05 impulses/s (P < 0.05). The onset latency of the renal sympathetic efferents responsive to tendon stretch averaged 1.6 ± 0.2 s (n = 12). There was a significant tendency for renal sympathetic efferents responsive to stretch to be nonresponsive to MLR stimulation (Table 1; P < 0.05). In addition, there was a significant tendency for renal sympathetic efferents responsive to stretch to be also responsive to static contraction (Table 1; P < 0.05). Tendon stretch significantly increased mean arterial pressure (from 112 ± 4 to 129 ± 5 mmHg, P < 0.05) and heart rate (from 179 ± 9 to 184 ± 9 beats/min, P < 0.05). Peak developed tension averaged 3.3 ± 0.2 kg.

Finally, the onset latencies of the renal sympathetic efferents responding to MLR stimulation were significantly shorter (P < 0.05) than the onset latencies of the renal sympathetic efferents responding to tendon stretch or static contraction. However, the onset latencies of the renal sympathetic efferents responding to tendon stretch were not significantly different from the onset latencies of the sympathetic efferents responding to static contraction.

DISCUSSION

The present study has shown for the first time that MLR stimulation and the muscle reflex activate, for the most part, separate populations of renal sympa-
thetic postganglionic efferents. In addition, static contraction while the triceps surae muscles were freely perfused, static contraction during circulatory arrest, and stretch of the calcaneal tendon. Histograms show discharge of a sympathetic efferent before, during (horizontal filled bar, 60 s), and after stimulation of the MLR (A), stretch of the calcaneal tendon (B), and static contraction of the triceps surae muscles (C and D). Arrows on histograms correspond to point in neurogram (a–d). Right: arterial blood pressure (mmHg), renal nerve activity, and airflow (inspiration upward) during stimulation of the MLR (A), during tendon stretch (B), and during static contraction (C and D). In D, thin horizontal bar represents time when circulation was arrested and thick horizontal bar represents time when triceps surae muscles were contracted. Circulatory arrest was maintained during the contraction period. Time in D is longer than times in A–C. T, tension in kg.

Renal sympathetic nerves synapse onto three effectors in the kidney, namely, the juxtaglomerular apparatus, the distal and proximal tubules, and vascular smooth muscle (3). Activation of renal sympathetic efferents increases renin secretion from the granular cells in the juxtaglomerular apparatus, sodium reabsorption in tubule cells, and vascular resistance by contracting smooth muscle (4, 5, 12, 14, 22). Mild-to-moderate increases in RSNA activate the renin-angiotensin system and increase sodium reabsorption but do not cause vasoconstriction (5, 14). Larger increases in RSNA result in increased renal vascular resistance via vasoconstriction in addition to further increasing renin secretion and sodium reabsorption (12).

The link between the intensity of RSNA and its effects on the kidney has led to the theory that each renal sympathetic efferent is capable of causing renin secretion, sodium reabsorption, and vasoconstriction but that the specific effect is determined by its discharge pattern (8). Studies using a constant voltage to stimulate the renal nerve, a maneuver that is expected to recruit a constant number of axons, have shown that the three renal effectors responded in a frequency-dependent manner. Specifically, low-frequency stimu-
ulation (0.25 – 1.5 Hz) resulted in an increased renin secretion (26); moderate-frequency stimulation (1.0–3.0 Hz) resulted in a further increase in renin secretion and a decrease in sodium excretion; and high-frequency stimulation (3–7 Hz) further increased renin secretion, further decreased sodium excretion, and decreased renal blood flow (2, 13).

Using anatomic tracing techniques, Barajas and Muller (1) found instances where a single renal sympathetic fiber synapsed with juxtaglomerular cells, renal tubules, and renal arterioles as well as instances where a single renal sympathetic fiber synapsed onto only one renal effector. Unfortunately, the sample sizes were small, and little quantification was provided by the authors. However, they were able to show that the renal vasculature had 4.5 times more neuroeffector junctions than did the renal tubules. The findings of Barajas and Muller suggested that the renal vasculature receives far more innervation than do the other renal effectors. The fact that the renal vasculature appears to have the greatest level of innervation, yet requires the highest frequency of nerve activity to change renal vascular resistance, suggests that the activation threshold of each renal effector differs substantially.

Although there have been several attempts to define in humans the roles played by central command and the muscle reflex in the control of sympathetic outflow to limb skeletal muscle (27–29, 33, 35, 36) and skin (27, 36, 37), there have been few indirect attempts to define the roles played by these neural mechanisms in the control of sympathetic outflow to the kidney. For example, Middlekauff et al. (20) found that static handgrip decreased renal cortical blood flow and increased renal cortical vascular resistance. The early aspect of these renal cortical vascular responses to exercise was attributed to central command or muscle mechanoreflex. The late aspect was attributed to a muscle metaboreflex, which was evoked by postexercise circulatory arrest. Recently, the mechanoreflex was found to be exaggerated in heart failure (19). In addition, Tidgren et al. (32) found that dynamic exercise increased the concentrations of norepinephrine and renin in the renal venous blood but were not able to identify the neural mechanisms causing these responses.

Studies in conscious animals support the notion that central command and the muscle reflex stimulate sympathetic postganglionic discharge to the kidney (15, 23–25). For example, O’Hagan et al. (24) observed in rabbits an increase in RSNA 2–4 s before the start of dynamic exercise; this increase was attributed to central command. Matsukawa et al. (15) showed that static contractions in operantly trained cats elicited an initial and a late component of RSNA. The initial component occurred at or before the development of force and lasted for ~10 s. Matsukawa et al. attributed this component to central command but could not rule out the muscle mechanoreflex as a contributing factor once force development occurred. The late component occurred at 14 s and was sustained for the remainder of the contraction. The authors attributed this component to the muscle metaboreflex.

The present study has two limitations. First, we could not determine the function(s) of the sympathetic efferents from which we recorded. We assume that at least a portion were vasomotor in nature. Second, the levels of stimulation evoking central command and the exercise pressor reflex were relatively low. Specifically, during MLR stimulation (i.e., central command), it is not uncommon to evoke pressor responses of 60 mmHg (6), whereas in our studies we evoked pressor responses averaging 27 mmHg. This lower pressor response is due to lower frequency and current of stimulation as well as denervation of one kidney. High levels of MLR stimulation may have activated efferent fibers that only responded to reflex activation. Nevertheless, the pressor responses evoked in our experiments would be consistent with changes in blood pressure in humans during routine behavior.

Our use of a decerebrate preparation in which a central command to locomote and the exercise pressor reflex were elicited separately has yielded some unique insights about how the sympathetic nervous system might be controlled during exercise. Previously, we found that the sympathetic outflow to the vasculature of hindlimb skeletal muscle was activated by the exercise pressor reflex but not by central command (9). Subsequently, we found that the sympathetic outflow to the vasculature of the hairy skin was activated by central command but not by the exercise pressor reflex (10). Presently, we found that the sympathetic outflow to the kidney was activated by central command and the exercise pressor reflex but that the two neural mechanisms were linked to separate populations of renal postganglionic efferent fibers. Considered together, these findings raise the possibility that central command and the exercise pressor reflex do not function in a redundant manner. Nevertheless, redundancy may occur at high levels of exercise or at autonomic outflows other than those investigated in the present and prior studies (9, 10). Obviously, a thorough test of this redundancy hypothesis awaits the examination of single-fiber sympathetic discharge in response to near-maximal levels of central command and elicitation of the exercise pressor reflex.

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REFERENCES


