Spinoreticular neurons that receive group III input are inhibited by MLR stimulation

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Degtyarenko, Alexandr M., and Marc P. Kaufman. Spinoreticular neurons that receive group III input are inhibited by MLR stimulation. J Appl Physiol 93: 92–98, 2002. First published March 22, 2002; 10.1152/japplphysiol.00072.2002.—In decerebrate paralyzed cats, we examined the responses of 18 spinoreticular neurons to electrical stimulation of the mesencephalic locomotor region. The activity of each of the spinoreticular neurons was recorded extracellularly from laminae IV through VI of the L7 and S1 spinal cord. In addition, each of the 18 spinoreticular neurons received group III afferent input from the tibial nerve. Spinoreticular projections were established for each of 18 neurons by antidromic invasion of the ventro lateral medulla at the P11 though P14 levels. The onset latencies and current thresholds for antidromic invasion from the ventro lateral medulla averaged 15.0 ± 3.8 ms and 117 ± 11 μA, respectively. Electrical stimulation of the mesencephalic locomotor region attenuated the spontaneous activity or the responses of each of the spinoreticular neurons to tibial nerve stimulation at currents that recruited group III afferents. Our data support the notion that thin-fiber muscle afferent input to the ventrolateral medulla is gated by a central command to exercise. cats evoked increases in mean arterial pressure, heart rate, and ventilation that were less than the sum of the increases evoked by separate activation of the two mechanisms (37, 40). A “ceiling effect” was not responsible for this finding because high-intensity electrical stimulation of the sciatic nerve evoked increases in these variables that were greater than the sum of the increases evoked by separate activation of central command and the exercise pressor reflex (37, 40).

One possible explanation for the finding that simultaneous activation of central command and the exercise pressor reflex did not sum algebraically may be that the former mechanism inhibited the latter. In addition, the anatomic site of this inhibition may be the dorsal horn of the spinal cord. Recently, we have provided support for this possibility by showing that stimulation of the MLR inhibited the activity of dorsal horn neurons receiving input from group III hindlimb muscle afferents (14, 15).

Further support for this possibility would include evidence that stimulation of the MLR inhibits the discharge of dorsal horn neurons that project to the ventrolateral medulla (VLM). The integrity of this brain stem area has been shown to be essential for expression of the exercise pressor reflex (28). Consequently, we recorded the impulse activity of spinoreticular neurons receiving input from group III hindlimb muscle afferents while stimulating the MLR. We found that stimulation of this midbrain site inhibited the discharge of spinoreticular neurons.

METHODS

General. Adult cats of either sex (2.2–3.5 kg) were anesthetized with a mixture of halothane (3–4%) and oxygen. Once anesthetized, the cats breathed the anesthetic gas mixture through a nose cone, while the trachea, one common carotid artery, and the left jugular vein were cannulated. The lungs were then ventilated with the anesthetic gas mixture through the tracheal cannula. The remaining carotid artery was ligated in preparation for the decerebration. Body temperature was maintained near 37°C with a heating pad and lamp. The cat was placed in a Kopf stereotaxic and spinal unit. The right hindlimb was extensively denervated, and the common peroneal nerve was dissected free and cut, and its
central end was mounted on bipolar stainless steel recording electrodes. The tibial nerve was dissected free, kept intact, and mounted on a stimulating electrode. The ipsilateral calcaneal (Achilles) tendon was cut. A laminectomy exposing spinal segments L4–S2 was performed and was followed by a precollicular postmammillary decerebration. All neural tissue rostral to the section was removed, bleeding was controlled, and the cranial vault was filled with agar. After the decerebration procedure was completed, the lungs were ventilated with a mixture of room air and oxygen. Skin flaps surrounding the spinal cord and the hindlimb nerves were used to construct warm (37°C) mineral oil pools. Arterial blood gases were measured and maintained within normal limits by either adjusting ventilation or injecting sodium bicarbonate (8.5% iv).

A stainless steel monopolar electrode was placed into the MLR (coordinates: P2, L4, HC-1) for delivery of monophasic electrical pulses (parameters of stimulation: 20–50 Hz; 0.2–0.5 ms; 100–150 μA). The optimal position of the MLR electrode was judged by the appearance of efferent activity in the peroneal nerve with a low intensity of stimulation. Extracellular impulses from dorsal horn neurons were recorded from each L7 or S1 spinal segments with tungsten microelectrodes (Frederick Haer) with tip impedances at 1,000 Hz of 4–12 MΩ. The electrodes were connected to a high-impedance probe, which in turn was connected to a preamplifier (PBA-1, Frederick Haer). Filters were set at 100–5,000 Hz. Electrodes recording efferent discharge from the muscle nerves were connected to a high-impedance probe (HIP 511, Grass), which in turn was connected to a preamplifier (511, Grass). Filters were set at 100–3,000 Hz. Arterial blood pressure was measured from the carotid arterial cannula, which in turn was connected to a Statham transducer (P23XL). All signals were written on a chart recorder (Gould) and also recorded on videotape after being digitized (Vetter). Activity from the dorsal horn was displayed on a storage oscilloscope. Extracellular records were superimposed offline by using a RC-Electronics program. We measured latency of responses of the dorsal horn neurons to electrical stimulation of the tibial nerve from stimulus onset.

Protocols. Cats were first paralyzed with vecuronium bromide (0.1 mg/kg iv), which was supplemented every 30 min. Then, activity of dorsal horn neurons that received group III afferent input was identified by stimulating (1 Hz; 0.25–0.5 ms) the tibial nerve at current intensities that were 8–30 times threshold. As the tibial nerve was stimulated, we advanced the recording electrode through the dorsal horn. In this way, we searched for both spontaneously active and silent neurons.

A minimum of 10 stimulus presentations were recorded if a neuron was suspected of receiving group III afferent input from the tibial nerve. We considered a neuron as receiving group III afferent input if the latency of response was at least 6–7 ms and the intensity for activation was greater than eight times threshold. Group III afferents are known to be stimulated by currents equal to eight times motor threshold, and, given that the conduction distance between the stimulating and recording electrode was ~150 mm, dorsal horn neurons that received group III inputs should respond with a latency of at least 6 ms. In some experiments, we either stretched the calcaneal tendon or gently squeezed the ipsilateral triceps surae muscles to help identify group III muscle afferent input to the dorsal horn neurons.

After identifying a dorsal horn neuron that received group III afferent input, we used the antidromic invasion technique to determine whether its axon projected to the VLM. To accomplish this, we inserted stainless steel monopolar electrodes into the contralateral and ipsilateral VLM (P11 through P14 levels). Pulses of no more than 50–150 μA (0.2–0.5 ms) with a frequency of 1–2 Hz were passed through the VLM electrodes in an attempt to antidromically invade dorsal horn neurons. If a constant onset latency was found in response to the pulses applied to the VLM electrode, frequency-following (i.e., 333 Hz) and collision tests were performed. For the latter, the antidromic impulse was evoked by VLM stimulation, and the orthodromic impulse was either spontaneous or, more frequently, evoked by tibial nerve stimulation at an intensity that recruited group III afferents.

Next, the effect of electrical stimulation of the MLR on the discharge of the neurons was examined. Typically, the MLR

![Fig. 1. Line drawings of the medulla showing the location of the stimulating electrode tips for each of the 18 spinoreticular cells antidromically activated from the ventrolateral medulla (VLM).](http://jap.physiology.org)
was stimulated for 5–30 s. If an effect on discharge of the cell was observed, the stimulus was turned off for 2–3 min and then repeated. The sequence was repeated two to four times until a clear pattern of effect emerged.

We used a conditioning-test protocol to determine the time course and efficacy of the MLR-induced inhibition of dorsal horn neuronal discharge. The conditioning stimulus was a brief train of 3–11 pulses (250–300 Hz) applied to the MLR. The test response was evoked from the dorsal horn neuron by stimulating the tibial nerve with a current intensity of at least eight times the threshold. At least five single stimuli (1 Hz) were applied to the tibial nerve to determine the firing index, which was calculated as the average number of impulses discharged by the dorsal horn neuron in response to tibial nerve stimulation. The ratio of the firing index of the neuron with and without the conditioning stimuli was used as a measure of the inhibition of the dorsal horn neuron’s responses to tibial nerve stimulation by the MLR. The conditioning-testing interval was the time period between the first pulse applied to the MLR and the pulse applied to the tibial nerve.

Silent neurons were activated electrically by stimulating the tibial nerve at 1 Hz. Current intensities and pulse duration were the same as those used when the neurons were initially identified. At the end of the experiment, electrolytic lesions were made to mark the position of the stimulating electrodes in the VLM. The electrode localizations were verified histologically. Values are reported as means ± SE. We performed paired t-tests to determine statistical significance. The criterion level was set at P < 0.05.

RESULTS

We recorded the impulse activity of 18 spinoreticular neurons, each of which was antidromically activated from stimulation sites in the VLM (Figs. 1 and 2). Anatomically, most of these sites were in the lateral reticular nucleus (LRN). Five of the 18 stimulation sites in the VLM were contralateral to the recording sites in the dorsal horn. The latency and threshold current for antidromic activation averaged 15.0 ± 3.8 ms and 117 ± 11 μA, respectively (n = 18). The conduction velocity of each of the axons of these spinoreticular neurons was calculated and averaged 31.1 ± 3.9 m/s (range: 4.5–57.5 m/s) (Fig. 3). The recording sites were located on average 1.85 ± 0.06 mm from the surface of the L7 or S1 spinal cord. Most of these locations corresponded to laminae V and VI of the dorsal horn (Fig. 4).

Each of the 18 spinoreticular neurons was activated by electrical stimulation of the ipsilateral tibial nerve. The onset latency and current intensity, respectively, averaged 7.8 ± 0.4 ms and 11.6 ± 0.9 times that needed to elicit a muscle twitch (n = 18), which indicated that these dorsal horn neurons received group III afferent input. We attempted to mechanically activate
Fig. 5. Effect of MLR stimulation on the responses of a spinoreticular neuron to stimulation (1 Hz) of the tibial nerve at a current intensity that recruited group III fibers. Onset and offset of MLR stimulation is depicted by the downward and upward arrows, respectively. Large vertical lines in the middle extracellular (EC) trace represent stimulus artifacts and are superimposed onto the impulse activity of the spinoreticular cell. In bottom EC (traces appearing vertically), 1 represents 10 superimposed stimulus presentations to the tibial nerve before MLR stimulation (note that the 10-stimulus presentations evoked a total of 10 impulses); 2 represents 6 superimposed stimulus presentations to the tibial nerve −2 s after MLR stimulation commenced (note that the 6 presentations evoked only 1 impulse; 3 represents 10 superimposed stimulus presentations to the tibial nerve −10 s after MLR stimulation commenced (note that the 10 presentations evoked a total of 7 impulses); and 4 represents 10 superimposed stimulus presentations to the tibial nerve beginning −1 s after MLR stimulation ended. Also note that the 10 presentations evoked 10 impulses. Consequently, the spinoreticular neuron’s response to tibial nerve stimulation was strongly inhibited in 2 but only weakly in 3. Per, neuronal discharge from the cut central end of the common peroneal nerve.

Fig. 6. Stimulation of the MLR inhibits both the spontaneous (A) and evoked (B) activity of a spinoreticular neuron. Note that in both A and B, the inhibition induced by MLR stimulation lasted longer than the stimulus applied to this midbrain site. Note that the large vertical lines in B arise from the stimulus artifact applied to the tibial nerve. Also note that A and B are from the same spinoreticular neuron. ABP, arterial blood pressure.
10 of the 18 spinoreticular neurons. We were successful in eight and found that three responded to stretching the calcaneal tendon, two responded to gently squeezing the triceps surae muscles, and three responded to both maneuvers.

Stimulation of the MLR inhibited the discharge of each of the 18 spinoreticular neurons, regardless of whether this discharge was spontaneous or was evoked by stimulation of group III afferents in the tibial nerve. Two patterns of MLR-induced inhibition of discharge were seen. In the first, which was shown by three dorsal horn neurons, the evoked responses to tibial nerve stimulation were inhibited only during the initial period of MLR stimulation (Fig. 5). In the second pattern, which was shown by 15 neurons, the MLR-induced inhibition lasted for at least as long as the stimulation period (n = 12) and sometimes outlasted the stimulation period by 10–20 s (n = 3) (Fig. 6).

In two spinoreticular neurons, we determined the minimum conditioning-test interval needed to elicit maximum MLR-induced inhibition of the cells’ responses to tibial nerve stimulation. The minimum interval for the two neurons was 17 and 40 ms. The duration of this inhibition was 232 and 40 ms (Fig. 7).

DISCUSSION

We have shown that the stimulation of the MLR, a maneuver that elicits a central command to exercise (18), inhibited the evoked discharge of spinoreticular neurons that receive group III afferent input. This finding is consistent with previous findings from our laboratory in which we have shown that MLR stimulation inhibited group III and IV muscle afferent input onto dorsal horn neurons located in both the superficial and deep laminae of the dorsal horn (14–16). We have also shown that GABA functions as one of the neurotransmitters causing this MLR-induced inhibition of dorsal horn neuronal discharge (17).

When attempting to antidromically invade neurons, one must always be concerned about spread of the electrical stimulus. The mean currents that we used to antidromically invade spinoreticular neurons (i.e., 117 ± 11 μA) were considerably less than those used by others to invade these neurons in cats (7, 22, 38). Previously, Bagshaw and Evans (3) found that a 100-μA pulse, which approximates the average current used in our experiments, spread over a radius of 0.5 mm. This finding offers us a useful context with which to judge stimulus spread. Consequently, axons that were antidromically activated in our experiments were not far from the stimulus site within the VLM. Despite the fact that we used relatively small currents to antidromically invade spinoreticular neurons, the calculated conduction velocities that we reported were remarkably similar to those reported by others (7, 22, 38).

There is strong neuroanatomic evidence that dorsal horn neurons project to the VLM (13, 33). Indeed, the largest projection is believed to arise from the dorsal horn of the spinal cord (13). Spinoreticular neurons are widely distributed throughout the entire length of the cord. The projection is both crossed and uncrossed, and cell bodies of spinoreticular neurons are found in laminae II through VIII of the spinal gray matter (13, 33).

Spinoreticular neurons projecting to the VLM may have a particular significance for the exercise pressor reflex. Specifically, the integrity of the VLM is essential for the full expression of the exercise pressor reflex (26, 28) as well as for the pressor reflex evoked by electrical stimulation of the sciatic nerve (11). Moreover, medullary neurons in the VLM have been shown to respond to static muscular contraction. Some of these projected to the spinal cord and also discharged in synchrony with the sympathetic nerves (4, 5, 25). Finally, studies using anatomic techniques involving either metabolism-induced uptake or gene expression have shown that neurons in the VLM are activated by static muscular contraction (27, 32).

Our findings might explain the report by Arshavsky et al. (2) that the responses of VLM neurons to high-intensity electrical stimulation of the radial nerve were suppressed by actual locomotion. Likewise, our find-
ings might explain the report by Eldridge et al. (18), who found that the cardiovascular and ventilatory responses to spontaneous locomotion were not different from the cardiovascular and ventilatory responses to fictive locomotion. We speculate that activation of the MLR during actual locomotion suppressed transmission from thin-fiber afferents to spinoreticular neurons in the data reported by both Arshavsky et al. (2) and Eldridge et al. (18).

The pathways responsible for MLR-induced suppression of transmission from group III muscle afferents to spinoreticular neurons are not known. However, electrical stimulation of the ventromedial reticular formation has been shown to inhibit transmission from thin-fiber muscle and skin afferents to dorsal horn neurons with ascending projections (19). Moreover, the time course of the inhibition of transmission from thin-fiber afferents to ascending spinal pathways (19) was similar to that reported by us when we stimulated the MLR. Furthermore, this midbrain region has been shown electrophysiologically to have short latency connections with ventromedial neurons having descending spinal projections (23).

From a classical point of view, the LRN is thought of as a brain stem area that projects solely to the ipsilateral cerebellum (9, 10). This projection can be the source of some confusion because the exercise pressor reflex arc is known to include the area in and surrounding the LRN (26), but it does not include the cerebellum (28). Although many of our antidromic stimulation sites were located anatomically within the cytoarchitectural boundary of the LRN, we would prefer to call this area the VLM because of its relevance to the exercise pressor reflex arc.

Nevertheless, the LRN, named for its location rather than for its projection to the cerebellum, is an important integratory site for control of the cardiovascular system. For example, electrical stimulation of the LRN increases arterial pressure and heart rate (11). In addition, bilateral lesion of the LRN eliminated the pressor responses to stimulation of the carotid sinus nerve (11), to stimulation of the sciatic nerve (11), to stimulation of the posterior hypothalamus (11), and to the static contraction of hindlimb muscles (26). Fictive locomotion induced by MLR stimulation has been shown to activate LRN neurons discharging with ventilatory- and/or locomotor-related rhythms (20). Other electrophysiological studies have shown a high degree of convergence of posterior hypothalamic, carotid chemoreceptor, and sciatric nerve input onto single LRN neurons (11). Finally, neurons in the LRN have been shown to project to the intermediolateral horn of the spinal cord (24) as well as to receive projections from the nucleus tractus solitarius (36).

In cats, both thoracic and lumbar spinoreticular neurons appear to project heavily to the medial reticular formation (8, 21). These neurons have been found to be responsive to nociceptive inputs that arise from the heart (7, 8) as well as from hindlimb skin (21). We suggest that this population of spinoreticular neurons that project to the medial reticular formation of the medulla are distinct from those found by us as well as those found by Thies (38), all of which projected to the VLM. In addition, we offer the speculation that dorsal horn neurons projecting to the medial reticular formation participate in transmitting the sensation of pain, whereas dorsal horn neurons projecting to the VLM participate in regulating the cardiovascular system.

Our studies are based on the assumption that both the MLR (i.e., central command) and the exercise pressor reflex function during exercise. Obviously, both mechanisms must be operative for them to interact, which is the underlying rationale of our studies. The finding that group III and IV muscle afferents are stimulated by low levels of dynamic exercise (1) supports the notion that the reflex mechanism is functional. Likewise, the recent finding that cells in the cuneiform nucleus are activated by dynamic exercise is consistent with the notion that the MLR functions as part of a central command to exercise (29). We think it reasonable, therefore, to speculate that the MLR and the exercise pressor reflex interact. The nature of this interaction might well be that the MLR gates the discharge of spinoreticular neurons stimulated by the exercise-induced stimulation of group III and IV muscle afferents.

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