Effect on airway caliber of stimulation of the hypothalamic locomotor region

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Effect on airway caliber of stimulation of the hypothalamic locomotor region. J. Appl. Physiol. 84(4): 1388–1394, 1998.—Airway dilation is one of the many autonomic responses to exercise. Two neural mechanisms are believed to evoke these responses: central command and the muscle reflex. Previously, we found that activation of central command, evoked by electrical and chemical stimulation of the mesencephalic locomotor region, constricted the airways rather than dilated them. In the present study we examined in decerebrate paralyzed cats the role played by the hypothalamic locomotor region, the activation of which also evokes central command, in causing the airway dilator response to exercise. We found that activation of the hypothalamic locomotor region by electrical and chemical stimuli evoked fictive locomotion and, for the most part, airway constriction. Fictive locomotion also occurred spontaneously, and this too, for the most part, was accompanied by airway constriction. We conclude that central command plays a minor role in the airway dilator response to exercise.

DYNAMIC EXERCISE EVOKES a large number of cardiovascular and respiratory responses (19), one of which is airway dilation (8, 15, 27). The neural mechanism responsible for the exercise-induced airway dilation is not known, but central command is an important candidate and is defined as the parallel activation of locomotor, ventilatory, and autonomic circuits at the onset of exercise (12). Central command is not dependent on feedback from the periphery (5).

In animals, central command can be simulated by activation of two sites: the cuneiform nucleus of the midbrain (5, 6, 21) and in or near the H2 field of Forel of the posterior hypothalamus (5, 23). The former site has been termed the mesencephalic locomotor region and the latter the hypothalamic locomotor region (HLR) (25). In a recent study our laboratory showed that electrical and chemical stimulation of the mesencephalic locomotor region increased total lung resistance, an effect that was caused by the activation of cholinergic receptors on airway smooth muscle (16).

This finding was surprising because it provided no support for the hypothesis that central command contributed to the airway dilation evoked by dynamic exercise. In the present study we have sought support for this hypothesis by stimulating the other central command site, i.e., the HLR. In addition, we have examined the effect of fictive locomotion, occurring spontaneously, on airway caliber.

METHODS

General. For anesthesia, cats were placed in a plastic box into which flowed a halothane (5%-nitrous oxide and oxygen gas mixture. After they were anesthetized, the cats were removed from the box and inhaled the gas mixture through a nose cone. The cervical trachea was cannulated, and the lungs were ventilated mechanically with a halothane (3%)–nitrous oxide and oxygen gas mixture. One common carotid artery and jugular vein were cannulated. The chest was opened by placing bilateral incisions between the ribs. The expiratory outlet of the ventilator was placed under 1–2 cm H2O to prevent collapse of the lungs. The cats were placed in a Kopf stereotaxic instrument and were then decerebrated by a blunt spatula passed at a 70° angle through the brain stem. The incision started 7 mm anterior to the sulcus between the superior and inferior colliculi. All neural tissue rostral to the section was removed, bleeding was controlled, and the cranial vault was filled with agar. After the decerebration the halothane and nitrous oxide were removed from the gas mixture ventilating the lungs and were replaced with room air, which was supplemented with oxygen.

The biceps femoris nerve was exposed and sectioned. The central cut end of this nerve, which supplies the hamstring muscles of the hindlimb, was covered with petroleum jelly and mineral oil and was placed on a bipolar hook recording electrode. Similarly, the C5 rootlet of the phrenic nerve was exposed and cut, and its central end was placed on a bipolar hook recording electrode. Both electrodes were connected to high-impedance probes (model HIP511, Grass), which in turn were connected to preamplifiers (model P511, Grass). The signals from both nerves were viewed on an oscilloscope and integrated (see below).

Airflow was measured by a heated pneumotach (no. 00, Fleisch) that was placed between the ventilator and the tracheal cannula. The pneumotach was connected to a differential pressure transducer (model DP45-24, Validyne). Transpulmonary pressure was measured with a differential pressure transducer (model DP45-14, Validyne), one end of which was connected to a side port in the tracheal cannula; the other end was left open to the atmosphere. Total lung resistance and dynamic compliance were calculated breath by breath with a P-one-mah digital acquisition, analysis, and archive system (version 1.0). The method of Amdur and Mead (2) formed the basis for these calculations. Arterial blood pressure was measured by connecting the cannula in the carotid artery to a transducer (model P23XL, Statham). Heart rate was calculated beat to beat from the arterial pressure pulse by the Po-ne-mah system.

Protocols. Before attempting to collect any data, we paralyzed the cats with pipercuronium bromide (0.2 mg/kg iv). We then stimulated the HLR (10–20 Hz, 0.75 ms, 50–150 µA) and selected the minimum current intensities that produced fictive locomotion. Our index of airway caliber was total lung resistance. The HLR was stimulated with a monopolar stainless steel electrode (model SNE-100, Rhodes); the indifferent electrode was an alligator clip attached to the scalp. A Grass S88 stimulator attached to a PSIU-6 constant-current unit.
was used to pass current through the stimulating electrode. At the end of the study, anodal current (4 mA, 10 s) was passed through the electrode tip to mark stimulation sites. The Prussian blue reaction was used to visualize sites.

In three cats the HLR was stimulated chemically as well as electrically. Specifically, picrotoxin (8 mM) was microinjected through one barrel of a double-barreled glass pipette, which in turn was connected to a picospritzer (General Valve). In two cats the volume microinjected into the HLR was 200 nl, and in the remaining one it was 100 nl. The interval between microinjection of picrotoxin and subsequent electrical stimulation was ~20 min. The other barrel of the pipette contained Chicago sky blue (2%), which was microinjected (100 nl) at the end of the study to mark the site. The HLR was identified using functional criteria, i.e., the elicitation of rhythmic bursts of activity from the biceps femoris nerve (i.e., fictive locomotion) and increases in arterial pressure, heart rate, and phrenic nerve discharge when the posterior hypothalamus was stimulated chemically or electrically (5, 25). In some instances the tibial nerve was stimulated electrically (10–20 Hz; 0.75 ms; 8 mA) and total lung resistance was assessed.

Data analysis. Control values for total lung resistance and dynamic compliance were determined by averaging values “breath by breath” for the 20 ventilatory cycles preceding the onset of stimulation. The peak response to stimulation was determined by averaging five consecutive “breaths” that displayed the largest change from control values during fictive locomotion. Control values for arterial pressure and heart rate were taken as the steady-state values; peak responses were taken as the highest values observed during fictive locomotion. Phrenic nerve discharge was integrated and quantified using the method described by Eldridge (4).

RESULTS

Electrical stimulation of the HLR. Electrical stimulation of the posterior hypothalamus evoked fictive locomotion in 23 paralyzed, decerebrate cats. On average, total lung resistance increased during fictive locomotion from a baseline value of 29.0 ± 1.4 to a peak value of 31.6 ± 1.8 cmH₂O·l⁻¹·s⁻¹ (P < 0.01, n = 23). Dynamic compliance decreased during fictive locomotion in these 23 cats from a baseline value of 4.08 ± 0.18 to a peak value of 3.87 ± 0.19 ml/cmH₂O (P < 0.01). As expected, mean arterial pressure increased (from 143 ± 5 to 194 ± 5 mmHg, P < 0.01) as did heart rate (from 210 ± 1389

**Fig. 1.** Airway, phrenic nerve, and cardiovascular responses to electrical stimulation of hypothalamic locomotor region. A: data from 16 cats in which stimulation of hypothalamic locomotor region caused airways to constrict. B: data from 7 cats in which stimulation of this region caused airways to dilate. RL, total lung resistance; Cdyn, dynamic compliance; MAP, mean arterial pressure; HR, heart rate; Loc, locomotion; bpm, beats/min. *Significantly different (P < 0.05) from baseline.
9 to 244 ± 8 beats/min, P < 0.01) and phrenic nerve discharge (187 ± 15% of baseline, P < 0.01).

In 16 of the 23 cats studied, total lung resistance increased during fictive locomotion, whereas in the remaining 7 cats, total lung resistance decreased (Figs. 1, 2, A and B). The delay between the onset of fictive locomotion and the onset of the increase in total lung resistance (n = 16) was 2.1 ± 0.9 s. Similarly, the delay between the onset of fictive locomotion and the onset of the pressor response in cats displaying an increase in total lung resistance was only 0.1 ± 0.3 s.

The delay between the onset of fictive locomotion and the onset of the decrease in total lung resistance (n = 7) was −1.9 ± 1.1 s. In other words, the decrease in total lung resistance began 1.9 ± 1.1 s before the onset of fictive locomotion in the cats showing a decrease in total lung resistance. Moreover, the increase in arterial pressure began 3.3 ± 1.7 s before the onset of fictive locomotion.

We examined the effects of nadolol (1 mg/kg iv), a β-adrenergic antagonist, followed by atropine methyl nitrate (1 mg/kg iv), a muscarinic antagonist, on the airway, phrenic, and cardiovascular responses to fictive locomotion. These antagonists were given sequentially to 8 of the 16 cats showing an increase in total lung resistance in response to fictive locomotion (Fig. 4) as well as to 4 of the 7 cats showing a decrease in total lung resistance (Fig. 5). We found that nadolol increased the airway constriction evoked by fictive locomotion in the former group of cats and converted the airway dilation evoked by fictive locomotion to a constriction in the latter group. Atropine abolished all airway effects evoked by fictive locomotion.

Chemical stimulation of the HLR. In three cats, we compared the airway, phrenic, and cardiovascular responses to microinjection of picrotoxin (8 mM) into the HLR with the responses to electrical stimulation of the
HLR. In each of the three cats tested, picrotoxin microinjection and electrical stimulation increased total lung resistance, increased mean arterial pressure, increased heart rate, increased phrenic nerve discharge, and decreased dynamic compliance (Fig. 2, C–F). The picrotoxin-induced fictive locomotion was long lasting and averaged 23.7 ± 3.7 min. Fictive locomotion started 7 ± 1.7 min after the last microinjection of picrotoxin.

Spontaneous fictive locomotion. In seven paralyzed cats, fictive locomotion occurred spontaneously, i.e., without electrical or chemical stimulation of the HLR. In five of these cats, total lung resistance increased significantly (*P*, 0.05; Table 1, Fig. 3C), whereas in
two it decreased slightly (data not shown). Fictive locomotion evoked by electrical stimulation of the HLR changed total lung resistance in the same direction as did spontaneous fictive locomotion in six of the cats (Fig. 3B). In the one remaining cat we were unable to evoke fictive locomotion by electrical stimulation of the HLR, even though it occurred spontaneously. In each of the seven cats studied, spontaneous and electrically induced fictive locomotion increased mean arterial pressure, heart rate, and phrenic nerve discharge. The magnitude of these increases appeared to be the same as in cats displaying an increase in total lung resistance and in cats displaying a decrease in total lung resistance.

Histology. Histological analysis revealed that all sites, stimulated electrically or chemically, were in the HLR as defined previously (5, 25) (Fig. 6).

Table 1. Effects of spontaneous fictive locomotion and fictive locomotion induced by electrical stimulation of HLR on airway, ventilatory, and cardiovascular function

<table>
<thead>
<tr>
<th></th>
<th>SF (n = 5)</th>
<th>HLR (n = 4)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Peak response</td>
</tr>
<tr>
<td>( R_L ), cmH(_2)O·l(^{-1})·s(^{-1})</td>
<td>30.9 ± 4.5*</td>
<td>35.1 ± 5.8</td>
</tr>
<tr>
<td>( C_{dyn} ), ml/cmH(_2)O</td>
<td>4.17 ± 0.65*</td>
<td>3.97 ± 0.60</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>147 ± 12.4*</td>
<td>173 ± 9.4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>227 ± 7.6*</td>
<td>246 ± 18.6</td>
</tr>
<tr>
<td>PNA, %baseline</td>
<td>100</td>
<td>134 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE from 5 cats displaying an increase in total lung resistance (RL) during spontaneous fictive (SF) locomotion; in 1 cat, electrical stimulation did not evoke fictive locomotion; thus data from hypothalamic locomotor region (HLR) are from 4 cats. \( C_{dyn} \), dynamic compliance; MAP, mean arterial pressure; HR, heart rate; PNA, phrenic nerve activity. *Significantly different (\( P < 0.05 \)) from peak response.

DISCUSSION

We have examined the role of the HLR in the control of airway caliber. Specifically, we tested the hypothesis that activation of this region is responsible for the airway dilation seen during dynamic exercise (8, 15, 27). Our requirements for the successful activation of the HLR, and therefore central command, were based on functional criteria. These included increases in mean arterial pressure, increases in heart rate, increases in phrenic nerve discharge, and fictive locomotion, each of which must have occurred for the data to be included in our analysis.

Overall, our data were not supportive of the hypothesis that the HLR plays an important role in causing the airway dilation evoked by dynamic exercise. For example, in ~70% of the 23 cats tested, electrical...
stimulation of the HLR constricted the airways. When dilation of the airways did occur (i.e., in ~30% of cats), its magnitude was modest. In addition, the airway constriction evoked by electrical stimulation did not appear to be an artifact caused by the activation of fibers of passage, because the same effect was caused by microinjection of picrotoxin, a GABA antagonist, which functions by blocking chloride iontophores on cell bodies and dendrites (22).

When stimulation of the HLR did evoke airway dilation, the response was converted by β-adrenergic blockade to airway constriction and subsequently was abolished by atropine. We can offer two explanations for the mechanism causing the airway dilator response to HLR stimulation. First, stimulation of the HLR activated β-adrenergic receptors located on airway smooth muscle. This activation could occur by the release of transmitter from sympathetic postganglionic nerves or by the release of epinephrine from the adrenal gland (24). Second, the pressor response to HLR stimulation evoked the baroreflex, one component of which is airway dilation (20). We do not know which explanation is correct, but when airway dilation was evoked by HLR stimulation, the pressor response was, on average, 18 mmHg greater than that when airway constriction was evoked by HLR stimulation (Fig. 2).

We speculate that in the seven cats showing a dilator response to HLR stimulation, the large pressor effect may have been sufficient to overwhelm the airway constriction usually observed when this maneuver is initiated.

Nevertheless, electrical stimulation of the HLR for the most part evoked airway constriction, as evidenced by an increase in total lung resistance. This increase was not effected by β-adrenergic blockade but was abolished by muscarinic blockade. This finding is consistent with previous reports that the constrictor response to stimulation of the brain stem is caused by the activation of cholinergic pathways to airway smooth muscle (7, 17).

If central command is not the major neural mechanism causing the exercise-induced airway dilation, then what is? Two candidates come to mind: the Hering-Breuer reflex and the muscle reflex. Both evoke bronchodilation by withdrawal of cholinergic tone to airway smooth muscle (3, 10, 11, 13, 28). Slowly adapting stretch receptors comprise the afferent arm of the Hering-Breuer reflex arc. The activity of these slowly adapting receptors will be increased by exercise-induced increases in tidal volume and the rate of breathing. Similarly, group III and IV afferents comprise the afferent arm of the muscle reflex arc (14). The activity of these thin fiber afferents is known to be increased by dynamic exercise (1, 18).

Our conclusion that activation of the HLR is not the major mechanism causing exercise-induced airway dilation applies to the tracheobronchial tree but not to the upper airways. In our experiments the cats were paralyzed, which prevented neurotransmission from the HLR to the skeletal muscles. In addition, the trachea was cannulated below the larynx, which removed the upper airways from our calculations of total lung resistance and dynamic compliance. Consequently, the role of central command in the control of the upper airways during exercise remains to be determined.

The fact that the cats used in our experiments were paralyzed and ventilated had another consequence; i.e., feedback from pulmonary stretch receptors to brain stem neurons controlling airway caliber and breathing was abnormal. In turn, afferent input from these receptors reached the brain stem out of phase with central respiratory drive. Therefore, we cannot exclude the possibility that this factor was responsible for our inability to evoke major dilation of the airways during stimulation of the HLR. We were, however, able to evoke airway dilation in our preparation when the tibial nerve was stimulated electrically (present results) or when the triceps surae muscles were contracted statically (16).
There is no doubt that central command plays an important role in the control of cardiovascular and ventilatory function during exercise. Indeed, central command is thought to be the dominant mechanism causing the ventilatory response to exercise (9, 26). Nevertheless, central command appears to play a minor role in the airway smooth muscle dilation known to occur during dynamic exercise. This conclusion is based on the assumption that the hypothalamic and mesencephalic locomotor regions of the decerebrate unanesthetized cat comprise the neuroanatomic substrate for central command. Other sites in the brain stem may also be involved in the generation of central command, and their effects on airway caliber will need to be investigated as these sites are identified.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-40910.

Received 9 September 1997; accepted in final form 5 December 1997.

REFERENCES