Role of caudal ventrolateral medulla in reflex and central control of airway caliber

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PADRID, PHILIP A., JAMES R. HASELTON, AND MARC P. KAUFMAN. *Role of caudal ventrolateral medulla in reflex and central control of airway caliber.* J. Appl. Physiol. 71(6): 2274–2282, 1991.—We investigated the role played by the caudal ventrolateral (CVL) medulla in the reflex and central neural control of airway caliber in chloralose-anesthetized dogs. Changes in total lung resistance were evoked by four different stimuli. These changes were compared before and after bilateral injection of either ibotenic acid (75 nl; 100 mM) or cobalt chloride (75 nl; 50 mM) into the CVL medulla. The four stimuli used to change lung resistance were static muscular contraction, electrical stimulation of thin fiber afferents in the sciatic nerve, electrical stimulation of the posterior diencephalon, and hypoxia. The first three stimuli have been shown to decrease total lung resistance, whereas the latter stimulus has been shown to increase resistance. We found that injection of both ibotenic acid, which destroys cell bodies but not fibers of passage, and cobalt, which prevents synaptic transmission, either abolished or greatly attenuated the decrease in total lung resistance evoked by static contraction, by sciatic nerve stimulation, and by posterior diencephalic stimulation. We also found that injection of ibotenic acid and cobalt attenuated the reflex increase in lung resistance evoked by hypoxia. In control experiments, we found that bilateral injection of ibotenic acid into the dorsal medulla had no effect on the changes in total lung resistance evoked by these four stimuli. We conclude that the CVL medulla plays an important role in the reflex and central control of airway caliber.

airway smooth muscle; dogs; total lung resistance; ibotenic acid; cobalt chloride; brain stem

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THE ROLE of the caudal ventrolateral (CVL) medulla in the control of the cardiovascular system in anesthetized animals has been extensively investigated. For example, chemical stimulation of cells within the CVL medulla has been shown to decrease arterial pressure and heart rate (3, 22); in a few instances, however, chemical stimulation of this area has been shown to increase these variables (6, 9). In addition, electrolytic destruction of the CVL medulla has been shown to greatly attenuate the reflex pressor responses either to electrical stimulation of the sciatic nerve (4) or to static muscular contraction (8).

Stimulation of the CVL medulla also has effects on airway caliber. Using injections of DL-homocysteic acid, Connelly et al. (5) found that stimulation of the CVL medulla dilated the airways by withdrawing cholinergic tone. Little is known, however, about the role played by this area in the reflex and centrally evoked bronchodilations arising from contraction of skeletal muscle (11, 17), from electrical stimulation of thin fiber afferents in the sciatic nerve (18), or from stimulation of the posterior diencephalon (13). We examined, therefore, the effects of bilateral injections into the CVL medulla of either ibotenic acid or cobalt chloride on the bronchodilation evoked by each of these stimuli. Although both substances inactivate cell bodies but not fibers of passage, the effects of cobalt chloride are reversible (12), whereas those of ibotenic acid are not (19). In addition, we have examined the effect of injection of these substances into the CVL medulla on the bronchoconstriction evoked by ventilating the lungs with hypoxic gas mixtures (15). We used total lung resistance, calculated "breath-by-breath" as a functional index of airway caliber.

METHODS

General. Dogs, weighing 8–22 kg, were initially anesthetized with sodium thiamylal (10 mg/kg iv) followed by α-chloralose (60–80 mg/kg iv). Additional α-chloralose (10–20 mg/kg iv) was given hourly or sooner if signs of discomfort were present. The effectiveness of the anesthesia was periodically verified by firmly pinching one of the dog's toes. If an increase in blood pressure was evoked or if the dog withdrew its limb, additional α-chloralose (10–20 mg/kg iv) was given.

An endotracheal tube was inserted with its balloon placed immediately below the larynx; the lungs were ventilated with a mixture of oxygen and room air. The volume of the ventilator was set at 10–15 ml/kg, and the rate was adjusted to maintain arterial blood gases and pH within normal limits. Sodium bicarbonate was given, when necessary, to correct for metabolic acidosis. The expiratory outlet of the ventilator was placed under 1–2 cmH_2O, and a pneumothorax was established by inserting tubes bilaterally in the fifth intercostal space. The chest was opened to prevent any stimulus-induced (see below) increases in lung volume or breathing frequency from causing reflex bronchodilation (20). A catheter was placed in the common carotid artery to measure arterial pressure and to obtain samples to determine blood gases. A venous catheter was placed in one forelimb for delivering anesthesia, sodium bicarbonate, and lactated Ringer solution. Body temperature was maintained at 37 ± 1°C with a heating pad.

Airflow was measured with a heated pneumotach (Fleisch no. 0), which was placed between the ventilator and the tracheal cannula and was connected in turn to a differential pressure transducer (Validyne DP45-24). Transpulmonary pressure was measured with a differential pressure transducer (Validyne DP45-14), one end of which was connected to a side port in the tracheal cannula, while the other end was left open to the atmosphere. Our measure of transpulmonary pressure included the resistance of the ventilatory circuit and, therefore, introduced a small and constant error into our calculations of total lung resistance. Arterial pressure was measured by connecting the carotid catheter to a transducer (Statham P23 XL). Heart rate was calculated beat-to-beat from the arterial pressure pulse. Total lung resistance and dynamic compliance were calculated breath-by-breath by a Buxco pulmonary mechanics analyzer (model 6) by using the methods described by Amdur and Mead (1) and von Neegaard and Wirz (16). In our experiments, the calculations of total lung resistance were performed at 70% of tidal volume and were averaged over inspiration and expiration. Moreover, these calculations reflect both airflow resistance and tissue resistance, of which the latter is assumed to be constant throughout the course of the experiment. Total lung resistance and dynamic compliance, as well as arterial pressure, heart rate, airflow, tidal volume, and transpulmonary pressure, were recorded using a printer. The resistance of the endotracheal tube was not subtracted from the calculations of total lung resistance.

The dog's head was placed in a Kopf stereotaxic instrument. The brain stem was exposed by removing the basioccipital bone and by opening the atlantooccipital membrane. The surface of the brain stem was covered with warm mineral oil. In some dogs, both tibial nerves were exposed. A shielded stimulating electrode was placed on each tibial nerve, which is a branch of the sciatic nerve. In these dogs, both tibial nerves were electrically stimulated (40 Hz; 0.03 ms; 2–3 times motor threshold) to cause the gastrocnemius and flexor digitorum superficialis muscles to contract statically for 60 s. Any autonomic responses evoked by stimulation of the tibial nerves at these current intensities have been shown to be caused reflexly by muscle contraction and not by electrical activation of afferent fibers (10, 15). In other dogs, only one sciatic nerve was exposed and cut. A shielded stimulating electrode was placed on its central cut end. The sciatic nerve was electrically stimulated (20 Hz; 0.75 ms) for 60 s at current intensities (7 mA) shown previously to recruit C-fiber discharge (18).

Experimental protocols. In 10 dogs, we examined the effects of bilateral injection of ibotenic acid (75 nl; 100 mM) into the CVL medulla on the bronchomotor and cardiovascular responses to four stimuli: static muscular contraction, electrical stimulation of the sciatic nerve, electrical stimulation (80 Hz; 0.5 ms; 250 μA) of the posterior diencephalon, and ventilation of the lungs with 5% O_2-95% N_2 for ~2 min. The first three stimuli cause the airways to dilate (10, 11, 13, 18), whereas the last stimulus causes the airways to constrict reflexly because of peripheral chemoreceptor stimulation (15). Likewise, in four dogs, we examined the effects of bilateral ibotenic acid injections into the dorsal medulla on the bronchomotor responses to these four stimuli. The order of presentation of the four stimuli was varied randomly. Not every dog received each of the four stimuli.

We statically contracted the muscles of both hindlimbs because in preliminary experiments we found that the bronchodilator response to static contraction occurred more frequently when both hindlimbs were contracted than when one hindlimb was contracted. After injecting the ibotenic acid bilaterally, we waited ~75–90 min to allow adequate cell body destruction and then performed each of the maneuvers again. The effects of ibotenic acid were not reversible (19). Therefore, cobalt chloride injections were performed in four dogs to show that any attenuation of autonomic responses to these stimuli was not the result of the preparation deteriorating over time.

In each of the four dogs, we examined the effects of bilateral injections of cobalt chloride (75 nl; 50 mM) into the CVL medulla on the airway and cardiovascular responses to one of the four stimuli described above. Cobalt chloride prevents presynaptic release of neurotransmitter by blocking calcium channel conductance. Its effect is believed to last for ~30–60 min (12). Therefore, bilateral injection of this compound into the CVL medulla afforded us the opportunity to show that disruption of synaptic transmission in this area attenuated in a reversible manner the bronchomotor and cardiovascular responses to each of the four maneuvers. After obtaining a control response to a stimulus, we then injected bilaterally the cobalt chloride. We waited 15–20 min and then repeated this stimulus. In an attempt to show reversibility, we repeated these stimuli again ~60–70 min after cobalt injections. All injections into the medulla were done with a Kopf microinjector unit (model 5000) that contained a 1-μl syringe and a 26-gauge needle.

Stimulation sites in the posterior diencephalon were marked by passing anodal direct current (300 μA; 10 s). Injection sites in the CVL medulla were marked by using ibotenic acid in 2% fast green dye. After the end of the experiment, the dog was killed with an overdose of pentobarbital sodium (60 mg/kg iv) followed by saturated KCl solution (iv). The brain was removed, placed in Formalin (10%) for 24 h, and then transferred to Formalin (10%)-sucrose (30%) solution for 24 h. The brains were then frozen, sectioned coronally (40 μm), mounted on slides, and stained for cell bodies by using 1% neutral red. Using a microprojector, we made drawings of tissue sections containing either stimulation or injection sites.

Data analysis. We averaged total lung resistance breath-by-breath for the 60-s control period preceding each stimulus. We compared this control value with the peak change in total lung resistance evoked by the stimulus; this peak change consisted of the average of five consecutive ventilatory cycles occurring during the stimulus. Control dynamic compliance was averaged for the first five ventilatory cycles immediately preceding a stimulus. This was done because the compliance averaged over five cycles was almost identical to that averaged over a 60-s control period. The peak change in dynamic compliance consisted of five ventilatory cycles occurring during the stimulus. The control values for mean arterial pressure and heart rate were their respective steady-state levels,

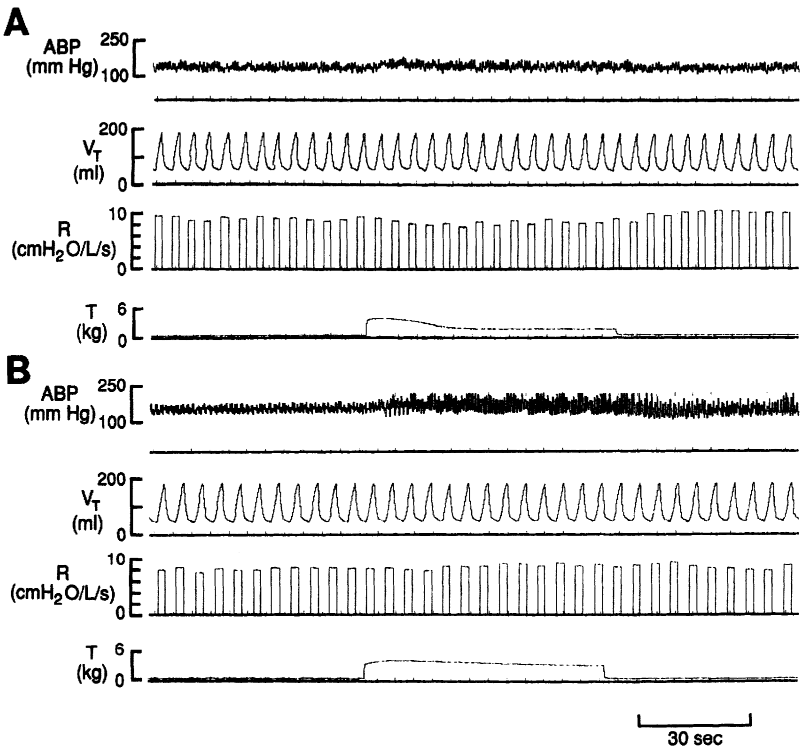

FIG. 1. Record from dog in which bilateral injections of ibotenic acid into caudal ventrolateral medulla abolished reflex bronchodilator response to static muscular contraction. Note that contraction decreased total lung resistance before ibotenic acid injections (*A*; 75 nl; 100 mM) but had no effect 75 min later (*B*). Also note that tidal volume (VT) did not change because chest was opened and lungs were ventilated mechanically. ABP, arterial blood pressure; R, total lung resistance; T, tension.

whereas peak values were selected during the stimulus. A two-by-two within-subjects analysis of variance (14) was used to determine statistical significance, for which the criterion level was set at $P < 0.05$. The significance of the interaction representing the difference between the lung resistance response to a maneuver before and after ibotenic acid injection was the statistic used to test the hypotheses (see Figs. 2, 5, 7, and 8).

RESULTS

Effects of ibotenic acid and cobalt injections on the responses to static contraction. Bilateral injections (75 nl; 100 mM) of ibotenic acid into the CVL medulla abolished the decrease in total lung resistance evoked by static muscular contraction in each of eight dogs tested (Figs. 1 and 2). In six of these dogs, ibotenic acid injections converted the decrease in total lung resistance evoked by static muscular contraction to an increase. Neither mean arterial pressure nor heart rate was significantly changed by static muscular contraction either before or after ibotenic acid injection (Fig. 2). Histological analysis of the dye marks in the CVL medulla revealed that the injection sites were located at the level of the obex and were centered dorsolateral to the lateral reticular nucleus (Figs. 3 and 4).

In three other dogs, we bilaterally injected ibotenic acid (75 nl; 100 mM) into the dorsal medulla at the level of the obex (Fig. 3). This control procedure had no effect on the decrease in total lung resistance evoked by static contraction. Before these control injections, contraction decreased resistance from 9.5 ± 2.5 to 8.4 ± 2.4 $cmH_2O \cdot l^{-1} \cdot s$; after the injections, contraction decreased resistance from 8.9 ± 0.9 to 8.2 ± 1.0 $cmH_2O \cdot l^{-1} \cdot s$. After these injections, static contraction decreased total lung resistance in each of the three dogs tested. By contrast, after bilateral injection of ibotenic acid into the CVL medulla, static contraction no longer decreased lung resistance in any of the eight dogs tested.

In another dog, we bilaterally injected cobalt chloride (75 nl; 50 mM) into the CVL medulla (Fig. 3). In this dog, we found that cobalt injections abolished in a reversible manner the decrease in lung resistance evoked by static contraction. Thus, before the injections of cobalt, contraction decreased resistance from 9.8 to 8.9 $cmH_2O \cdot l^{-1} \cdot s$, whereas 13 min after the injections, contraction increased resistance from 9.6 to 12.2 $cmH_2O \cdot l^{-1} \cdot s$. Seventy minutes after the injections, contraction again decreased resistance from 11.6 to 10.8 $cmH_2O \cdot l^{-1} \cdot s$.

Effects of ibotenic acid and cobalt injections on the responses to sciatic nerve stimulation. Bilateral injections of ibotenic acid (75 nl; 100 mM) into the CVL medulla of eight dogs abolished the decrease in total lung resistance evoked by electrical stimulation of the central cut end of the sciatic nerve. These injections, however, had no effect on either the pressor or the tachycardiac responses to electrical stimulation of the sciatic nerve (Fig. 5). Histological analysis of the dye marks revealed that the injection sites were located at the level of the obex and were centered dorsolateral to the lateral reticular nucleus (Fig. 3).

In three other dogs, we bilaterally injected ibotenic acid (75 nl; 100 mM) into the dorsal medulla at the level of the obex (Fig. 3). This control procedure had no effect on the decrease in total lung resistance or on the increase in arterial pressure evoked by electrical stimulation of the central cut end of the sciatic nerve. Thus before these control injections, stimulation of the sciatic nerve decreased resistance from 9.3 ± 1.2 to 8.2 ± 1.2

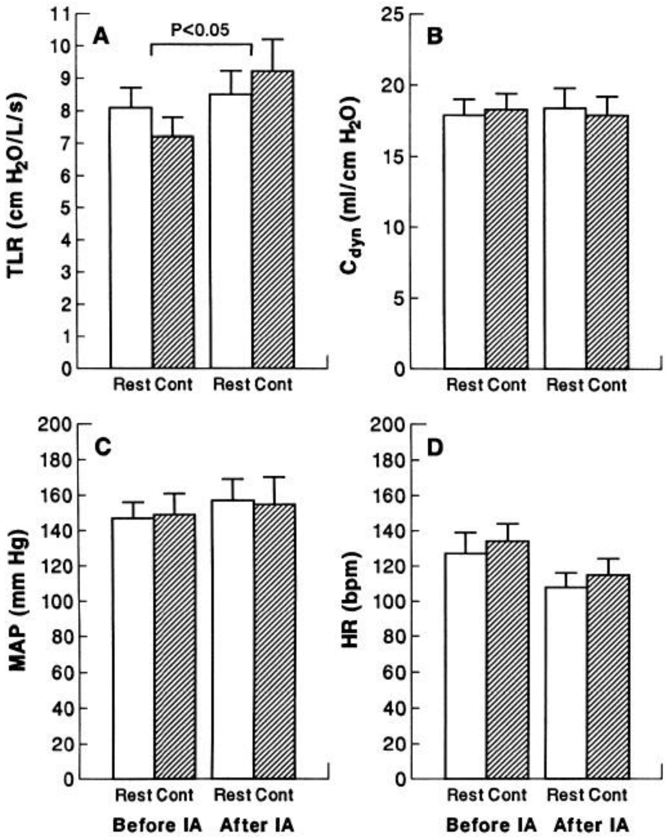

FIG. 2. Summary data from 8 dogs showing the effects of bilateral injection of ibotenic acid (75 nl; 100 mM) into caudal ventrolateral medulla on respiratory and cardiovascular responses to static muscular contraction. Bracket signifies that static contraction decreased total lung resistance (TLR) from resting value before injection of ibotenic acid but had no effect on resistance after injection of ibotenic acid. Cdyn, dynamic compliance; MAP, mean arterial pressure; HR, heart rate; IA, ibotenic acid; Cont, static contraction.

$cmH_2O \cdot l^{-1} \cdot s$; afterwards, stimulation still decreased resistance from 8.9 ± 0.5 to 7.7 ± 0.7 $cmH_2O \cdot l^{-1} \cdot s$. Stimulation of the sciatic nerve decreased resistance in each of the three dogs tested both before and after these injections into the dorsal medulla. Likewise, before the injections, stimulation of the sciatic nerve barely increased mean arterial pressure from 148 ± 29 to 150 ± 34 mmHg. After the injections, stimulation still barely increased mean arterial pressure from 178 ± 42 to 182 ± 63 mmHg.

In one dog, we injected bilaterally cobalt chloride (75 nl; 50 mM) into the CVL medulla (Fig. 6). We found that 14 min after these injections, stimulation of the central cut end of the sciatic nerve decreased total lung resistance from 7.9 to 7.3 $cmH_2O \cdot l^{-1} \cdot s$, whereas before the injections stimulation decreased resistance from 7.7 to 6.3 $cmH_2O \cdot l^{-1} \cdot s$. Seventy-four minutes after the injections, stimulation decreased resistance from 7.8 to 6.8 $cmH_2O \cdot l^{-1} \cdot s$, a finding that suggested that the attenuating effects of cobalt chloride were starting to reverse (Fig. 6). In addition, before the cobalt injections, stimulation increased mean arterial pressure from 154 to 162 mmHg; 14 min later, stimulation had almost no effect on arterial pressure, and after 74 min, stimulation increased pressure from 157 to 166 mmHg. The effects of cobalt on the pressor response to sciatic nerve stimulation are difficult to interpret because of the change in baseline mean arterial pressure in this one dog.

Effect of ibotenic acid and cobalt injections on the responses to posterior diencephalic stimulation. Bilateral injections of ibotenic acid (75 nl; 100 mM) into the CVL medulla of eight dogs abolished the decrease in total lung resistance evoked by electrical stimulation (80 Hz; 0.5 ms; 250 μA) of the posterior diencephalon (Fig. 7). These injections had no effect on the depressor response evoked by posterior diencephalic stimulation (Fig. 7). Histological analysis revealed that the stimulation sites were widely distributed throughout the posterior diencephalon. Four sites were in the ventroposterior thalamus, two were in the subthalamic nucleus, one was in the dorsomedial posterior thalamus immediately lateral to the habenular nucleus, and the last was on the border of the midbrain and was near the red nucleus.

In three other dogs, we injected bilaterally ibotenic acid (75 nl; 100 mM) into the dorsal medulla at the level of the obex (Fig. 3). This control procedure appeared to increase the bronchodilator response to stimulation of the posterior diencephalon. Hence, stimulation before the bilateral injections of ibotenic acid decreased total lung resistance from 9.4 ± 1.4 to 8.1 ± 1.2 $cmH_2O \cdot l^{-1} \cdot s$, whereas after the injections, stimulation decreased resistance from 10.0 ± 1.2 to 7.6 ± 0.04 $cmH_2O \cdot l^{-1} \cdot s$. Likewise, the depressor response to this maneuver also appeared to increase after ibotenic acid injections (i.e., from 149 ± 31 to 137 ± 29 mmHg before the injections to 175 ± 37 to 144 ± 36 mmHg after injections), but this is difficult to interpret because of the change in baseline mean arterial pressure.

In one dog, we injected bilaterally cobalt chloride (75 nl; 50 mM) into the caudal ventrolateral medulla (Fig. 3).

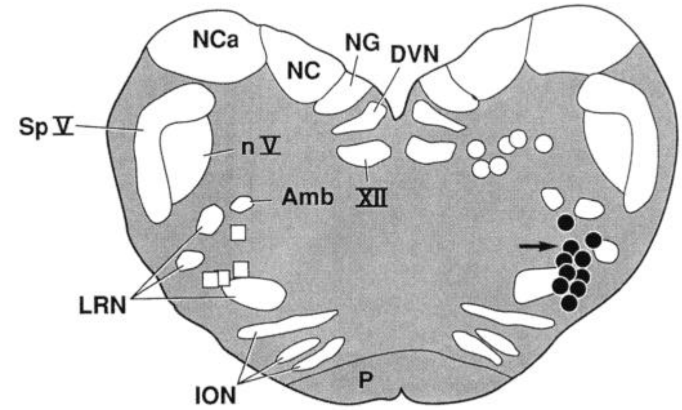

FIG. 3. Line drawing of coronal section of medulla at level of obex showing injection sites of either IA (○, ●) or cobalt chloride (□). Filled circles represent injection sites that attenuated or abolished changes in TLR evoked by static contraction, sciatic nerve stimulation, posterior diencephalic stimulation, and hypoxia. ○, Injection sites that had no effect on changes in TLR evoked by these stimuli. Arrow points to injection site shown in Fig. 4. Note that in every case injections were bilateral but are represented by one symbol on one side of medulla for purposes of illustration. Amb, nucleus ambiguus; DVN, dorsal vagal nucleus; ION, inferior olivary nucleus; LRN, lateral reticular nucleus; NC, cuneate nucleus; NCa, accessory cuneate nucleus; NG, nucleus gracilis; nV, nucleus of spinal tract of fifth nerve; P, pyramidal tract; SpV, spinal tract of fifth nerve; XII, hypoglossal nucleus.

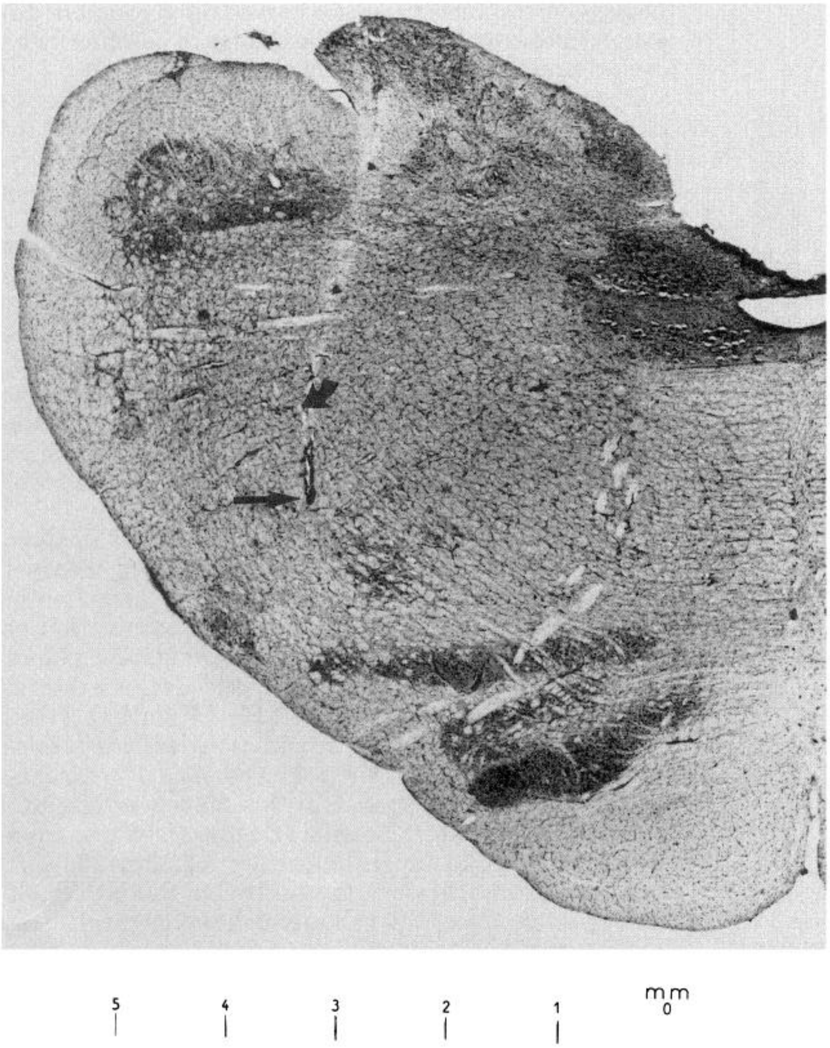

FIG. 4. Coronal section of medulla taken at level of obex. Straight thin arrow points to injection site of IA, and curved thick arrow points to nucleus ambiguus.

We found that 13 min after these bilateral injections, electrical stimulation of the posterior diencephalon had little effect on total lung resistance, decreasing it from 13.0 to only 12.8 $cmH_2O \cdot l^{-1} \cdot s$. By contrast, stimulation of the posterior diencephalon before the cobalt injections decreased resistance from 12.5 to 11.5 $cmH_2O \cdot l^{-1} \cdot s$. Likewise, stimulation of the posterior diencephalon 60 min after cobalt injections also decreased resistance from 12.7 to 11.3 $cmH_2O \cdot l^{-1} \cdot s$. Before cobalt injections, the depressor response to posterior diencephalic stimulation was 24 mmHg, whereas 13 and 60 min after these injections, the depressor to stimulation was 16 and 49 mmHg, respectively.

Effects of ibotenic acid and cobalt chloride injections on the responses to hypoxia. Bilateral injections of ibotenic acid into the CVL medulla of nine dogs attenuated by 72% the increase in total lung resistance evoked by ventilating the lungs with 5% O_2 in nitrogen (Fig. 8). These injections, however, had no statistically significant effect ($P > 0.05$) on the pressor response to hypoxia. In addition, heart rate did not change in response to hypoxia either before or after the ibotenic acid injections. Before the ibotenic acid injections, changing the gas mixture ventilating the lungs from room air to 5% O_2 in N_2 decreased arterial P_{O_2} from 151 ± 11 to 29 ± 1 Torr, decreased arterial pH from 7.44 ± 0.01 to 7.38 ± 0.02, and had no effect on arterial P_{CO_2} (35 ± 3 vs. 34 ± 2 Torr). After the ibotenic acid injections, changing the gas mixture from room air to 5% O_2 decreased arterial P_{O_2} from 141 ± 14 to 31 ± 2 Torr and had no significant effects on either pH (from 7.35 ± 0.03 to 7.38 ± 0.02) or arterial P_{CO_2} (from 35± to 36 ± 1 Torr).

In two dogs, we injected bilaterally ibotenic acid (75 nl; 100 mM) into the dorsal medulla at the level of the obex (Fig. 3). These control injections had no effect on either the bronchoconstrictor or the pressor responses to hypoxia. Before ibotenic acid injections, ventilating the lungs with 5% O_2 in N_2 increased total lung resistance from 10.7 ± 2.9 to 18.6 ± 9.5 $cmH_2O \cdot l^{-1} \cdot s$ and increased mean arterial pressure from 123 ± 11 to 133 ± 15 mmHg. After these injections, ventilating the lungs with 5% O_2 still increased both lung resistance (from 11.7 ± 2.0 to 18.4 ± 4.4 $cmH_2O \cdot l^{-1} \cdot s$) and mean arterial pressure (from 130 ± 7 to 151 ± 5 mmHg).

In one other dog, we injected bilaterally cobalt chloride (75 nl; 50 mM) into the CVL medulla. Before cobalt injections, hypoxia increased total lung resistance by 2.9 $cmH_2O \cdot l^{-1} \cdot s$; 13 min after the injections, hypoxia in-

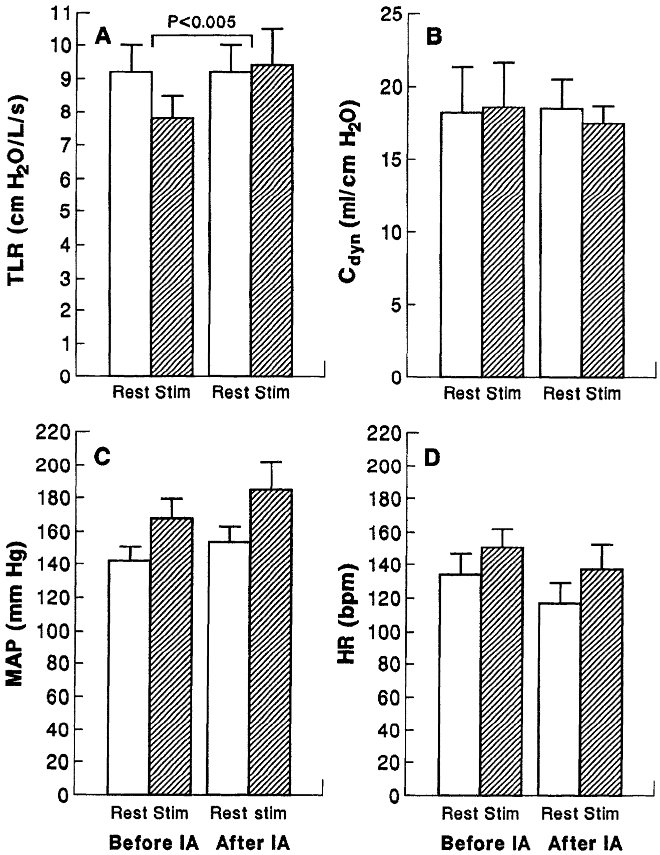

FIG. 5. Summary data from 8 dogs showing effects of bilateral injections of IA (75 nl; 100 mM) into caudal ventrolateral medulla on respiratory and cardiovascular responses to electrical stimulation of central cut end of sciatic nerve. Bracket signifies that electrical stimulation decreased TLR from resting value before injection of IA but had no effect on resistance after injection. Also note that electrical stimulation increased MAP both before and after IA injection ($P < 0.001$) but that magnitudes of these increases before and after injection were not significantly different from each other.

creased lung resistance by 1.9 $cmH_2O \cdot l^{-1} \cdot s$, and 60 min after injection hypoxia increased resistance by 4.3 $cmH_2O \cdot l^{-1} \cdot s$. The pressor response to hypoxia appeared to be unchanged by these cobalt injections.

Effects of ibotenic acid and cobalt chloride on baseline values. In the 10 dogs used in the experiments described above, bilateral injection of ibotenic acid into the CVL medulla had no statistically significant effect on baseline total lung resistance. Thus, before injection of ibotenic acid, baseline resistance averaged 9.0 ± 0.6 $cmH_2O \cdot l^{-1} \cdot s$; likewise, 60–90 min after injection, baseline resistance averaged 8.9 ± 0.7 $cmH_2O \cdot l^{-1} \cdot s$. In addition, bilateral injections of ibotenic acid into the CVL medulla significantly increased mean arterial pressure from 140 ± 6 to 155 ± 9 mmHg ($P < 0.05$) 60 min after this excitotoxin was administered. Bilateral injection of ibotenic acid had no statistically significant effect on baseline heart rate, decreasing it from 127 ± 9 to 115 ± 10 beats/min; likewise, injection had no effect on dynamic compliance, averaging 22.4 ± 2.8 before and 21.8 ± 2.4 ml/cmH_2O after injection.

Bilateral injections of cobalt chloride (75 nl; 50 mM) into the CVL medulla had no effect on baseline total lung resistance in the four dogs tested. Thus resistance averaged 9.4 ± 1.3, 9.5 ± 1.5, and 9.6 ± 1.8 $cmH_2O \cdot l^{-1} \cdot s$ before, 12–15 min after the injections, and 60–70 min after the injections, respectively ($P > 0.05$). Mean arterial pressure appeared to be decreased by injections of cobalt chloride; however, the effect was not significant ($0.05 < P < 0.10$). Thus, arterial pressure averaged 136 ± 11, 124 ± 9, and 134 ± 10 mmHg before, 12–15 min after the injections, and 60–70 min after the injections, respectively. Heart rate averaged 145 ± 31, 122 ± 24, and 143 ± 28 beats/min before, 12–15 min after, and 60–70 min after the injections, respectively ($P > 0.20$). The effect of cobalt injections on baseline compliance was not calculated.

Effect of atropine on baseline total lung resistance. In 9 of the 10 dogs in which we injected ibotenic acid into the CVL medulla, we examined the effect of atropine methylnitrate (1 mg/kg iv) on baseline total lung resistance. This muscarinic blocking agent, which was always injected as the last procedure in the experiment, decreased total lung resistance in each of the nine dogs tested. On average, atropine decreased resistance from 8.8 ± 0.8 to 6.0 ± 0.4 $cmH_2O \cdot l^{-1} \cdot s$ ($P < 0.005$). We did not give atropine to the dogs in which we injected cobalt chloride into the CVL medulla.

DISCUSSION

We have shown that ibotenic acid-induced destruction of cell bodies in the CVL medulla of anesthetized dogs abolished the bronchodilation evoked by the following stimuli: static contraction of hindlimb muscles, electrical stimulation of Aδ- and C-fibers in the sciatic nerve, and electrical stimulation of the posterior diencephalon. In addition, we have shown that destruction of cell bodies in the CVL medulla attenuated the bronchoconstrictor response to ventilating the lungs with hypoxic gas mix-

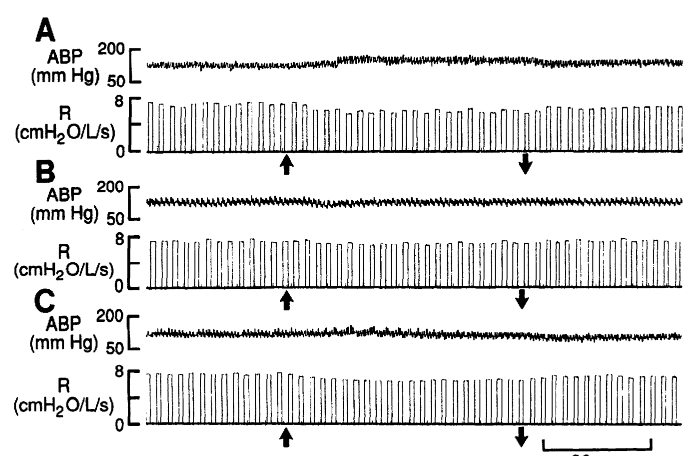

FIG. 6. Record from dog in which bilateral injection of cobalt chloride into caudal ventrolateral medulla attenuated reflex bronchodilator response to electrical stimulation of central cut end of sciatic nerve. *A*: sciatic nerve was stimulated electrically (20 Hz; 0.75 ms; 7 mA) for 60 s before bilateral injection of cobalt chloride (75 nl; 50 mM). *B*: 14 min after injection, sciatic nerve was stimulated electrically again. Note that bronchodilator response to stimulus was smaller in *B* than in *A*. *C*: 74 min after bilateral injection of cobalt chloride, sciatic nerve was stimulated a third time. Note that bronchodilator response to stimulation was greater in *C* than in *B*. *A–C*: electrical stimulation started at upward arrow and ended at downward arrow.

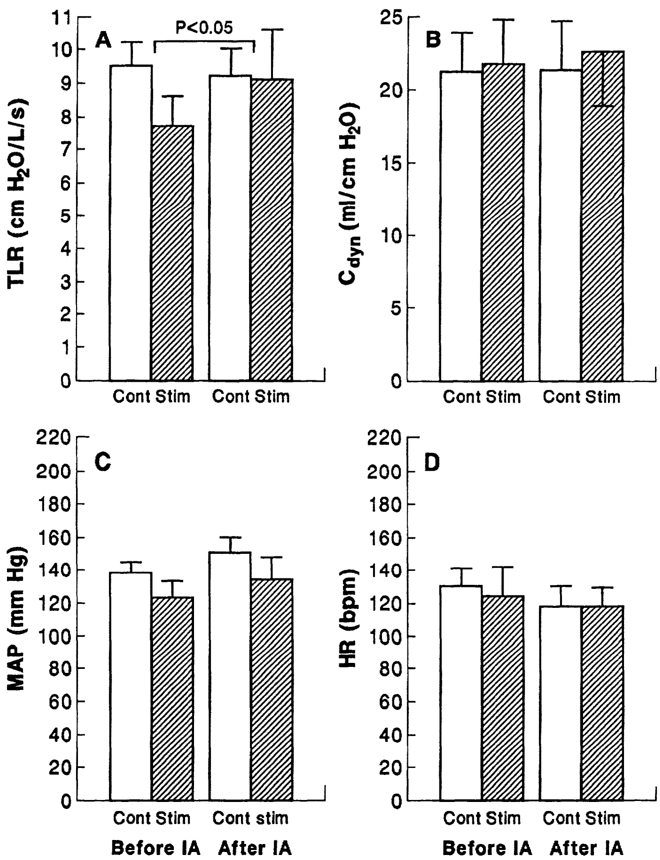

FIG. 7. Summary data for 8 dogs showing effects of bilateral injection of IA (75 nl; 100 mM) into caudal ventrolateral medulla on respiratory and cardiovascular responses to electrical stimulation of posterior diencephalon. Bracket signifies that stimulation decreased TLR from control value before injection of IA but not after. Also note that stimulation decreased MAP both before and after injection of IA.

tures. In contrast, ibotenic acid-induced destruction of cell bodies in the dorsal medulla had little if any effect on the bronchomotor responses to the four stimuli used to alter airway caliber in our experiments. These findings are consistent with the hypothesis that an intact CVL medulla is needed for the expression of reflex and centrally arising mechanisms that change airway caliber.

The possibility exists that ibotenic acid abolished or attenuated the changes in airway caliber in our experiments by destroying the cell bodies the axons of which comprised the vagal preganglionic outflow to the lungs. We think this possibility is unlikely for two reasons. First, bilateral injections of ibotenic acid into the CVL medulla did not decrease baseline total lung resistance, a finding that should occur if this excitotoxin had destroyed vagal preganglionic cell bodies. Second, intravenous atropine, given after ibotenic acid and injected into the CVL medulla, markedly decreased total lung resistance. This second finding suggested that after ibotenic acid injection the vagal outflow to airway smooth muscle was still intact.

Ibotenic acid injections into the CVL medulla converted the bronchodilator response to static muscular contraction into a bronchoconstrictor response (see Fig. 2). This bronchoconstriction might have been caused by an increase in arterial P_{CO_2} (15), which in turn was caused by an increase in muscle metabolism in dogs in which ventilation was fixed. We did not measure arterial P_{CO_2} during static contraction and, therefore, this explanation must be viewed as speculation.

We were surprised that the ibotenic acid-induced destruction of cell bodies in the CVL medulla attenuated the bronchoconstriction evoked by hypoxia. Stimulation of cell bodies in this area with DL-homocysteic acid, a powerful glutamate analogue, has been shown to dilate the airways (5, 7). We can only speculate why the destruction of the CVL medulla attenuated the bronchoconstriction evoked by hypoxia. This area of the medulla might contain interneurons that received peripheral chemoreceptor input and which, in turn, activated vagal preganglionic cell bodies the axons of which innervate airway smooth muscle. We further speculate that these interneurons were either not stimulated by DL-homocysteic acid or that their bronchoconstrictor action was overwhelmed by interneurons causing bronchodilation when this excitatory amino acid was injected into the CVL medulla.

Because electrical stimulation of the brain does not distinguish between activating cell bodies and activating fibers of passage, any interpretation of our experiments in which we showed that ibotenic acid-induced lesions of

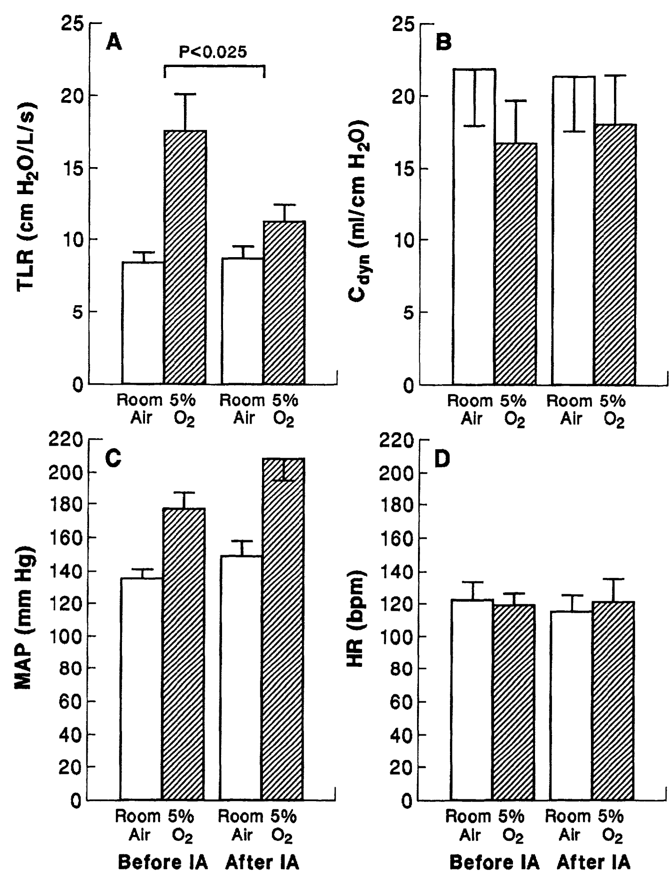

FIG. 8. Summary data for 9 dogs showing effects of bilateral injection of IA (75 nl; 100 mM) into caudal ventrolateral medulla on respiratory and cardiovascular responses to ventilating lungs with gas mixture containing 5% O_2-95% N_2. Bracket signifies that increase in TLR evoked by hypoxia was greater before than after injection of IA. Also note that increase in MAP evoked by hypoxia was no different before than after injection.

the CVL medulla abolished the bronchodilation evoked from the posterior diencephalon must be quite limited. Nevertheless, we believe that the value of these experiments lies in the fact that they are the first pieces of evidence suggesting that an intact CVL medulla is needed not only to express changes in airway caliber caused by reflex mechanisms but also to express changes in airway caliber arising from the brain itself. At this point, we can offer no information about what role, if any, these widely distributed sites in the posterior diencephalon might play in causing bronchodilation in an intact behaving animal.

In our experiments, destruction of cell bodies in the caudal ventrolateral medulla attenuated the bronchomotor but not the pressor responses to hypoxia and to electrical stimulation of the sciatic nerve. Before the advent of excitotoxins, which destroy cell bodies but not fibers of passage, Ciriello and Calaresu (4) reported that electrolytic destruction of the caudal ventrolateral medulla abolished the reflex pressor response to electrical stimulation of the sciatic nerve. Subsequently, Stornetta et al. (21) attributed this abolition of the reflex to the destruction of fibers of passage. Our finding that ibotenic acid injections into the caudal ventrolateral medulla did not attenuate the reflex pressor responses to sciatic nerve stimulation is consistent with the view stated by Stornetta et al. (21). At this point, we cannot exclude the possibility that the blood pressure effects caused by these stimuli would have been attenuated also had we used large injection volumes of ibotenic acid and cobalt chloride to inactivate large portions of the CVL medulla.

Because static muscular contraction did not increase mean arterial pressure in our experiments, we were not able to compare our findings with previous ones showing that inactivation of the CVL medulla attenuated the reflex pressor responses to this maneuver (2, 8). We speculate that static muscular contraction did not reflexly increase arterial blood pressure in our experiments, because either the intensity of contraction or the mass of muscle engaged was below the threshold required to activate the sympathetic vasomotor outflow in our anesthetized preparation. If this speculation proves to be true, it then follows that the threshold required during exercise to reflexly dilate the airways is lower than the threshold required to reflexly increase arterial blood pressure.

Ibotenic acid destroys cell bodies (19), and therefore its effect is not reversible. Hence, we needed to show that the ibotenic acid-induced attenuation of bronchomotor responses to the four stimuli used in our experiments was not caused by the degradation of our anesthetized preparation over time. We controlled for this possibility in two ways. First, we showed that bilateral injections of ibotenic acid into the dorsal medulla had no effect on the bronchomotor responses to the four stimuli. Second, we showed that bilateral injections of cobalt chloride into the CVL medulla reversibly attenuated these responses. Although the number of dogs used in our experiments with cobalt were small, they nevertheless provided a useful adjunct to our experiments with ibotenic acid, because we obtained similar results with two substances having different mechanisms of action.

In summary, we have found that inactivation of the CVL medulla with ibotenic acid and cobalt chloride prevented both the reflex and centrally evoked bronchomotor responses to static contraction, to electrical stimulation of thin fiber afferents in the sciatic nerve, to electrical stimulation of the diencephalon, and to hypoxia. Our findings suggest that the CVL medulla is an important site of integration for neural events controlling airway caliber.

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