Breathing of awake goats during prolonged dysfunction of caudal M ventrolateral medullary neurons


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Breathing of awake goats during prolonged dysfunction of caudal M ventrolateral medullary neurons. J. Appl. Physiol. 84(1): 129–140, 1998.—Cooling the caudal M ventrolateral medullary (VLM) surface for 30 s results in a sustained apnea in anesthetized goats but only a 30% decrease in breathing in awake goats. The purpose of the present study was to determine, in the awake state, the effect of prolonged (minutes, hours) caudal M neuronal dysfunction on eupneic breathing and CO₂ sensitivity. Dysfunction was created by ejecting excitatory amino acid receptor antagonists or a neurotoxin on the VLM surface through guide tubes chronically implanted bilaterally on a 10- to 12-mm² portion of the caudal M VLM surface of 12 goats. Unilateral and bilateral ejections (1 μl) of selective antagonists for N-methyl-D-aspartic acid or non-N-methyl-D-aspartic acid receptor had no significant effect on eupneic breathing or CO₂ sensitivity. Unilateral ejection of a nonselective excitatory amino acid receptor antagonist generally had no effect on eupneic breathing or CO₂ sensitivity. However, bilateral ejection of this antagonist resulted in a significant 2-Torr hypoventilation during eupnea and a significant reduction in CO₂ sensitivity to 60 ± 9% of control. Unilateral ejection of the neurotoxin kainic acid initially stimulated breathing; however, breathing then returned to near control with no incidence of apnea. After the kainic acid ejection, CO₂ sensitivity was reduced significantly to 60 ± 7% of control. We conclude that in the awake state a prolonged dysfunction of caudal M VLM neurons results in compensation by other mechanisms (e.g., central chemoreceptors, wakefulness) to maintain near-normal eupneic breathing, but compensation is more limited for maintaining CO₂ sensitivity.

METHODS

Ejecting the neurotoxin kainic acid (KA) unilaterally on the caudal M-rostral S ventrolateral medullary (VLM) surface or injecting KA into the retrotrapezoid nucleus (RTN) of anesthetized or decerebrate mammals briefly stimulates but then depresses breathing/phrenic nerve activity, often to terminal apnea (28, 29, 31, 32). Likewise, bilateral neuronal dysfunction induced through surface cooling of this VLM area causes sustained apnea in anesthetized mammals (6, 7, 12, 33, 34). These and other findings have led to postulates that this VLM area is the site of 1) respiratory rhythmogenesis, 2) facilitation of more dorsal respiratory neurons, 3) chemoreception, and/or 4) chemoreceptor integration (29, 31, 35, 38).

The data from recent studies on awake goats differ from the data on anesthetized goats. Specifically, in the awake state, 30 s of cooling the caudal M-rostral S surface does not cause apnea but causes only about a 30% reduction in breathing (12, 33, 34). Hence, neurons near this VLM surface are critical for respiratory rhythm in the anesthetized but not the awake state. Nevertheless, these VLM neurons contribute to the control of breathing in the awake state; VLM cooling over several awake conditions suggests that these neurons have a chemoreceptor-dependent and a chemoreceptor-independent facilitatory-like effect on breathing (12, 13).

To gain further insight into the caudal M contribution to the control of breathing in the awake state, we recently addressed three specific questions. 1) Is the contribution of this VLM area required for normal eupneic breathing in the awake state, or can other control mechanisms compensate for prolonged (minutes, hours) VLM neuronal dysfunction? To study this question, we created prolonged neuronal dysfunction by ejecting excitatory amino acid (EAA) receptor antagonists or a neurotoxin on the caudal M VLM of awake goats. 2) Can widespread brain chemoreceptors or other mechanisms compensate for prolonged dysfunction of presumed caudal M VLM chemoreceptor neurons? To study this question, the goats were studied after the VLM ejections, while breathing room air and while breathing CO₂-enriched gas mixtures. 3) What EAA receptor subtype mediates the caudal M VLM effect on breathing in the awake state? To study this question, we ejected N-methyl-D-aspartic acid (NMDA) or non-NMDA receptor agonists on the caudal M VLM surface to identify specific sites of respiratory neurons with these receptor subtypes, and subsequently at these sites we ejected NMDA or non-NMDA receptor antagonists or a nonselective EAA receptor antagonist.

Data were obtained on 10 female and 2 castrated male adult goats weighing 35–78 kg. Goats were housed in an environmental chamber with the ambient temperature and photoperiod adjusted seasonally. They had free access to hay and water except for periods of study. They were trained to relax in the sternal position in a stanchion.

Surgical Procedures

An initial surgery was performed to elevate a carotid artery. Anesthesia was induced with ketamine (Ketaset, 15 mg/kg im). After intubation, anesthesia was maintained with 1–1.5% halothane in oxygen. Under sterile conditions, a 5-cm segment of the left carotid artery was elevated. An antibiotic (chlorotetracycline, Biomycin) was administered daily for 1 wk after surgery.

Three weeks later, surgery was performed for chronic implantation of thermodes and guide tubes on the VLM.
Under anesthesia, the goat was positioned on its left side with the head rotated to a supine position. A midline incision exposed the trachea, which was retracted laterally, and the foramen magnum was visualized. The basilar occipital bone was partially removed, exposing the dura, which was cauterized and removed for visualization of cranial nerves VI and XII.

Once the medulla was exposed, we determined the effects of discrete bilateral VLM cooling (33). Thermodes for cooling were constructed from 19-gauge hypodermic tubing formed into a "horseshoe" shape (~3.5 × 3.5-mm outside dimensions). Two of these thermodes were tied together for bilateral cooling ~4–8 mm lateral to the midline. A thermocouple was soldered to one thermode for monitoring of temperature. Flexible tubing was attached to the thermodes for inflow and outflow of ice water. After 1 min for establishing the baseline condition, ice water was rapidly infused to reduce thermode temperature to 20°C within 3–4 s. Thereafter, flow was adjusted to maintain the thermode at 20°C for 30 s. In each goat we identified a site ~3–6 mm caudal to the exit of cranial nerve VI, where cooling under anesthesia resulted in a sustained apnea. This site corresponds to the caudal M chemosensory area in cats (35). At this site we then chronically implanted the thermodes, to which we had attached three to six stainless steel guide tubes (19 gauge) in the center of the horseshoe loop (Fig. 1). These devices rested on the VLM surface and were fixed to the basilar occipital bone using bone screws and dental acrylic. Teflon tubing was attached to the guide tube and exteriorized for insertion of ejection tubes. The opening created by the partial removal of the basilar occipital bone was entirely sealed, and the overlying soft tissue was closed.

VLM cooling in goats can result in upper airway obstruction (12). Accordingly, to circumvent this potential complication, a tracheotomy was created. The goats then recovered and were returned to the laboratory. Brain edema was controlled by dexamethasone (0.4 mg·kg\(^{-1}\)·day\(^{-1}\) iv initially, then 0.05 mg·kg\(^{-1}\)·day\(^{-1}\) iv). To minimize infection, the exit tubes were carefully cleaned daily with alcohol and wrapped in a sterile glove. Chloromycetin was administered daily as an antibiotic, and buprenorphine was administered every 12 h for 2 days.

Procedures and Protocols

Studies were always initiated at least 72 h after surgery, because this amount of time was usually required for the goats to regain their appetite and to cease mild hyperventilation. It has been our experience that goats are in good health for only ~10–14 days after VLM surgery. Body (rectal) temperature of ~39°C, arterial PSCO\(_2\) (PaCO\(_2\)) of ~35 Torr, arterial Pao\(_2\) of ~90 Torr, arterial pH of ~7.46, and normal appetite were our criteria for good health. To maintain the goats' good health and physiological conditions, we were limited in the number of studies we could complete each day. Accordingly, we were limited in the total number of studies we could complete on each goat.

For all studies a breathing valve was attached to auffed endotracheal tube placed through the tracheotomy for measurement of breathing using a pneumotachograph connected to a recorder. The elevated carotid artery was catheterized to monitor arterial blood pressure (ABP) and to obtain blood samples for pH, arterial Pao\(_2\), and Paco\(_2\), determination (model 278, Ciba-Corning). ABP was monitored, because VLM sites are also important in its control (14, 22). Rectal temperature was measured after each blood sample was taken. Measurements were made during four different protocols.

Protocol 1: VLM cooling. The initial studies on all awake goats involved cooling the VLM via the thermodes to establish that the thermodes (with guide tubes) were placed over respiratory neurons. In addition, the final studies in some goats involved cooling the VLM in the awake and anesthetized states. The protocol for these studies was exactly as described for cooling during surgery. We were unable to use cooling to create a prolonged dysfunction in the awake state, because after ~30 s of cooling, awake goats became agitated, and breathing had a definite behavioral component (12).

Protocol 2: EAA receptor agonist ejections. After the initial VLM cooling in the awake state, we determined the effects on breathing and ABP of ejecting on the VLM either NMDA (8 goats) or a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (7 goats). These ejections were made to establish whether NMDA and non-NMDA receptor subtypes exist on caudal M VLM respiratory neurons of adult goats. We chose not to completely characterize the awake goats’ responses to EAA receptor agonists, because that would have required several of the few available days for study on each goat and because we believed that, for our purposes, it was necessary to demonstrate only that breathing was affected when the agonists were ejected on the VLM surface.

While the goats were breathing room air, breathing and blood pressure were continuously monitored and blood was withdrawn periodically over several hours. After a control period of at least 15-min, a preloaded 31-gauge stainless steel ejection tube was inserted into one guide tube for the exact (known) length of the tube, resulting in ejection on the VLM surface. NMDA and AMPA were dissolved in mock cerebrospinal fluid (CSF) with a composition (in mM) of 124 NaCl, 2.0 KCl, 2.0 MgCl\(_2\), 1.3 NaHPO\(_4\), 2 CaCl\(_2\), 26 NaHCO\(_3\), and 11 glucose. Before ejection the solutions were equilibrated (at 39°C) in a tonometer with a 45 Torr PCO\(_2\), 100 Torr PO\(_2\)-balance N\(_2\) gas mixture. Ejection volumes were usually 1 µl, with 10-µl volumes on a few occasions as indicated. Ejections were made over a 30-s interval. If there was a sustained ventilatory response, blood was withdrawn at 10- to 30-min intervals until breathing returned to control levels. If there was no ventilatory response after the ejection, then 28–30 min later, blood was withdrawn and an ejection was made at another guide tube. The total number of ejections in 1 day was 1–10. On any single day, only NMDA (250 or 500
mM) or AMPA (20–40 µM) was ejected. In two goats a selective antagonist of NMDA, 2-amino-5-phosphonooxalate (AP-5, 5 mM), was ejected 15 min before an NMDA ejection at the site where previously there was a ventilatory response to NMDA.

**Protocol 3: EAA receptor antagonist ejections.** In 10 of the goats, for several days usually after the agonist injections or after the initial cooling studies, we determined in the awake state the effects on breathing and ABP of ejecting on the VLM surface mock CSF or (in mock CSF) 250 mM kynurenic acid (KynA), 2 mM AP-5, and 300 µM 2,3-dihydroxy-6-nitro-7-sulfamozlbenzo (F) quinoxaline (NBQX). KynA is a nonselective EAA receptor antagonist (39), AP-5 is a selective NMDA receptor antagonist (9), and NBQX is a selective non-NMDA receptor antagonist (37). These antagonists are effective for several minutes (27); thus these ejections provided a means of creating prolonged neuronal dysfunction. The concentration of the antagonist was based primarily on what others have shown to be effective in other preparations (27, 36).

The protocol for all studies lasted ~105 min. During the initial 15 min (control period), breathing and ABP were continuously monitored while the animals breathed room air. Two 2-ml samples of arterial blood were withdrawn over the last 2 min of this period. Mock CSF with or without an EAA receptor antagonist was then ejected over 30 s as described for agonist ejections. Breathing and ABP were continuously monitored, and arterial blood was withdrawn 15, 30, and 60 min after the ejection. Then a second ejection identical to the first was made. After 15 min, CO2 sensitivity was assessed by increasing inspired CO2 in 2.5% increments every 5 min to 7.5%. Breathing and ABP were continuously monitored, and arterial blood was sampled over the last 2 min at each level of inspired CO2. At least 2 h were allowed for recovery before a second and final protocol was completed on the same day.

The substance and volume ejected varied within and between goats. For six of the goats, NMDA elicited a hyperpnea and hypocapnia when ejected into one guide tube. Initially, on these goats, mock CSF alone or with AP-5 was ejected at only the guide tube where NMDA had elicited a hyperpnea. Subsequently, in some of these goats, KynA or NBQX was also ejected only at this guide tube. In other goats the initial studies always involved ejection of mock CSF alone or with KynA in a single guide tube. Subsequently, unilateral or bilateral ejections (1 or 10 µl) of mock CSF alone or with one of the three EAA receptor antagonists were made into multiple guide tubes. For each goat the mock CSF and each antagonist were always ejected into the same guide tubes, including the tube where single, unilateral ejections were made. During any single study the maximum number of ejections was 12, which were completed in ~15 min.

**Protocol 4: Ejection of the neurotoxin KA.** The final ejection studies in six goats involved ejecting 5 mM KA (in mock CSF) unilaterally on the VLM surface. KA intensely stimulates one class of EAA receptors in the brain, resulting in sustained depolarization and eventually cell death (24, 36). As others have in the past (11, 14, 21, 28, 31, 32), we administered a neurotoxin to destroy cell bodies at the site of application without disrupting axons of passage. However, it is now known that KA also causes cell death distant to the ejection site (36). The distant effects occur hours after the local effect; thus, by continuously monitoring the goats, we were able to ascertain the local (caudal M VLM) effect on breathing. Breathing and ABP were monitored for at least 5 min before and continuously after the ejections. Arterial blood was withdrawn at 15- to 30-min intervals. A second ejection into a contralateral guide tube was made in all goats, a third ejection in two goats, and a fourth ejection in one goat. The time between ejections ranged from 30 min to 4 days. All goats were studied while breathing room air for at least 1 h at 24 h after the initial KA ejection, and three of the goats were studied periodically over the subsequent 3 days. In four of the goats, ventilatory responsiveness to CO2 was assessed as previously described 24 h or 4 days after the initial ejection.

When studies in the awake state were completed, the goats were anesthetized. We then ejected mock CSF with Alcian blue dye on the VLM surface, or we advanced the ejection tube such that the ejection was made into the tissue 1–2 mm below the surface. The goat was then killed, and the brain was perfused with phosphate-buffered saline (pH 7.3) and 4% paraformaldehyde fixative and then removed. Frozen, transverse sections (40 µm) were cut and examined for blue dye.

Signals from the recorder were stored in a Citus 486 computer for subsequent computerized computation of pulmonary ventilation (V˙I), tidal volume (Vt), breathing frequency (f), inspiratory time (Ti), expiratory time (Te), mean inspiratory flow rate (V˙I/Ti), mean ABP (MABP), and heart rate (HR). One-way analysis of variance for repeated measures was used to establish whether the mock CSF ejections had an effect on the physiological variables. Linear regression analysis was used to calculate ventilatory CO2 sensitivity (ΔV˙I/ΔPaCO2). Statistical significance was accepted for P < 0.05.

**RESULTS**

**Effect of VLM Cooling**

In all 12 goats, bilateral cooling of the caudal M VLM in the anesthetized state resulted in apnea (Fig. 2A). The apnea began on average 4.7 ± 6.5 s after the onset of cooling and was sustained on average for 26.3 ± 3.1 s. In the awake state, apnea did not occur in any goat with caudal M VLM cooling (Fig. 2). However, breathing was significantly decreased in all goats, reaching a nadir 27 ± 10% below control after 10–20 s of cooling. Unilateral cooling always had less effect than bilateral cooling. The unilateral cooling effect was not always the same on both sides, probably because the thermodes were not placed at exactly the same site bilaterally.

**Effects of NMDA Ejections**

In seven of eight goats, V˙I increased significantly 160 ± 25% after unilateral ejection of NMDA into one guide tube placed on the caudal M VLM. The hyperpnea began 1–7 min after the ejection and usually peaked 10–15 min after ejection, but the return to control V˙I was highly variable, occurring 30 min–4 h after the ejection (Fig. 3, A and B). The NMDA hyperpnea was a result of significantly increased V˙I/Ti and reduced Ti and Te (Table 1). In six of seven goats, PaCO2 decreased as expected from the hyperpnea, but in one goat, PaCO2 increased during the hyperpnea (Fig. 4). The NMDA ejections did not significantly alter MABP or HR (Table 1).

There were three additional important findings. 1) In two goats, ejections made at the same site on two different days elicited a similar response (Fig. 4B). 2) In two goats the NMDA hyperpnea did not occur when the selective EAA receptor antagonist AP-5 was ejected 15 min before the NMDA ejection (Fig. 3C). 3) The effective site for eliciting a hyperpnea appeared quite focal:
of the seven goats, none of the AMPA ejections altered breathing beyond the variation observed after ejection of mock CSF.

Effect of EAA Receptor Antagonist on Eupneic Breathing

There were no significant changes in $\dot{V}_i$ or $P_{aCO_2}$ (Figs. 6 and 7, A and B) or in any other measured parameter when mock CSF or 5 mM AP-5 was ejected into the single guide tube of the goats, where on a previous day an ejection of NMDA had elicited a hyperpnea. Likewise, there was no significant change in $V_i$ (Fig. 6), $V_i/T_i$, $T_i$, or $T_E$ (Table 2) when mock CSF or one of the three EAA receptor antagonists was ejected in multiple guide tubes unilaterally or bilaterally. Similarly, $P_{aCO_2}$ did not change with multiple

Effects of AMPA Ejections

In four of seven goats, $\dot{V}_i$ clearly increased after unilateral ejection of AMPA into one guide tube placed on the caudal M VLM. One of these goats (Fig. 5A) demonstrated a brief but definite hyperpnea after most AMPA ejections. In the other three of these four goats the AMPA ejections elicited a similar brief hyperpnea when ejected into some guide tubes but a prolonged hyperpnea when ejected into other guide tubes (Fig. 5, B–D). The AMPA hyperpnea in these four goats was a result of significantly increased $V_i/T_i$ and decreased $T_E$ (Table 1). These AMPA ejections did not significantly change MABP, but the ejections significantly increased HR by 18 beats/min (Table 1). Finally, in the other three
ejections of mock CSF, 5 mM AP-5, or 300 µM NBQX (Fig. 7, A–C). However, with bilateral ejections of 250 mM KynA, there was a small (1.9 ± 1.0 Torr) but significant increase in PaCO2 (Fig. 7D). This slight effect was not dose dependent; increasing the number or volume of ejections did not enhance the hypercapnia when an effect was observed with one 1-µl ejection on each side.

There was no significant effect on MABP or HR with multiple ejections of any antagonist (Table 2).

| Table 1. Ventilatory and circulatory effects of ejecting NMDA, AMPA, or KA on caudal M VLM of awake goats |
|-----------------|-----------------|-----------------|-----------------|
|                 | NMDA            | AMPA            | KA              |
|                 | Before          | After           | Before          | After           | Before          | After           |
| V˙I, l/min      | 6.60 ± 0.68     | 17.00 ± 2.35*   | 6.62 ± 0.90     | 19.20 ± 2.90*   | 9.54 ± 1.14     | 19.19 ± 3.53*   |
| Vr/Ti, l/s      | 0.30 ± 0.03     | 0.63 ± 0.07*    | 0.32 ± 0.05     | 0.89 ± 0.18*    | 0.38 ± 0.08     | 0.76 ± 0.14*    |
| Tr, s           | 1.27 ± 0.13     | 0.84 ± 0.07*    | 1.29 ± 0.37     | 1.29 ± 0.43     | 0.86 ± 0.18     | 0.46 ± 0.07*    |
| Te, s           | 2.13 ± 0.15     | 0.90 ± 0.07*    | 2.47 ± 0.44     | 1.70 ± 0.47*    | 1.71 ± 0.26     | 0.73 ± 0.14*    |
| MABP, mmHg      | 106.40 ± 2.70   | 113.00 ± 6.10   | 114.00 ± 6.20   | 117.30 ± 4.10   | 123.30 ± 2.20   | 126.30 ± 5.70   |
| HR, beats/min   | 103.00 ± 10.70  | 113.70 ± 15.70  | 96.00 ± 23.00   | 114.00 ± 26.00* | 87.10 ± 10.80   | 109.30 ± 17.50* |

Values are means ± SE of goats whose individual pulmonary ventilation (V˙I) data are presented in Figs. 3, 5, 9, and 10. Data represent average minute values 5 min before ejections and peak 1-min values. NMDA, N-methyl-D-aspartic acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; KA, kainic acid; VLM, ventrolateral medulla; Vr/Ti, mean inspiratory flow rate; Ti, inspiratory time; Te, expiratory time; MABP, mean arterial blood pressure; HR, heart rate. *Statistically significant difference from control.

Fig. 4. Effect on arterial PCO2 (PaCO2) of ejecting (dashed line) NMDA on caudal M VLM of 7 adult, awake goats. Each symbol represents data from a single goat, with symbols corresponding to those in Fig. 3, except ■ is added for a 7th goat. All ejections but one resulted in hypocapnia, and temporal pattern of hypocapnia differed among goats (cf. A and B). Also, response was reproducible, as indicated in B by a similar PaCO2 temporal pattern of 2 separate ejections at same guide tube in goats represented by ■ and Δ.

Fig. 5. Effect on V˙I of ejecting 1 µl of 20–40 µM α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) on caudal VLM of 4 awake goats. A–D represent data from a single goat. In A, B, and D, data are presented from 3 goats that also responded to NMDA ejections in Figs. 2 and 3; in 2 of these goats, AMPA and NMDA increased V˙I when ejected into same guide tube. Goat represented by data in C did not respond to NMDA when ejected into any guide tube including guide tube that elicited an AMPA hyperpnea. Arrows, times of ejections. There appeared to be a short-lasting hyperpnea after some ejections, but after 3 ejections there was a prolonged hyperpnea.
Effect of EAA Receptor Antagonists on CO₂ Sensitivity

The slopes of the CO₂ response curves were linear for all goats over all conditions. The squared correlation coefficients ($r^2$) between $V'_I$ and $PaCO_2$ exceeded 0.95 for all the data presented.

Ventilatory sensitivity to CO₂ ($l \cdot min^{-1} \cdot Torr^{-1}$) was tested after unilateral ejections only at the site where NMDA had previously elicited a hyperpnea. CO₂ sensitivity was 0.82 ± 0.06 and 0.88 ± 0.09 after ejections of mock CSF and the NMDA antagonist AP-5, respectively ($P > 0.05$).

CO₂ sensitivity was also not altered after any single unilateral ejection of any EAA antagonist. However, in one goat, increasing the total volume ejected (10 µl in each of 3 unilateral tubes) attenuated CO₂ sensitivity by ~33%.

Bilateral ejections of 1 µl of the selective antagonists (AP-5 or NBQX) at one or more guide tubes did not consistently alter CO₂ sensitivity (Table 3). However, bilateral ejections of 1 µl of KynA at one or more guide tubes significantly reduced CO₂ sensitivity to 60 ± 9% of control (Table 3). In those goats in which one 1-µl bilateral ejection attenuated CO₂ sensitivity, neither increasing the volume of the ejection to 10 µl nor increasing the number of 1-µl ejection sites further attenuated CO₂ sensitivity. The attenuation of CO₂ sensitivity by KynA was through an effect on $V_T$, $f$, $V_T/TI$, $T_i$, and $TE$ (Fig. 8).

Effect of KA Ejections

A hyperpnea was elicited by 10 of the 16 unilateral KA ejections on the caudal M VLM surface of six awake goats.
goats (Figs. 9 and 10). The onset of the hyperpnea usually occurred within 2–5 min after the ejection. The hyperpnea often lasted for 2–5 min, but after three ejections the hyperpnea was sustained for 60–180 min (Fig. 9, A–C). The magnitude of the hyperpnea varied from a 25% (Fig. 10B) to a >150% (Fig. 9B) increase in \( V_{\text{I}} \) above control. The hyperpnea was a result of a significant increase in \( V_{\text{T}}/T_{\text{I}} \) and a decrease in \( T_{\text{E}} \) (Table 1).

Usually after the hyperpnea, \( V_{\text{I}} \) returned to control levels for several hours. However, in one goat there was a transient hypopnea after the hyperpnea (Fig. 10A). In addition, \( V_{\text{I}} \) was below control 24 h (Fig. 9E) and 72 h (Fig. 10F) after the ejections in two goats. Usually, \( P_{\text{aCO}_2} \) was at or below control after the KA ejections (Figs. 9 and 10, D and H).

Induction of anesthesia 24–96 h after KA ejections resulted in a decrease in \( V_{\text{I}} \), but an apnea was never observed (Fig. 10F).

The \( V_{\text{I}} \) response to \( \text{CO}_2 \) was significantly attenuated to 60 ± 7% of control 1–4 days after bilateral KA ejection on the caudal M VLM (Table 3). This attenuation was a result of a significant increase in \( V_{\text{T}}/T_{\text{I}} \).

By 24 h after the KA ejection or sometime thereafter, the goats became lethargic, lost appetite, salivated excessively, and seemed to lack coordination for swallowing and maintenance of normal posture. Other researchers observed some of these changes in rats after systemic and/or intracerebral KA administration (15, 36).

Identification of Ejection Site

On necropsy the implantation site was identified by a slight indentation of the VLM surface and/or by fibrotic, necrotic tissue at the site of implantation.

Attempts at specific identification by ejecting blue dye were not successful. When in some goats dye was ejected on the VLM surface in a manner similar to the ejections of the EAA receptor antagonists, we never found dye in the underlying tissue. When in other goats the ejection tube was further advanced into the tissue, histological analysis identified the ejection site by the ejected dye and by the damaged tissue. However, this information provided only an indication of the ejection site; it did not indicate the diffusion pattern when EAA receptor antagonists were ejected on the surface. Accordingly, Fig. 1 shows the topographical site of the implanted thermodes and guide tubes.

**DISCUSSION**

There were three major findings of the present study.

1) In the caudal M area of the VLM surface, where NMDA and non-NMDA receptors exist on respiratory neurons, neither unilateral nor bilateral ejection in the awake state of nonselective or selective EAA receptor antagonists altered eupnic \( V_{\text{I}} \); only the selective antagonist caused a slight hypoventilation. 2) Neither unilateral nor bilateral ejections of the selective EAA receptor antagonists on this VLM surface altered ventilatory \( \text{CO}_2 \) sensitivity, but multiple unilateral or bilateral ejections of the nonselective antagonist attenuated \( \text{CO}_2 \) sensitivity to ~60% of control. 3) Unilateral ejection of the neurotoxin KA on the caudal M VLM surface in the awake state initially stimulated breathing, but subsequently there was no apnea or hypoventilation. However, KA reduced \( \text{CO}_2 \) sensitivity to 60% of control.

### Table 2. Ventilatory and circulatory effects of ejecting EAA receptor antagonists on caudal M VLM of awake goats

<table>
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<tr>
<th>Goat Symbol</th>
<th>Mock CSF</th>
<th>AP-5</th>
<th>NBQX</th>
<th>KynA</th>
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<tr>
<td></td>
<td>( V_{\text{I}}, \text{l/min} )</td>
<td>( V_{\text{T}}/T_{\text{I}}, \text{l/s} )</td>
<td>( T_{\text{E}}, \text{s} )</td>
<td>( \text{MABP}, \text{mmHg} )</td>
<td>( \text{HR}, \text{beats/min} )</td>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
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<tr>
<td>( \square )</td>
<td>8.99 ± 2.51</td>
<td>9.09 ± 2.49</td>
<td>6.74 ± 1.09</td>
<td>7.39 ± 1.22</td>
<td>7.03 ± 1.99</td>
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Values are means ± SE corresponding to \( V_{\text{I}} \) and \( P_{\text{aCO}_2} \) data in Figs. 6 and 7. Data represent average minute values 5 min before ejections and internal 25–30 min after ejections. CSF, cerebrospinal fluid; AP-5, 2-amino-5-phosphonovalerate; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo (F) quinoxaline; KynA, kynurenic acid. There was no statistically significant effect of any excitatory amino acid (EAA) receptor antagonist on any variable.

### Table 3. Slope of \( \text{CO}_2 \) response curve when mock CSF or EAA receptor antagonists were ejected bilaterally on caudal M VLM surface

<table>
<thead>
<tr>
<th>Goat Symbol</th>
<th>Mock CSF</th>
<th>AP-5</th>
<th>NBQX</th>
<th>KynA</th>
</tr>
</thead>
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<tr>
<td></td>
<td>( \Delta V_{\text{I}}/\Delta \text{PCO}_2 )</td>
<td>( \Delta V_{\text{T}}/\Delta \text{PCO}_2 )</td>
<td>( \Delta T_{\text{E}}/\Delta \text{PCO}_2 )</td>
<td>( \Delta \text{MABP}/\Delta \text{PCO}_2 )</td>
</tr>
<tr>
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<tr>
<td>( \Delta )</td>
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</table>

Values are expressed in \( \text{l} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1} \). *CO2 sensitivity after KynA and KA ejections was significantly different from that after mock CSF ejection.
Critique of the Methods

In our laboratory, CO2 sensitivity of unoperated goats is 1.3–1.9 l·min⁻¹·Torr⁻¹. In only three goats with implanted thermodes and guide tubes was CO2 sensitivity within this range. Indeed, in two of the goats studied here, the CO2 sensitivity approximated 1.5 and 1.0 l·min⁻¹·Torr⁻¹ before and 6 days after implantation, respectively. It is possible that the pressure of the implanted device on the VLM (which in most goats created a slight indentation) may have been sufficient to attenuate CO2 sensitivity, or the fibrotic/necrotic tissue observed in some goats (at the site of the implant) may represent destruction of neurons involved in intracranial chemoreception. However, three goats had minimal VLM compression and no necrosis, yet they had reduced CO2 sensitivity; thus some factor other than compression and tissue damage must have contributed to the low CO2 sensitivity.

Even though CO2 sensitivity may have been lower than normal in most goats, we are confident in concluding that KynA attenuated CO2 sensitivity. In five of six goats, bilateral ejections of KynA reduced CO2 sensitivity, and this effect was observed in goats that had normal CO2 sensitivity as well as goats that had relatively low CO2 sensitivity when mock CSF was ejected. Moreover, this effect was observed in goats that had minimal compression of the VLM surface and no deterioration of the VLM tissue.

We demonstrated that the Vt response to NMDA was blocked by a prior ejection of the selective antagonist AP-5; thus we are confident that we used an appropriate concentration of AP-5. However, we never attempted to block the response to AMPA with a prior ejection of NBQX; thus we assume that 300 µM NBQX is effective in awake goats, as demonstrated in other preparations (37). Despite these limitations, we believe that the interpretations/conclusions relative to questions 1 and 2 are warranted. However, some of the interpretations/conclusions regarding question 3 should be viewed as tentative until it is shown that 300 µM NBQX blocks non-NMDA receptors in awake goats.

As indicated earlier, we were able to identify the topographical location of the thermodes and guide tubes (Fig. 1). However, we were unable to identify the specific nucleus/cell groups that were affected by the VLM surface ejections, and we do not know how much of the ejectate actually was delivered and diffused into

Fig. 8. Relationship of Vt, tidal volume (VT), breathing frequency (f), mean inspiratory flow rate (VT/TI), inspiratory time (Ti), and expiratory time (Te) to PaCO2 when inspired CO2 is elevated. Values are means of 5 goats (1st 5 symbols in Table 3) after bilateral ejections of mock CSF (○) or 250 mM kynurenic acid (●) on caudal M VLM surface. Reduced CO2 sensitivity after kynurenic acid ejection was due to reduced VT and f secondary to effects on VT/TI and Te.

Fig. 9. Vt and PaCO2 of 3 goats after unilateral ejections of 5 mM kainic acid on caudal M VLM. Arrows, time of each ejection. Data in E–H were obtained 24h after data obtained in A–D. Note prolonged hyperpnea in all goats after a kainic acid ejection; subsequently, there was no apnea in any goat. Also, PaCO2 was at or near control during and after hyperpnea.
the tissue. As a result, conclusions from our data are limited in some respects. Nevertheless, by cooling and by ejection of an EAA receptor agonist, we documented that the ejections of the EAA receptor antagonist were at a site accessible to glutaminergic respiratory neurons. Moreover, the NMDA responses were reproducible, a majority of the KA ejections elicited a hyperpnea, and multiple bilateral ejections of KynA resulted in a significant hypoventilation and attenuated CO₂ sensitivny. These data indicate that fluid was probably delivered with each ejection, and it did diffuse into the tissue. Therefore, we believe that the limitations of the study did not prevent achievement of the stated objectives, and we believe that the stated conclusions are warranted. Finally, we previously (Fig. 8 in Ref. 34) provided information on the neuroanatomic structures in the caudal M VLM of adult goats, and additional unpublished data indicate that our guide tubes were placed over the rostral RTN, which is within 1–2 mm of the ventral surface in goats. Thus the data presented here provide insight into the contribution to the control of breathing by respiratory neurons near the VLM surface and possibly neurons in the RTN.

Is the Caudal M VLM Ventilatory “Drive” Required to Maintain Normal Eupneic Breathing in the Awake State?

Neuronal dysfunction induced by 30 s of cooling the caudal M VLM surface in the awake state reduces breathing by ~30% (Fig. 2) (12, 33, 34). These findings indicate that neurons at this site provide a significant drive to breathe during awake, eupneic conditions. However, this drive is not required to maintain a near-normal level of eupneic breathing during prolonged caudal M VLM neuronal dysfunction in the awake state. Neither selective nor nonselective EAA receptor antagonists ejected bilaterally on this VLM surface significantly altered V̇, and only the nonselective antagonist caused a mild hypoventilation. Moreover, bilateral ejection of the neurotoxin KA did not consistently reduce breathing or result in hypoventilation in awake goats.

If the brief initial intense stimulation by KA administration observed under anesthesia results in cell death (24, 36), then cell death should similarly occur after the hyperpnea in the awake state. Why does this presumed cell death not cause terminal apnea, the need for assisted ventilation for survival, or even a hypopnea? Also, why do EAA receptor antagonists not have a sustained effect on eupneic breathing? Obviously, excitatory input to the rhythm generator from carotid and widespread brain chemoreceptors, sources associated with wakefulness, and/or other sources compensate for attenuated VLM stimulation. In contrast, in anesthetized animals, when these other inputs are reduced or absent, the rhythm generator is critically dependent on caudal M VLM excitatory input; thus neurotoxic destruction of these neurons in the anesthetized state often reduces excitation below the level needed to sustain respiratory rhythm (28, 31, 32). However, recovery is possible after neurotoxin administration in the anesthetized state if breathing is supported through the period when excitation from wakefulness sources is attenuated (2, 11). Moreover, eventually with the compensation from these and possibly other sources or through reorganization within the central nervous system (plasticity), breathing is sustained during subsequent loss of wakefulness (see sustained breathing under anesthesia in Fig. 10F).

Can Widespread Brain Chemoreceptors Compensate for Prolonged Caudal M VLM Neuronal Dysfunction?

In anesthetized reduced preparations, chemoreceptors have been identified at widespread sites within the brain (3, 8, 16, 19, 20, 25). It is unclear, however, whether all these are capable of stimulating breathing during physiological conditions. Moreover, it is unclear where in the brain and how such potential widespread chemoreceptor input is “integrated” into an “appropriate” ventilatory response. Intriguing are the findings that in the anesthetized preparation a very focal acidosis (i.e., stimulation of relatively few chemoreceptors) can provide a hyperpnea that is ~60% of a global acidosis (8). On the other hand, a very focal lesion near the caudal M VLM surface or in the RTN can greatly attenuate or eliminate CO₂ sensitivity (28, 29, 32).
Data from recent VLM surface cooling studies provided some insights into the VLM contribution to CO₂ sensitivity in the awake state (12, 13). Specifically, at 10–30 s of VLM cooling, breathing was attenuated more during hypercapnic than during eupneic, hypoxic, and exercise conditions. These data indicate a dysfunction of chemoreceptor-related neurons and suggest that presumed chemoreceptors in this VLM area contribute to the CO₂ hyperpnea. However, the CO₂ hyperpnea was attenuated only ~60% when all S and M areas were cooled. Therefore, chemoreceptors at other sites must be capable of stimulating breathing in the awake state. Moreover, because the hyperpnea was not eliminated, area S of the VLM does not appear to be the site of a neurological integrator and/or a site of convergence of all chemoreceptor afferents, as has been previously postulated (35).

In this study, we investigated whether, in the awake state with prolonged VLM chemoreceptor dysfunction, other chemoreceptors would maintain CO₂ sensitivity near normal levels. We found that CO₂ sensitivity was not altered from control when EAA receptor antagonists were ejected unilaterally at a site where EAA receptor agonists stimulated breathing. Moreover, in most cases, multiple unilateral ejections of the antagonists did not alter CO₂ sensitivity. If it is assumed (from cooling studies) that chemoreceptors in this region normally stimulate breathing during hypercapnia, these data suggest that “compensation” can occur when a portion of VLM chemoreceptor activity is eliminated. However, with multiple large unilateral ejections of EAA receptor antagonists and with bilateral ejection, CO₂ sensitivity was significantly attenuated. In addition, bilateral ejections of KA also significantly attenuated CO₂. Accordingly, there appears to be a limit to the compensation that can occur for a dysfunction of a portion of the chemoreceptor neurons. On the other hand, there also seems to be a maximum that CO₂ sensitivity can be reduced by VLM dysfunction. For example, bilateral ejection of 1 and 10 µl of an EAA receptor antagonist equally attenuated CO₂ sensitivity in some goats. Moreover, in our previous study (13), CO₂ sensitivity was reduced by ~60% when virtually all VLM M and S areas were cooled, and it was presently attenuated in several goats to nearly the same degree after the nonselective EAA antagonist KA was ejected on only the caudal M VLM surface.

The entire meaning of a chemoreceptor system that appears to have a limited compensatory capacity but also limits the amount that CO₂ sensitivity can be reduced by dysfunction of VLM chemoreceptor neurons is unclear. Seemingly compatible with these characteristics is a system with an integrator, a system that provides a coordinated, appropriate response to input from widespread chemoreceptors. However, our previous data on VLM cooling in the awake state (13) do not support the concept of an integrator near the VLM surface. Conceivably, the more dorsally located premotor neurons are capable of chemoreceptor integration (37).

During bilateral caudal M VLM neuronal dysfunction, the attenuation of CO₂ sensitivity to 60% of control contrasts with the nearly complete compensation of eupneic breathing. These findings indicate that the mechanism of compensation for eupneic breathing is not available, nor is it capable of similar compensation for CO₂ sensitivity. In addition, these findings emphasize that eupneic breathing is neither determined solely by nor is it critically dependent on CO₂ sensitivity.

What EAA Receptor Subtype Mediates the Caudal M VLM Effect on Breathing in the Awake State?

A hyperpnea was elicited in seven of eight awake goats by ejection of NMDA, in four of seven goats by ejection of AMPA, and in six of six goats by ejection of KA on the caudal M VLM. These data indicate that all three EAA ionotropic receptor subtypes are located on neurons near the caudal M VLM surface. The prolonged hyperpnea in response to some ejections may suggest that metabotropic receptors were also activated by some ejections. Nattie and Li (30) provided data supportive of metabotropic receptors in the RTN of anesthetized rats. An alternative explanation is that some ejections excited neurons that activated a neuronal network that mediated a prolonged hyperpnea. Several other investigators applied EAAs to area S or the more rostral VLM surface or into RTN tissue of anesthetized mammals and found stimulation of breathing (5, 14, 18, 22, 32). However, we (see Fig. 3 legend and others (5, 22) also found no effect or a depressant effect of EAA receptor agonist ejections or injections at some VLM sites on breathing (5, 22).

Ejections of neither the selective NMDA antagonist (AP-5) nor the non-NMDA antagonist (NBQX) consistently altered eupneic breathing or CO₂ sensitivity. However, the nonselective antagonist (KynA) caused a slight hypoventilation and attenuated CO₂ sensitivity. These findings are somewhat analogous to those of Abrahams et al. (1). They injected into the subretrofacial nucleus of anesthetized rats and found that KynA induced apnea, an NMDA antagonist slightly decreased breathing, and a non-NMDA antagonist had a minimal effect on breathing. Jung et al. (17), Nattie et al. (27), and Dillon et al. (10) also attenuated breathing by injecting KynA into tissue near the VLM surface. Nattie et al. also found that selective EAA receptor antagonists injected into the RTN attenuated phrenic nerve activity, and Jung et al. attenuated breathing when a non-NMDA antagonist was injected into the caudal VLM. Dillon et al. and Jung et al. found increased breathing or no effect of EAA receptor antagonist injected at some VLM sites.

Accordingly, several investigators (1, 5, 10, 14, 17, 18, 22, 27, 30, 32) found a site near the VLM surface where EAA receptor agonists and antagonists had an effect on breathing, but there is no clear consensus on the site or EAA receptor subtype that affected breathing. These varying results may reflect differences in preparations between studies (23), or there might be an intermingling of inhibitory and excitatory glutaminergic neurons that could account for the differences in findings of the various investigators.
A conclusion from our studies is that the caudal M VLM drive to breathe is not critically dependent on any single EAA receptor subtype. Moreover, our data do not provide support for the concept of a specialized function in the control of breathing of the different classes of glutamate receptors (4). Indeed, we found that ejections of EAA agonists stimulated breathing by increasing VT/TI and shortening T1 and TE. In addition, ejection of KynA attenuated CO2 sensitivity by an effect on VT/TI, T1, and TE. It seems that several EAA receptor subtypes have the capability to alter drive and timing components of breathing. This would allow compensation by one receptor subtype when another subtype is dysfunctional.

Summary

With prolonged dysfunction of neurons near the caudal M VLM surface, other mechanisms compensate to maintain near-normal, awake, eupneic breathing. However, equivalent dysfunction does not result in compensation to maintain awake CO2 sensitivity. With more limited caudal M neuronal dysfunction, normal CO2 sensitivity is maintained. On the other hand, there appears to be a maximum that CO2 sensitivity can be attenuated by caudal M neuronal dysfunction. Finally, our data do not support the concept of a specialization of function by NMDA and non-NMDA receptors on respiratory neurons in this VLM region.

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