

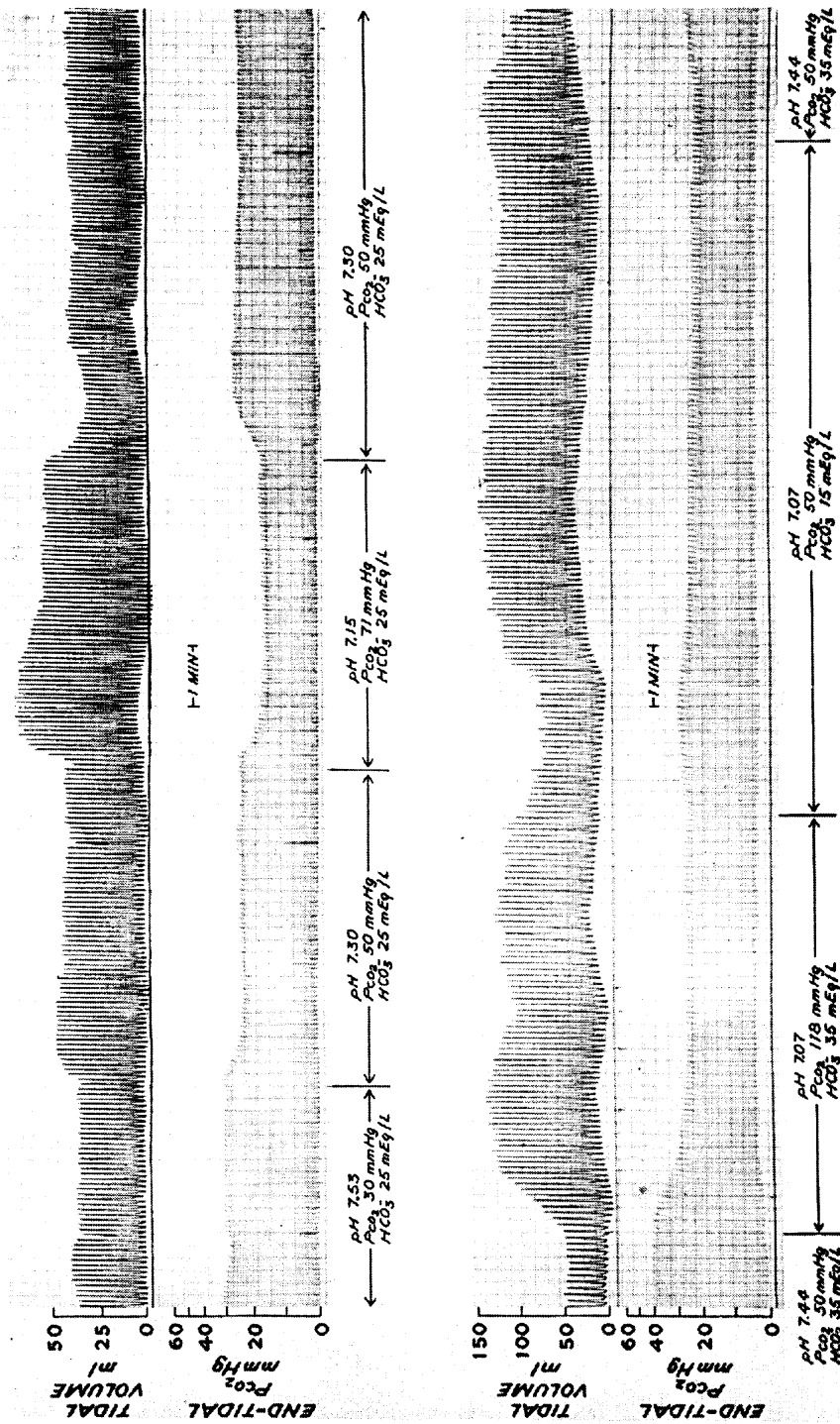
## Central Chemoreceptor Location and the Ventrolateral Medulla

EUGENE E. NATTIE, AIHUA LI, and E. LEE COATES

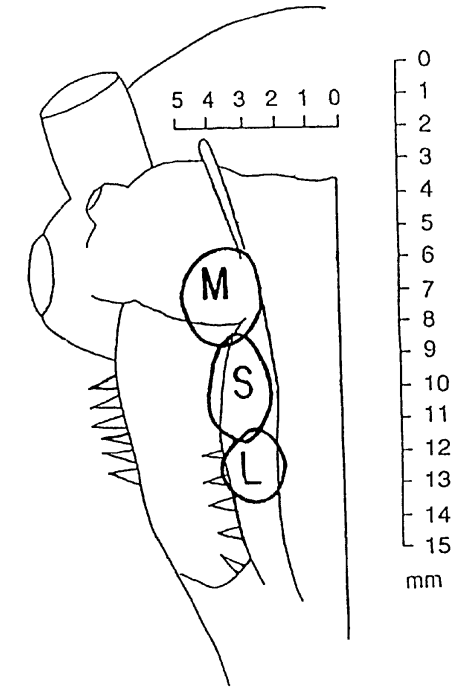
Dartmouth Medical School  
Lebanon, New Hampshire

### I. Introduction

Historically, central chemoreceptors have been located at or within a few hundred micrometers of the ventral surface of the medulla (1-6). Mitchell et al. (3,4) stimulated ventilation by bathing the subarachnoid region of the ventrolateral medulla (VLM) directly with acidic solutions (Fig. 1). The direct application of acidic or basic artificial cerebrospinal fluid to specific locations on the VLM surface using small cotton pledgets or a superfusion apparatus delineated a rostral chemosensitive area, referred to as Mitchell's area (M), and a caudal chemosensitive area, referred to as Loeschcke's area (L) (4,5) (Fig. 2). Tonic nonrespiratory modulated medullary units located just beneath the VLM surface at these chemosensitive areas have been shown *in vivo* (7-10) and *in vitro* (11-15) to increase firing with increased CO<sub>2</sub>. However, it is difficult to prove that these units are actually chemoreceptive and responsible for changes in breathing. An intermediate area (IMA), lying between the M and L areas and referred to as Schläfke's area (S), was also described (Fig. 2). The IMA was not thought to be chemosensitive, but cooling of this area produced depression of ventilation and of chemosensitivity (6,16-18). This paper will



**Figure 1** Effects on ventilation and end-tidal PCO<sub>2</sub> of changing the hydrogen ion concentration and PCO<sub>2</sub> of artificial cerebrospinal fluid introduced into the subarachnoid space of two anesthetized cats with vagal and carotid sinus nerve section. (From Ref. 3.)



**Figure 2** The topographical locations of the rostral (Mitchell's or M) and caudal (Loeschcke's or L) chemosensitive areas and the intermediate (Schláfke's or S) area in the cat. The vertical rule is in millimeters caudal to the foramen cecum; the other in millimeters lateral to the midline.

expand on these traditional concepts on central chemoreceptor location and on the role of the IMA.

## II. Central Chemoreceptors

This set of locations for central chemoreceptors, at or within a few hundred micrometers of the VLM surface at rostral and caudal sites, has received widespread but not universal acceptance. In rats, for example, some have found ventilatory responses to VLM acid application (19) and others have not (20). One problem is that substances applied to the VLM surface can actually be transported more deeply within the medulla by numerous blood vessels that penetrate from the ventral surface (7). For example, both large (21) and small radiolabeled molecules (22-24) applied to the VLM surface have been shown to penetrate within minutes to a depth of up to 2 mm. These observations suggest that the precise location of central chemoreceptors cannot be accurately deduced from surface application studies.

Others have argued for the presence of central chemoreception at sites well distant from the surface of the VLM. In vivo studies have shown that a high proportion of respiratory-modulated medullary single units in the pons and dorsal medulla (25,26) increase their firing rate with systemic hypercapnia. Injections of CO<sub>2</sub>-laden saline into the vertebral artery (27-29) have shown the presence of CO<sub>2</sub>-responsive tonic units located near the rostral aspect of the ventral respiratory group (VRG) and ventral to the nucleus tractus solitarius (dorsal respiratory group, DRG) as well as near the VLM surface. In these experiments however, the increase in unit discharge may not reflect chemosensitivity per se, it may merely reflect a respiratory system response—i.e., the unit is part of the respiratory control system responding to the chemoreceptor stimulation. In vitro studies have also shown cells responsive to acidic stimulation at more dorsal locations near the nucleus tractus solitarius (13,14,30,31) as well as in parts of the slice not usually associated with respiratory control (14). These in vitro studies indicate that there are many neurons within the brainstem that are excited by CO<sub>2</sub>/H<sup>+</sup>. Whether any of these responses represent ventilatory chemoreception is unknown, since no measure of ventilatory output could be included.

Non-electrophysiological approaches have also suggested that many parts of the brainstem participate in the response to hypercapnia. The immunohistochemical localization of the protein product of the *c-fos* oncogene describes neurons activated by a particular stimulus and has been applied to the study of chemoreception. Rats exposed to 13% to 15% CO<sub>2</sub> show *fos*-like immunoreactivity within 150 μm of the VLM surface at the rostral and caudal chemosensitive areas and at the IMA as well as more dorsally near the NTS (32). Similar studies have shown *fos*-like immunoreactivity at the locus ceruleus (33) and in the region ventral and ventromedial to the facial nucleus (34). This approach does not distinguish between receptor neurons and any other neurons activated in the response to hypercapnia.

We have searched for a method to produce a focal region of acidosis in the brainstem that might result in a respiratory system response. For example, we used a microdialysis system to dialyze focal brainstem regions with hypercapnic solutions. This approach did stimulate respiratory output but was limited by nonspecific effects, which were related to the size of the dialysis probe. In a study designed initially for a different reason, we noted that acetazolamide (AZ) applied to the surface of the VLM in anesthetized cats under isocapnic conditions increased phrenic amplitude and decreased medullary tissue pH (35). Surprisingly, we also found that microinjections (10 to 100 nL) of AZ unilaterally at putative VLM chemoreceptor sites also brought about large increases in phrenic activity (35). This observation suggested that we might use AZ injections as a probe to search for central chemoreceptor locations.

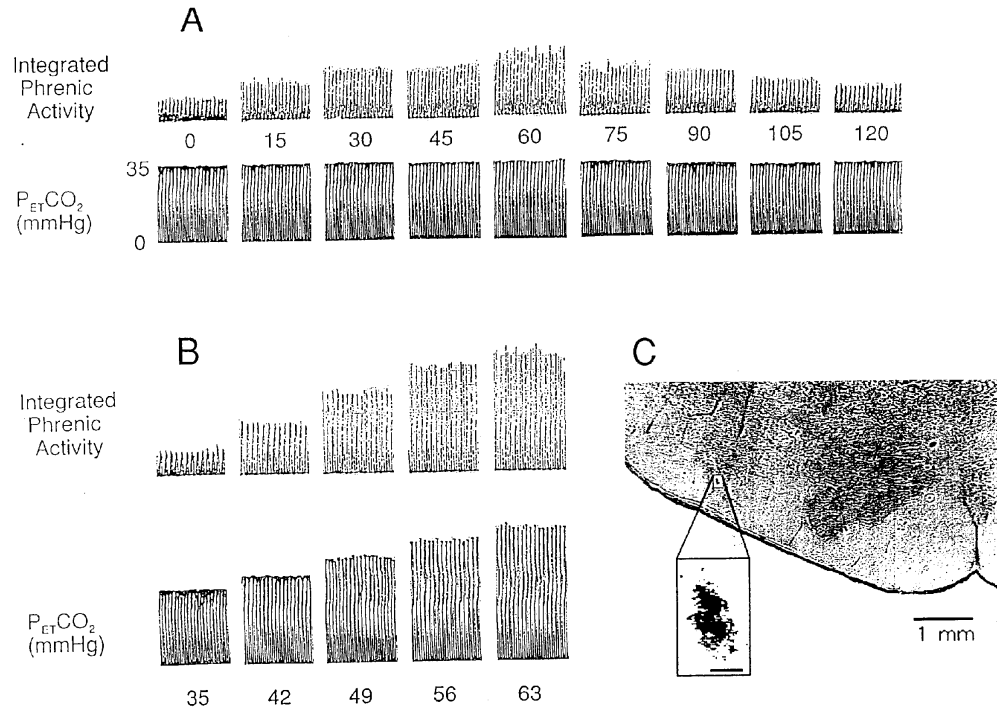
To determine the size of the region of tissue acidosis produced by AZ microinjections, we made 1-nL AZ ( $5 \times 10^{-6}$  M) injections in the medulla of

anesthetized rats and measured pH at varying distances from the AZ injection center (36). The measured pH change following AZ injection was normalized to that produced in each animal by a 20 mm Hg increase in end-tidal PCO<sub>2</sub>. At the center of the AZ injection, the average tissue pH change was equivalent to that produced by a 36 mm Hg increase in end-tidal PCO<sub>2</sub>. The medullary pH change produced by AZ rapidly decreased with radial distance away from the injection center, such that at distances greater than 350 μm, tissue pH was unaffected. We then used this injection volume with AZ concentrations of  $5 \times 10^{-6}$  to  $10^{-5}$  M as a probe to search for the locations of central chemoreceptor sites, knowing that the region of tissue acidosis would have a radius of 350 μm or less.

We made such 1-nL AZ injections to produce focal brainstem regions of decreased pH in anesthetized cats and rats and measured the response using the phrenic nerve as an index of the whole respiratory control system response to the focal pH change. Figure 3 shows a typical response. The nondiffusible fluorescent microbeads included with each injection mark the injection site, in this case in the region just dorsal to the caudal chemosensitive area. The prior measured tissue pH changes indicate that the entire region affected by the combination of the subsequent AZ diffusion from the injection site and the AZ effects on tissue pH lies within 350 μm of the injection center. While many neurons could be affected, the region of decreased tissue pH is small enough to allow the use of this approach to probe the brainstem for the location of central chemoreceptor sites.

Figure 3 also shows the time course of the increase in phrenic nerve activity, measured with constant end-tidal PCO<sub>2</sub>, which resulted from this injection. The response to increased systemic PCO<sub>2</sub> is shown and indicates the maximum response to stimulation of all central chemoreceptors in this cat. Note that at 60 min following the AZ injection, which stimulated only the chemoreceptors within the region of decreased pH produced by the injection, the phrenic nerve activity has increased to a level that is a large fraction of the maximum observed with systemic CO<sub>2</sub>. Overall in these experiments, 26 of 57 (46%) injections in 30 cats produced a significant increase in phrenic activity, which returned to baseline within 120 min. For these 26 positive injections, on average, the normalized phrenic activity increased from 35% of maximum to 56% of maximum following the AZ injection. The sites of these positive injections are shown on Fig. 4, and they can be grouped into three regions: first, sites within 800 μm of the VLM surface at the rostral and caudal chemosensitive areas and at the IMA; second, sites near the nucleus tractus solitarius; and third, sites more rostrally located in the vicinity of the locus ceruleus. Similar findings were obtained in rats.

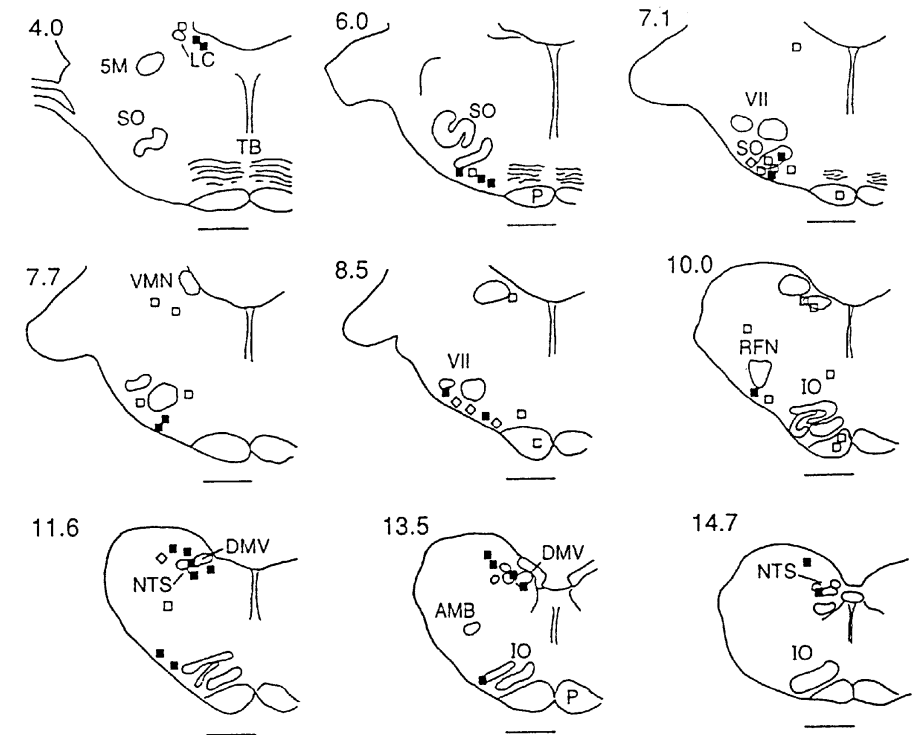
The use of 1-nL AZ injections to produce focal regions of decreased pH results in the stimulation of a sufficient population of chemoreceptors to produce a measurable control system output—i.e., a phrenic activity. This fortunate outcome allows us to know that the AZ injection did stimulate a ventila-



**Figure 3** (A) Changes in phrenic activity at times (shown in numbers below tracings) following a 1 nL injection of acetazolamide into the region shown on panel C. Note that end-tidal  $PCO_2$  is kept constant. (B) The response of the phrenic to increased end-tidal  $PCO_2$  (numbers below bottom tracing reflect mean end-tidal  $PCO_2$ ). (C) Cross section of medulla showing site of fluorescent beads marking this injection lateral to the inferior olivary nucleus. Inset shows a higher-power image of the fluorescent beads. Bar is 100  $\mu$ m. Note that the response to this single AZ injection is maximum at 60 min and that this maximum is a significant fraction of that observed with systemic hypercapnia. (From Ref. 37.)

tory chemoreceptor site; it did not merely excite a group of medullary neurons of unknown function. The results indicate that central chemoreception is located at widespread sites within the brainstem. In fact, there may well be other sites. For example, hypercapnia excites neurons in hypothalamic slices (37), and the approach of the AZ microinjection probe can be used to ask if local acidosis within the hypothalamus results in ventilatory stimulation. Such work is in progress.

One may ask if the AZ could possibly have an effect independent from that of the focal tissue acidosis. The presence of many ineffective injection sites and the absence of any response to injections of an inactive AZ analogue are helpful controls for the specificity of the AZ injection. Inhibition of brain carbonic anhydrase using acetazolamide administered systemically (38) and via



**Figure 4** A series of medullary cross sections of the cat showing microinjection sites determined by localization of the fluorescent beads injected with the AZ. Numbers at the top left of each section refer to millimeters caudal to the interaural plane. The solid squares represent AZ injection sites associated with an increased phrenic amplitude; open squares sites at which the AZ injection had no effect; and open diamonds sites of the injection of an AZ analogue which had no effect. Note the distribution of positive AZ injection sites along the VLM surface, in the vicinity of the NTS, and in the vicinity of the LC. Calibration bars are 2 mm. Abbreviations: AMB, nucleus ambiguus; DMV, dorsal motor nucleus of the vagus; LC, locus ceruleus; 5M, motor trigeminal nucleus; NTS, nucleus tractus solitarius; P, pyramidal tract; RFN, retrofacial nucleus; TB, trapezoid body; VMN, medial vestibular nucleus. (From Ref. 36.)

local application on, or microinjection into, the VLM (35,36) increases ventilatory output and decreases medullary tissue pH. In response to increased  $CO_2$ , the phrenic output at any level of  $CO_2$  is increased; i.e., the response is shifted upward. This reflects the additive effects of the greater baseline levels of ventilatory output produced by AZ injection and the stimulation of the hypercapnia (35,38). The slope of the steady-state phrenic nerve response to hypercapnic stimulation is unaffected by AZ treatment, although responses to step increases in  $PCO_2$  are delayed in reaching the steady state (35,39,40). These data suggest that the  $CO_2/H^+$  transduction process is unaffected by AZ, although the time course is slowed.

The exact function of carbonic anhydrase within the central nervous system is unknown. Carbonic anhydrase is located in cells within the VLM chemosensitive areas (41) and in neurons cultured from the medulla (42), many of which can be excited by hypercapnia (43). Recent *in vitro* evidence points to an important role for the enzyme in pH mediated events. Accidental dialysis of intracellular carbonic anhydrase via a whole cell patch pipette removes the pH sensitivity of some neurons and addition of the enzyme to the pipette restores it (44). Chesler and Kaila (45) have proposed that carbonic anhydrase is necessary for activity-dependent changes in extra- and intracellular pH in the nervous system. For example, in crayfish, the use of carbonic anhydrase inhibitors affects the GABA-induced increase in extracellular pH and decrease in intracellular pH. These activity-dependent changes in neuronal intra- and extracellular pH presumably modulate the effectiveness of neurotransmitters and perhaps neuromodulators.

Thus, while carbonic anhydrase appears to be required for some single neurons to be excited by hypercapnia *in vitro* and for activity-dependent pH changes to take place at the synaptic level, in contrast, the steady-state ventilatory response to hypercapnic stimulation of central chemoreceptors measured *in vivo* does not appear to require the presence of this enzyme. We conclude that the effect of AZ injections on phrenic activity is most likely due to the focal changes in tissue pH.

The location of central chemoreceptors at many sites together with the unexpectedly large response to any single injection when expressed as percent of the maximum response observed with stimulation of all central chemoreceptor sites leads to the following hypothesis. Central chemoreceptors exist at many locations within the brainstem to sense local tissue or cell pH changes. Acidosis at any of these locations can result in a ventilatory response, which would tend to correct or minimize this focal acidosis. Central chemoreceptors, then, would exist as a guardian of medullary pH balance, using the ventilatory system to regulate local pH quickly. We predict that studies in progress will show other brainstem locations with central chemoreceptor function.

### III. The Intermediate Area

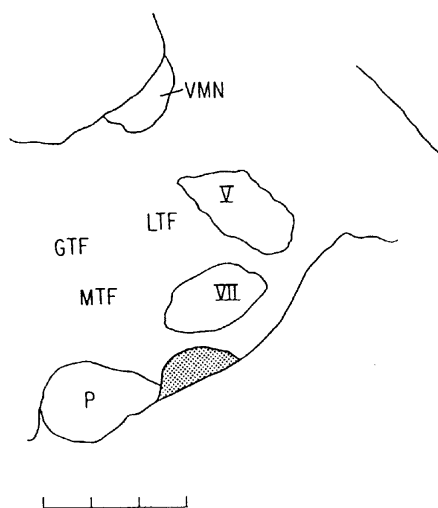
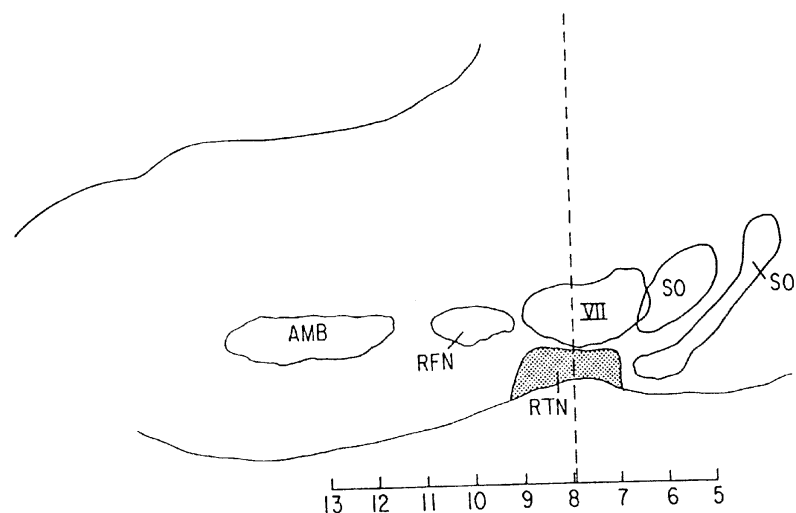
The IMA or Schläfke's area (3-6), located 3 to 4 mm lateral to the midline and rostral to the rootlets of the twelfth cranial nerve (Fig. 2), is remarkable in that surface cooling using temperatures that block neuronal function but not that of passing axons results in apnea (16,17). The use of a cooling probe at the IMA and, just rostrally, at the level of the caudal aspect of the rostral chemosensitive area also results in apnea and in decreased CO<sub>2</sub> sensitivity (18). Initially the IMA was proposed to be an integrative site not directly involved in chemoreception (46). The use of 1-nL injections of AZ to probe for central chemoreceptive locations (36) showed that chemoreception is present at regions

all along the VLM, including the IMA. However, this does not detract from the significance of the effects of IMA cooling on ventilatory output; it suggests that neurons within this medullary region may have many respiratory control functions. The probable anatomical substrates of the IMA include the retrotrapezoid nucleus, the retrofacial nucleus, the subretrofacial region, the nucleus paragigantocellularis lateralis, and perhaps aspects of the rostral VRG, including the Bötzing and pre-Bötzing regions.

Many recent studies have examined the possible role of these structures in the effects observed during IMA cooling. Within the retrofacial nucleus, lesions in the cat (47,48) and procaine injections in the rabbit (49) decrease phrenic nerve activity, sometimes to apnea, and CO<sub>2</sub> sensitivity. At sites approximately within the rostral VRG, 1.5 mm deep to the VLM surface, bilateral injections of glutamate receptor antagonists produce apnea in anesthetized cats (50,51) and rats (52,53). Small injections of excitatory amino acids at low concentrations (e.g., 1 to 100 mM for glutamate) within the dorsomedial and ventrolateral VRG also produced phrenic inhibition (54). The nucleus paragigantocellularis lateralis (PGCL) appears to be of particular importance. This structure in cat (55) and rat (56) lies essentially just dorsal to the center of the IMA. Afferent inputs converge there from a variety of sites (57,58), suggesting involvement of PGCL neurons in pain, analgesia, cardiovascular control, respiratory control, exteroceptive sensation, and arousal. A major source of efferents from the PGCL is the locus ceruleus, which itself has widespread inputs to the higher nervous system and is thought to be primarily involved in arousal and vigilance (59).

Studies in the *in vitro* neonatal rat brainstem preparation in a region that probably lies just deep to the IMA have provided evidence for the site of respiratory rhythmogenesis, at least for this rhythm in this preparation. Neurons located within a small region of the VLM just medial to the caudal aspect of the facial nucleus and close to the surface of the VLM (60) and a region just caudal to the level of the retrofacial nucleus (61) have been proposed for this function. This is a difficult preparation in that it has a clearly demarcated hypoxic central core of tissue, with the viable outside ring of medullary tissue being of the order of 800  $\mu$ m in thickness (62). So the importance of these findings for respiratory control in an adult with a completely viable brainstem is uncertain. Yet it is striking that both of these proposed regions for respiratory rhythmogenesis lie approximately within those anatomical regions which are substrates for the IMA in adult mammals, and that cooling or lesioning at sites within these regions in adult mammals have dramatic effects on ventilatory control.

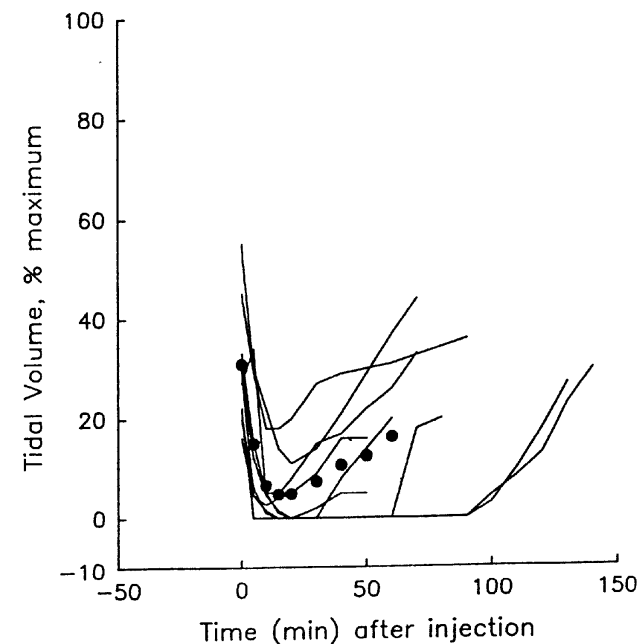
Recent work in my laboratory has focused on one of these neuronal groups lying dorsal to the IMA, the retrotrapezoid nucleus (RTN) (Fig. 5). Initial attempts in my laboratory to ask if neuronal cell bodies, not axons of passage, lying near to the VLM surface were involved in chemoreception involved the use of kainic acid, an excitatory amino acid analogue and neurotoxin. Applica-



**Figure 5** The location of the retrotrapezoid nucleus (RTN). The top panel is a parasagittal section 3.7 mm from the midline; the bottom panel a cross section at the dotted line in the top panel. The stippled area represents the RTN. The rules are in millimeters. Abbreviations not in Fig. 4 are: VII, facial nucleus; SO, superior olivary nucleus; LTF, lateral tegmental field; GTF, gigantocellular tegmental field; MTF, magnocellular tegmental field. (Modified from Fig. 1 in Ref. 69.)

tion of kainic acid to the VLM surface at the level of the rostral aspects of the IMA and caudal aspect of area M (63) and, subsequently, its microinjection within 800  $\mu\text{m}$  of the VLM surface specifically at one anatomical location (64) results in decreased phrenic activity, often to apnea, and an absent response to hypercapnia (Fig. 6). These effects last for many hours. The specific site of greatest effect was just ventral and ventromedial to the facial nucleus, a location that appears to be beneath the topographical border of area M and the rostral aspect of the IMA. Electrolytic as well as chemical lesions produced in decerebrate as well as anesthetized cats have similar effects (65) as they do in the anesthetized rat (82).

Following injection of retrograde tracers into the DRG and VRG, Smith et al. (66) described labeling in this same region, indicating the presence of monosynaptic anatomical connections between DRG and VRG and this location. They named this site lying ventral and ventromedial to the facial nucleus the retrotrapezoid nucleus. Studies have now shown the presence of respiratory-modulated single units in the retrotrapezoid nucleus of cat (67) and rat (68), some of which increase their firing rate with hypercapnia (8). The fact that rather small amounts of destruction produced unilaterally at this location can



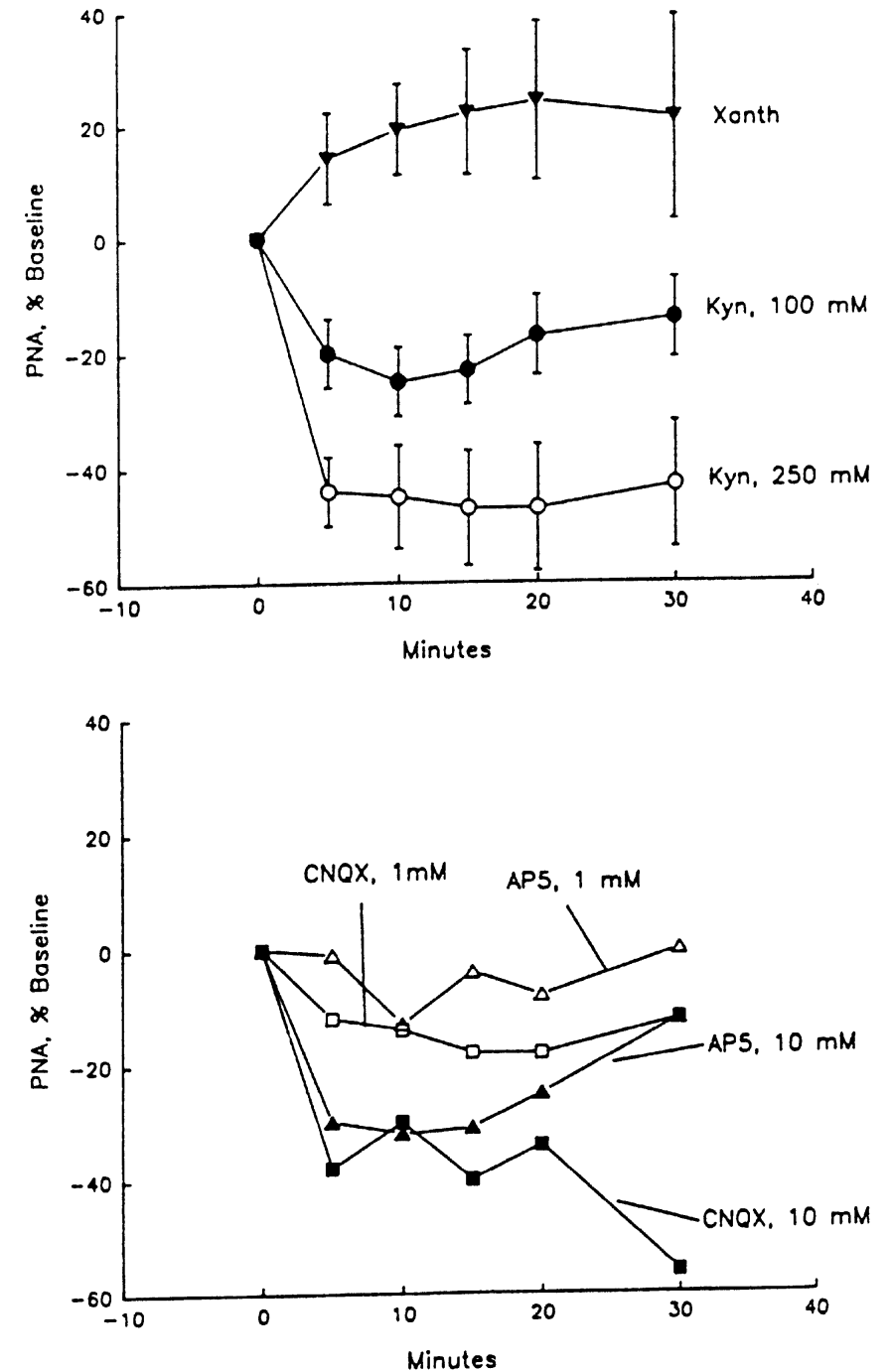
**Figure 6** The individual (thin lines) and average (solid symbols) responses of integrated phrenic amplitude ("tidal volume") to single, unilateral 10-nL injections of kainic acid (4.7 mM) into the RTN of the anesthetized cat. (Data from Ref. 64.)

result in long-lasting apnea and absent chemosensitivity suggests an important role for these respiratory neurons. The medial aspects of this region lying ventral to the facial nucleus can overlap with portions of the PGCL and the parapyramidal region of the midline raphe. Here we refer to the region of interest as the RTN, realizing that the precise identification of neurons in this area is a task for future experiments.

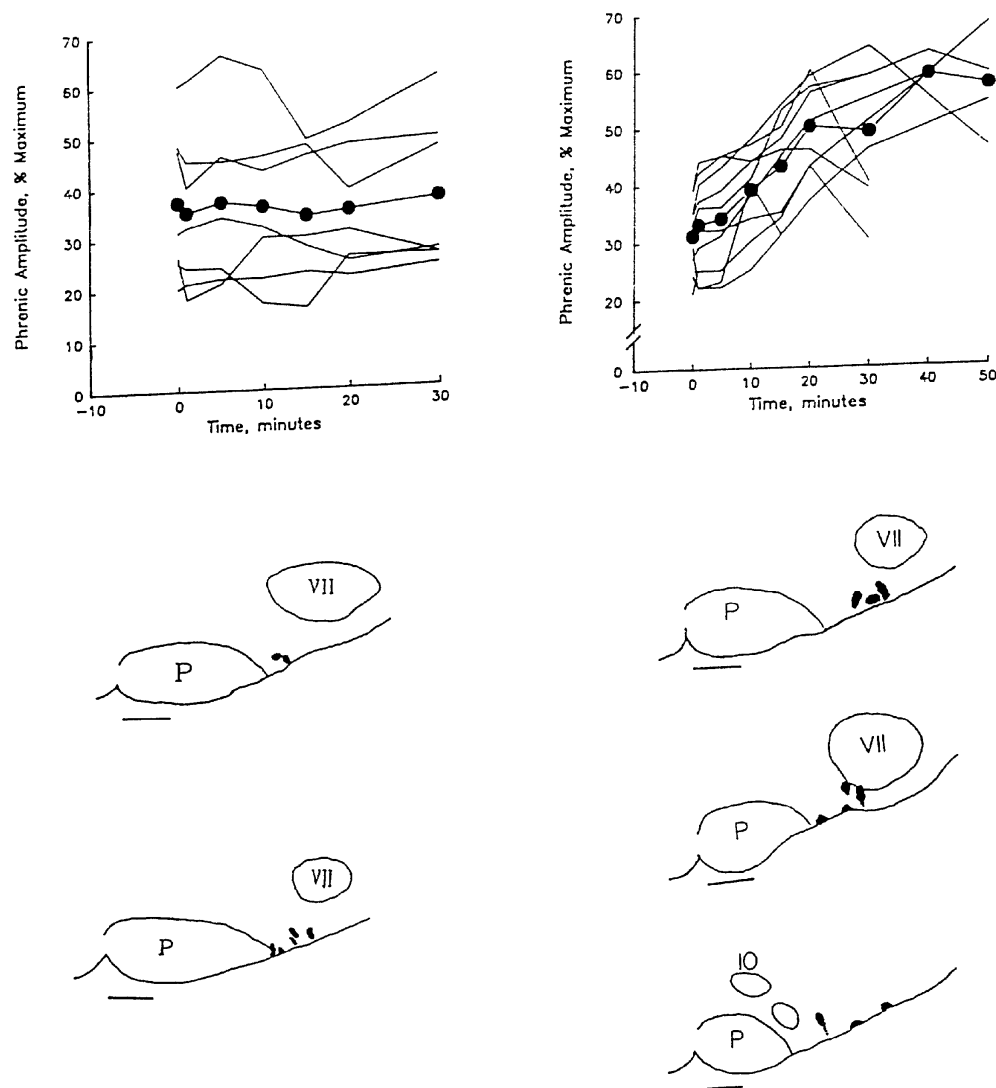
We have examined the effects of glutamate receptor antagonists injected into the RTN of the anesthetized cat (69) and rat (Nattie E.E., and Li A.; unpublished observations). Unilateral 10-nL injection of kynureate (a nonspecific glutamate receptor antagonist), CNQX (a non-N-methyl-D-aspartate [NMDA] receptor antagonist), or AP-5 (an NMDA receptor antagonist) into the RTN results in a decrease in the amplitude of the integrated phrenic signal (Fig. 7) and in the slope of the CO<sub>2</sub> response. These effects have a dose dependence, with an effective dose for kynureate of 100 mM; for CNQX and AP-5, of 10 mM. We concluded that endogenous glutamate input to RTN neurons is present under eucapnic conditions and that this input is necessary for expression of the normal CO<sub>2</sub> response. The source of this input is unknown. We do not attribute the decrease in CO<sub>2</sub> sensitivity to direct inhibition of the chemoreceptor sites in this region. Instead, we feel that it is due to inhibition of neurons that provide a tonic excitation of the respiratory system and whose function is necessary for the expression of a normal CO<sub>2</sub> response.

Direct injection (10 nL) into the RTN of glutamate itself (70) at the appropriate dose increases the amplitude of the integrated phrenic signal. A 100-mM dose produces this excitatory response, a 10-mM dose has no effect (Fig. 8), and a 1M-dose depresses phrenic amplitude, probably via depolarization block. In response to the 100-mM dose, we found, surprisingly and unexpectedly, that the duration of the phrenic stimulation was related to the duration of the glutamate injection (Fig. 8). In anesthetized cats (70) and rats (83) unilateral 10-nL RTN glutamate injection at 100 mM stimulates phrenic amplitude transiently if injected over 30 ms to 3 s. When the same volume and concentration is injected over 60 s, the phrenic amplitude is increased for 40 to 50 min. We compare this prolonged phrenic response to the long-term facilitation observed after repeated trains of carotid sinus nerve stimulation (71), suggesting that the 60 s of continuous glutamate injection mimics the release of glutamate in the RTN during the repeated stimulation of the nerve. The mechanism is uncertain. RTN single units do fire longer with 60-s versus 30-ms injections (70), with one unit observed having a sustained increase in firing rate over 40 min. We have suggested and are evaluating the possible role of metabotropic glutamate receptors in this long-term response.

We have observed a similar long-term stimulation of phrenic amplitude following unilateral RTN injection of thyrotropin releasing hormone (TRH) (0.5 mM; 10 nL) but this effect does not require long injection durations (72). This preliminary observation suggests an important role for TRH as a neuromodulator in the RTN region.



**Figure 7** Phrenic amplitude (mean  $\pm$  SEM;  $N=5$ ) of anesthetized cats following unilateral RTN injection (10 nL) of xanthurenic acid (an analogue of kynureate without glutamate receptor antagonist properties) and kynureate (top panel) and the non-NMDA antagonist CNQX or the NMDA receptor antagonist AP-5 (bottom panel). (From Ref. 69.)



**Figure 8** The individual (thin lines) and average (solid symbols) responses of phrenic amplitude in the anesthetized cat to the injection (10 nL) into the RTN of glutamate, 10 mM shown at left, 100 mM shown at right. At the bottom are panels depicting the location of the injections as marked by the use of fluorescent microbeads. The two sections at left represent the rostral and caudal aspects of the facial nucleus (VII); the sections at right, the rostral and caudal aspects of the facial nucleus and just caudal to the facial nucleus at the rostral level of the inferior olivary nucleus. (From Ref. 70.)

The studies cited above, which used surface cooling and lesions to examine the role of the IMA and the more specific anatomical sites beneath the IMA, were performed in anesthetized or decerebrate animals. Studies have also been conducted in conscious animals. Coagulation of the IMA bilaterally in cats (73, 74) results in a chronic loss of chemosensitivity in these animals as well as resting

hypoventilation. These findings have been related to "Ondine's curse" and to the sudden infant death syndrome (75). Forster and colleagues (76) have begun studies with cooling of the IMA in conscious goats. They have found severe depression of ventilation at rest, in response to hypercapnia, and in exercise during IMA cooling (Forster HV; personal communication). In conscious dogs, periods of cluster breathing can be seen after unilateral lesioning of the retrotrapezoid region by kainic acid injections (77). In my laboratory, we have found it difficult to keep rats alive after lesioning the retrotrapezoid nucleus bilaterally, an experience shared by Dormer et al. (77) and Forster (personal communication) in their experiments. These studies suggest an important role for this region of the VLM in the control of breathing, which is demonstrable in the more physiological conditions of a chronic, conscious animal preparation.

Neurons accessible from the IMA are very important in the control of breathing but are also involved in many other physiological functions. The focus of this chapter is on chemoreception and the control of breathing, but it is important to realize that neurons important in other physiological control systems lie close to and are, at some sites, probably intermingled with those whose primary purpose is in the control of breathing. For example, the sub-retrofacial pressor region (78), also called the C1 region because of the presence of many catecholamine-containing neurons (79), includes a large portion of the PGCL. This pressor region projects to the intermediolateral column of the spinal cord and is thought to be important in the origin and maintenance of cardiovascular sympathetic tone. Conditional pacemaker neurons, proposed as a source of cardiovascular sympathetic tone, have been reported here (80), and the link in which a portion of cardiovascular sympathetic tone is phasically related to phrenic nerve output apparently occurs here (69,81). The neurons of this rostral pressor region lie just caudal and dorsal to the retrotrapezoid nucleus, within the region ventral to the retrofacial nucleus. These neurons are accessible to surface manipulations at the IMA, and such studies often had blood pressure as well as respiratory effects, especially with manipulation of the caudal IMA. Hypercapnia increases cardiovascular sympathetic tone and blood pressure (69,81) as well as ventilation. The role of central chemoreceptors in these changes is unknown.

#### IV. Conclusions

1. Central chemoreceptors, as located by the use of tiny injections of acetazolamide to produce a small region of focal acidosis, are present in at least three brainstem sites; at or just deep to the VLM surface at the traditional rostral and caudal chemosensitive areas and the IMA, in the vicinity of the nucleus tractus solitarius, and in the region of the locus ceruleus.
2. Stimulation at one chemoreceptor site results in an increase in phrenic amplitude, which is 35% to 56% of that observed with maximum stim-

ulation of all central chemoreceptor sites. This suggests that central chemoreceptors serve to regulate regional brainstem pH.

3. Central chemoreceptors are present within the region just deep to the IMA. However, the IMA, at least the RTN portion of the IMA, appears to have many important functions in the control of breathing.
4. Unilateral RTN lesions result in depression of respiratory output and abolish central chemosensitivity. This suggests that neurons in the RTN are required to maintain normal eupneic ventilation and to allow expression of the CO<sub>2</sub> response.
5. Glutamate receptors of both the NMDA and non-NMDA subtypes are involved in normal RTN function presumably sensing glutamate released by afferents or interneurons.
6. Stimulation of the RTN by glutamate over long time periods (60 s) or TRH (30 ms) can result in prolonged stimulation of phrenic amplitude, raising the possibility that the RTN plays a role in long-term facilitation.

#### Acknowledgment

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