

The Role of the Ventral Medulla in Hypoxic Respiratory Depression and Sympathetic Excitation

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I. Introduction

In the absence of afferent inputs from peripheral chemoreceptors, systemic hypoxia triggers central mechanisms that depress breathing and produce sympathetic excitation (1–5). Several explanations have been proposed for the depressive actions of central nervous system (CNS) hypoxia on respiration. Hypoxic reduction of respiratory activity has, for example, been attributed to increased cerebral blood flow or changes in energy supply that interfere with the function of respiratory neurons (6–8).

Since hypoxia has a global action affecting different areas of the CNS (9, 10) and causing the release of either inhibitory or excitatory neurotransmitters, there may be specific sites in the brain where neurotransmitter release produces both sympathetic excitation and respiratory depression even before energy stores are exhausted. Our recent data suggest, as described below, that one of these sites could be the ventrolateral portion of the medulla oblongata (5,11, 12), a region that plays a pivotal role in the regulation of breathing and arterial blood pressure (13–18). There the excitation and depression may occur by a single mechanism at specific neuronal groups, which projects by multisynaptic

pathways to vasomotor and respiratory neurons within the ventrolateral medulla; or it is possible that respiratory depression and sympathetic activity occur by independent mechanisms.

The depression of breathing and the sympathetic excitation response may serve a common purpose. It may act on an emergency basis—like the diving reflex—to prevent further loss of available oxygen store while preserving the O₂ delivered to vital organs when O₂ is absent in the environment.

In this contribution we discuss some previous work on the effects of hypoxia of the CNS on breathing activity and sympathetic tone as well as our own recent studies on cardiorespiratory responses to hypoxia confined to caudal brainstem structures.

II. Hypoxic Depression of Respiration

Hypoxic depression of respiration occurs invariably in peripherally chemodenervated animals, but it can also be observed in animals and humans with intact peripheral chemoreceptors.

In an early study of hypoxic depression, we showed a depressive effect of systemic hypoxia on phrenic nerve activity in anesthetized, paralyzed, and artificially ventilated dogs (with chemoreceptors intact). These animals were exposed in separate trials to asphyxia, progressive hypercapnia under hyperoxic conditions, and hypoxia while hypercapnia was prevented by TRIS buffer infusion (19). The paralyzed dogs were mechanically ventilated with a constant volume so the effect of pulmonary stretch receptors and muscle proprioceptors were minimal. After an initial increase in fictive breathing, a clear depressive effect of hypoxia on phrenic activity could be observed during both isocapnic and hypercapnic conditions, while hypercapnia alone did not. The level of hypoxia at which phrenic nerve activity decreased from its peak was higher during hypercapnia than during isocapnic hypoxia. Tris buffer infusion, while it maintained isocapnia, regularly produced an increase in arterial pH despite the occurrence of hypoxia. In light of more recent experiments by Neubauer et al. (20) showing that prevention of acidosis largely prevented respiratory depression of cats exposed to a progressive increase in carbon monoxide level, it is possible that the Tris buffer alkalosis contributed to the greater resistance of the isocapnic animals to hypoxic depression.

Neubauer and coworkers have described different phases of hypoxic respiratory depression (20). An initial phase of depression is due to hypoxic augmentation of cerebral blood flow, which reduces PCO₂ (and [H⁺]) at central chemoreceptors. In a subsequent phase, the release of inhibitory neurotransmitters such as GABA, enkephalins, and adenosine (17,21–23) probably further diminish respiration, since the administration of the appropriate antagonist at least partially reverses the respiratory depression, even though the results reported in different studies have not been entirely consistent. When positive

effects have been seen, it is unclear whether these agents act on bulbar respiratory neurons or on neurons upstream that project to them. During this stage of hypoxic depression, excitatory responses to increments in CO₂ are maintained. Finally, the last stage of hypoxic depression of neuronal metabolism may be sufficiently severe that neuronal death is imminent.

It has been proposed that hypoxic depression of breathing, by decreasing neuronal metabolism, may serve a useful function by increasing the potential for survival during hypoxic stress. It could be that a beneficial object is also attained by the central excitatory effect of hypoxia on vasomotor activity. Differential changes in sympathetic discharge, by promoting perfusion of vital organs (analogous to the diving reflex), could have survival value.

III. Regional Differences in the Brain in Sensitivity to Hypoxic Depression

Respiratory neurons seem to be mainly concentrated in the medulla in two well-described aggregations of nerve cells in its ventral and dorsal portions (24–26). These groups are, in turn, reciprocally connected to a dorsally located pontine respiratory group in the parabrachial regions (27–29). In addition, evidence exists that, in several species, neurons sensitive to CO₂ are located superficial to the ventral respiratory group, particularly in rostral medullary regions. These respiratory neurons seem to form a population that is distinct from the vasomotor neurons (30–32). Hypoxic depression may not affect all respiratory outputs uniformly. Data obtained in the goat and the piglet show differential actions of hypoxia on upper airway dilating versus respiratory pumping muscles (16,33,34). Recently, we determined the contribution of brainstem hypoxia to the response of upper airway and chest wall muscles during early life. We perfused the brainstem through a vertebral artery intermittently with blood from an extracorporeal circuit in nine newborn piglets, ages 1 to 5 days. Brainstem perfusions were performed with hypoxemic blood (arterial PO₂ 32 ± 6 to 38 ± 8 Torr) with different levels of brainstem PCO₂ (28 ± 2, 37 ± 4, and 56 ± 5 torr) while systemic normocapnic hyperoxia was maintained (arterial PCO₂ 36 ± 3 to 40 ± 6 torr, arterial PO₂ 345 ± 73 to 392 ± 37 torr). Electromyograms (EMG) of alae nasi (AN), external intercostal (EI) muscles, and diaphragm (DIA) were recorded. Normocapnic hypoxia of the brainstem induced a sustained increase in AN EMG ($P < 0.01$, analysis of variance) and depression of EI and DIA EMG without a transient increase. These contrasting responses were also observed during hypocapnic and hypercapnic hypoxia of the brainstem and were not affected by inputs from the peripheral chemoreceptors or rostral cerebral structures that were not exposed to hypoxia. Thus, brainstem hypoxia elicits a selective rather than a generalized respiratory muscle depression (16). It may be that similar differences occur in regional sympathetic activity.

Studies by Korner stress the importance of higher brain centers in modulating the interplay of baroreceptor, lung stretch receptor, and peripheral chemoreceptor output in setting vasomotor and respiratory response during hypoxia (35). To a certain extent the adverse actions of hypoxia on nerve tissue seem to depend on differences in metabolic rates among brain tissues and vary with maturation (36,37).

Nonetheless, experiments in animals indicate that hypoxic depression of ventilation does not require brain tissue above the pons or lower mesencephalon. Brain transection experiments suggest that hypoxic depression arises from stimulation of the caudal mesencephalon or dorsolateral pons (38,39). These studies raise the possibility that hypoxia, rather than depressing, might excite certain brain areas that then have an inhibitory effect on breathing. It is possible that hypoxia, by stimulating these same areas, excites vasomotor- and inhibits respiratory-related neurons. Severe hypoxia is known to cause the release in brain slices of excitatory and/or inhibitory amino acids, which could form the basis of a mechanism for the excitatory and inhibitory effects of hypoxia (40-42).

The effects of central hypoxia on nerve activity require more time to develop than the effects of hypoxic stimulation through peripheral chemoreceptors, suggesting the involvement of relatively slow metabolic processes (8,43). Certain enzymes involved in the metabolism of neurotransmitters—like choline acetyl transferase, tyrosine, and tryptophan hydroxylase—are very sensitive to the effects of hypoxia (44). Much of the information on the cellular and membrane effects of hypoxia comes from studies of hippocampal slices (8).

Acidosis occurs during hypoxia because of augmented glycolysis. Recent studies of respiratory activity suggest that the depressive effects of hypoxia are largely abrogated if lactic acid production is reduced and acidosis prevented by systemic dichloroacetate (DCA) (20). It has been suggested that acidosis arising from hypoxia may interfere with the breakdown of neurotransmitters such as GABA, since the enzymes involved may be particularly sensitive to pH changes. GABA, for example, has inhibitory effects on ventilation when GABA acts on the structures near the ventral surface of the medulla (45).

IV. Effects of Hypoxia on the Vasomotor System

Brain ischemia, studied more extensively than hypoxia alone, produces sympathetic excitation, which can be eliminated by lesions in the rostral ventrolateral medulla (RVLM). However, ischemic hypoxia is complicated by simultaneously occurring loss of nutrient delivery and by hypercapnia (20,40,46,47). Hypercapnia may not contribute significantly to the ischemic reflex, since elimination of the excitatory effects of CO₂ by cooling of the intermediate area of Schläpke on the VMS, which overlaps a portion of the RVLM, does not prevent the pressor actions of cerebral ischemia (47).

The frequency content of sympathetic nerve activity is altered during cerebral ischemia. First there is accentuation of the normal 2-, 6-, and 10-Hz rhythmic fluctuations in the frequency content of sympathetic nerve, which arises, in large part, from the hindbrain, but later these cyclic changes become desynchronized. This desynchronization has been attributed to the central effects of hypoxia (48).

There are relatively fewer studies of the effect of CNS hypoxia alone on the cardiovascular system. Several investigators have observed an increase in sympathetic activity in heart rate and cardiac contractility with hypoxia in chemodenervated animals (4,49,50), although this is sometimes preceded by a transient decrease in activity. Hypoxia in peripherally chemodenervated cats acutely transected at C₁ increases the activity of preganglionic sympathetic nerve fibers (4). This suggests that hypoxia can excite preganglionic sympathetic neurons when input from the brain is eliminated. What remains unclear is whether the excitatory effects elicited by hypoxia are due to a direct action on the preganglionic neurons or are secondary to input from extracarotid chemoreceptors. In addition to the carotid and aortic bodies, oxygen-sensitive tissue is present in the thorax and abdomen, which is supplied by sympathetic afferents (1,51-55).

Pulmonary neuroepithelial bodies, composed of innervated clusters of amine- and peptide-containing cells, are widely distributed throughout the airway mucosa of human and animal lungs; they resemble chemoreceptors (such as carotid body, or taste buds) and may function as hypoxia-sensitive airway sensors (51). Recently, Youngston et al. cultured neuroepithelial bodies isolated from rabbit fetal lungs and identified voltage-activated potassium, calcium, and sodium currents using the whole-cell patch-clamp technique. Upon exposure to hypoxia, there was a reversible reduction (25% to 30%) in the outward potassium current, with no change in inward currents. In addition, it was demonstrated the expression of an oxygen-binding protein (b-cytochrome, NADPH oxidase) on the plasma membrane of these cells. The identification of an oxygen-sensing mechanism (namely the presence of an O₂-sensitive potassium channel coupled to an O₂ sensor protein) in the cells of pulmonary neuroepithelial bodies indicates that they are transducers of the hypoxia stimulus and hence may function as airway chemoreceptors in the regulation of respiration (55). It is possible that a spinal reflex involving these afferents contributes to hypoxic preganglionic neuron excitation.

Investigations by Arita and coworkers and Mitra et al. support a supraspinal pressor site sensitive to hypoxia (3,12,49). They found pressor responses to small injections of solutions equilibrated with N₂ injected into the vertebral artery in cats. Respiratory depression occurred simultaneously with blood pressure increases. Decerebration had no effect on the responses, suggesting that the vasopressor and respiratory inhibiting effects of N₂ were due to actions confined to hindbrain neurons.

V. Effects of Hypoxia on the Respiratory Modulation of Sympathetic Activity

A. The Effect of Hypoxic Loading of the Caudal Brainstem on Respiratory and Sympathetic Outflow

Sympathetic activity often has a pronounced respiratory modulation effect, particularly in nerves supplying the vasculature and the heart (2,56). Phasic increases occur in many sympathetic nerves synchronous with inspiration; these grow greater when respiration is stimulated (56). Haselton and Guyenet have reported respiratory modulation of the baroreceptor-sensitive neurons in the RVLM of vagotomized rats (57). Both hypoxia and hypercapnia heighten sympathetic activity, in part indirectly by increasing respiratory activity (due to spillover of central respiratory drive potentials to vasomotor neurons) but also in part independently of respiratory effects (58). Studies of the central effects of hypoxia in cats show that it is the inspiratory-related sympathetic activity that declines with respiratory depression (8). However, tonic activity of the sympathetic CNS generally grows greater in cats with central hypoxia, suggesting a more direct effect on vasomotor activity (59).

The effects of systemic hypoxia are complex and responses may differ from the site of action within the CNS. Because of that, we have studied the effect of hypoxia confined to the caudal brainstem using different techniques to localize hypoxic loading.

B. Cardiorespiratory Changes Induced by Vertebral Artery Injection of Sodium Cyanide

To assess the effects of hypoxia confined to the structures of the medulla on respiratory activity and sympathetic outflow, we utilized sodium cyanide (NaCN) loading (3,11,12).

Sodium cyanide-induced hypoxia was produced by administration of NaCN unilaterally into a vertebral artery at the spinomedullary junction or by topical application of NaCN. Unlike inhalation of hypoxic gas mixtures, local applications of sodium cyanide have the advantage of avoiding the systemic effects of hypoxia and limiting its effect to specific areas of the brain. The responses of phrenic and sympathetic activity in 14 anesthetized, paralyzed, and mechanically ventilated cats to different doses were compared.

Three dose levels of NaCN were used for the intravertebral injections (1, 10, and 20 μg) in animals ventilated with air. Similar doses, given systemically, had no effect on phrenic activity, sympathetic discharge, or blood pressure. Intravertebral injection of NaCN reduced integrated phrenic nerve amplitude, increased tonic sympathetic activity, and raised blood pressure, as shown in Figure 1. Phrenic amplitude decreased with increasing NaCN dose. Occasionally,

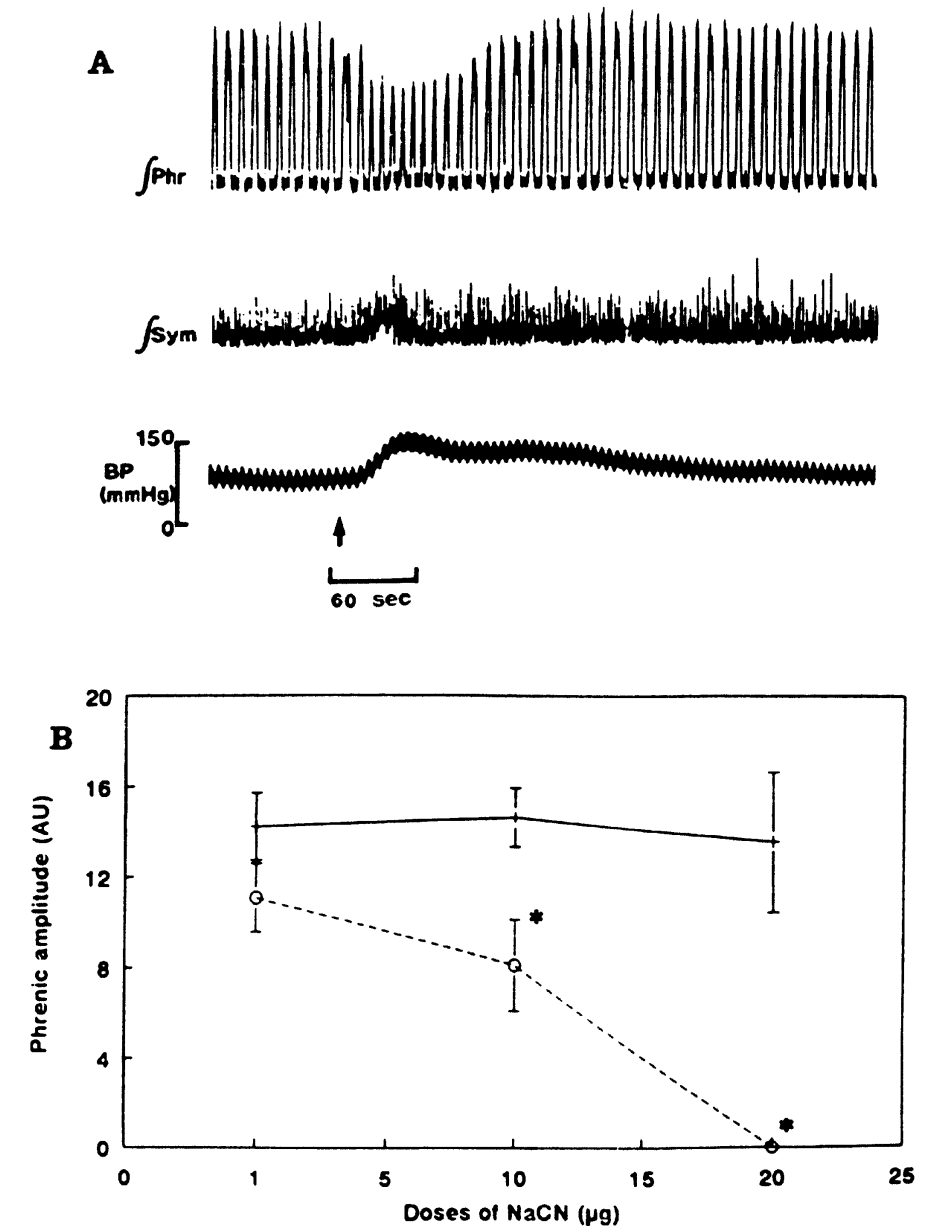
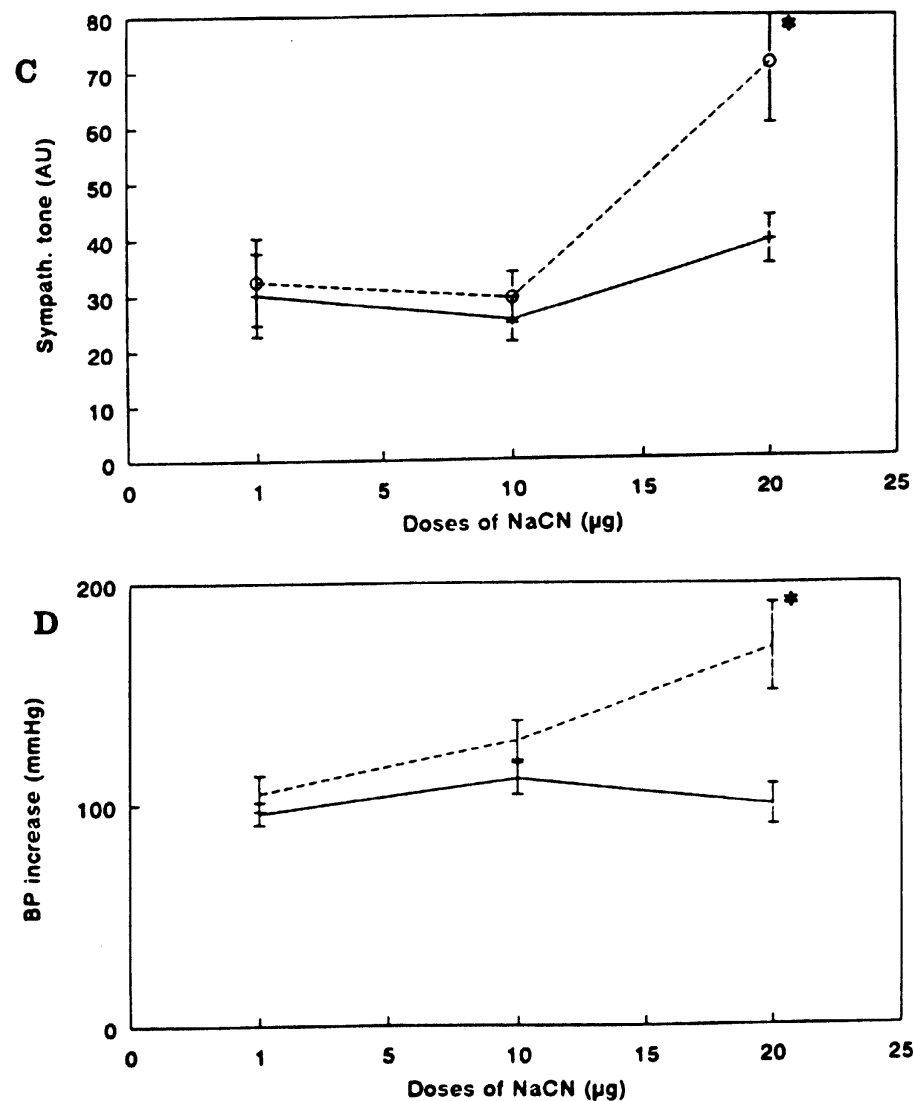


Figure 1 Panel A: Effect of intravertebral injection of NaCN (10 μg) in a single animal on integrated phrenic (Phr) and cervical sympathetic (Sym) activity. 1: injection of NaCN; BP: arterial blood pressure. (Modified from Ref. 12.) Panels B, C, and D: Mean changes in phrenic nerve amplitude, sympathetic tone, and blood pressure. *: $P < 0.05$.



respiratory frequency increased with depression of phrenic amplitude, but this was not a consistent finding. All animals became apneic after receiving the higher dose (20 μg) of NaCN. The average times for the onset of phrenic depression and apnea were 4.0 ± 1.0 , and 8.0 ± 2 s ($P < 0.05$), respectively, following injection (for all doses). The duration of apnea varied considerably (20.0 ± 10.0 s). The depression of phrenic nerve activity produced by NaCN was less in animals breathing 7% CO₂ in O₂ ($P < 0.05$).

It is known that phasic respiratory modulation of sympathetic nerve activity increases and decreases in parallel with changes in phrenic nerve activity (56,60). However, during hypoxic loading, we found that as phrenic nerve discharges decreased, the tonic discharge in sympathetic nerve increased, growing progressively with increasing dosages of NaCN. Although the increase in sympathetic activity was not statistically significant between the 1- and 10-μg dose levels, it was significant when the 10- and 20-μg dose levels were compared ($P < 0.05$). These changes were associated with an increase in arterial blood pressure and were the same with 7% CO₂ breathing.

From these experiments, it cannot be determined which neuronal population is responsible for respiratory depression, sympathetic excitation, and increased sympathetic nerve discharge.

C. Effect of NaCN Applied to VLM Surface and Microinjected into the Superficial Layer of the Ventral Medulla on Respiratory Activity

To ascertain the role of the ventrolateral medulla (VLM) in hypoxic depression of breathing, we examined the respiratory response to local application of cyanide to superficial structures of the ventrolateral medulla (Fig. 2).

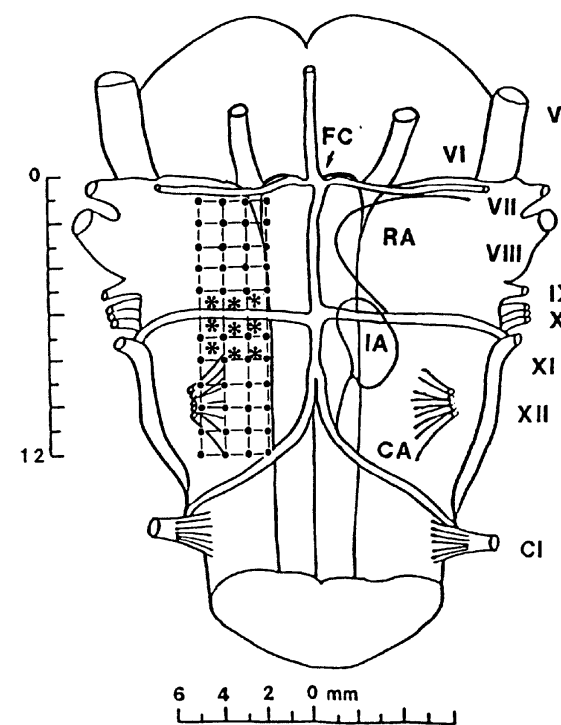


Figure 2 Diagrammatic sketch of cat's ventral medulla shows grid mark. Each dot in the grid represents an injection site. Typical response was observed when NaCN was applied 5 to 8.0 mm caudal to FC and 3.0 to 5.0 mm lateral to the midline.

In these experiments, cats were sedated with ketamine (10 mg/kg IV) and anesthetized with α -chloralose (60 mg/kg IV). To avoid superposition of secondary effects on the immediate respiratory changes induced by interventions, animals were mechanically ventilated with O_2 .

To determine whether changes of inspiratory discharge are related to overall inhibition of respiratory output or are caused by inhibition through tonically active expiratory neurons, we recorded EMG activity of inspiratory and expiratory chest wall pumping muscles (11). Topical application of cyanide (10 to 100 μ g) on the intermediate area of the ventral medullary surface (VMS) as described by Schläpke et al. (29) produced a consistent respiratory depression. In each animal, the electrical activity of the phrenic nerve and of all respiratory muscles studied began to decrease within 20 s from the application of cyanide. The maximal respiratory depressive nerve response occurred \sim 1 min after application of cyanide. The decrease in activity of the expiratory muscles exceeded that of the parasternal intercostal, which was in turn more pronounced than that of the diaphragm, but differences did not reach statistical significance ($P > 0.05$). However, doses of cyanide that had no effect on diaphragm activity and arterial blood pressure could cause a significant inhibition of transversus abdominis muscle activity. After the VMS was washed with warm buffered saline, the activity of abdominal expiratory muscle returned to its control level of activity later than that of the diaphragm and the parasternal intercostal muscle by \sim 60 to 90 s. An example of respiratory responses to topical application of cyanide in one cat (panel A) and results of individual animals (panel B) are presented in Figure 3. Topical application of cyanide to the VMS was associated with a decrease of phrenic nerve activity from 24 ± 3 to 10 ± 3 angstrom units (AU) ($P < 0.05$; $n = 4$) and diaphragm activity from 22 ± 1 to 7 ± 3 AU ($P < 0.005$; $n = 7$); inspiratory intercostal activity decreased from 22 ± 2 to 4 ± 1 AU ($P < 0.05$; $n = 6$) and expiratory activity of the transversus abdominis from 22 ± 7 to 1 ± 1 AU ($P < 0.05$; $n = 5$).

Respiratory depression was accompanied by a concomitant increase in arterial blood pressure. The peak pressure response occurred earlier than the maximal change in respiratory muscle activity and subsequently returned to the preapplication level. On average, mean arterial blood pressure increased by $12\% \pm 3\%$ ($P < 0.05$).

Results of this study showed for the first time that cyanide acting locally at the VMS inhibits both diaphragm and intercostal muscle activity. Effects on intercostal electrical activity were greater than the effects on diaphragm activity. Expiratory intercostal and abdominal muscle activities were very sensitive to cyanide applied to the intermediate area of the VMS. Doses of cyanide that induced only small alterations in diaphragm activity caused appreciable decreases in expiratory activity. Our findings indicate that cyanide acting on the VMS influences respiratory-related activity, but they do not permit us to determine whether these changes are due entirely to an action of cyanide on

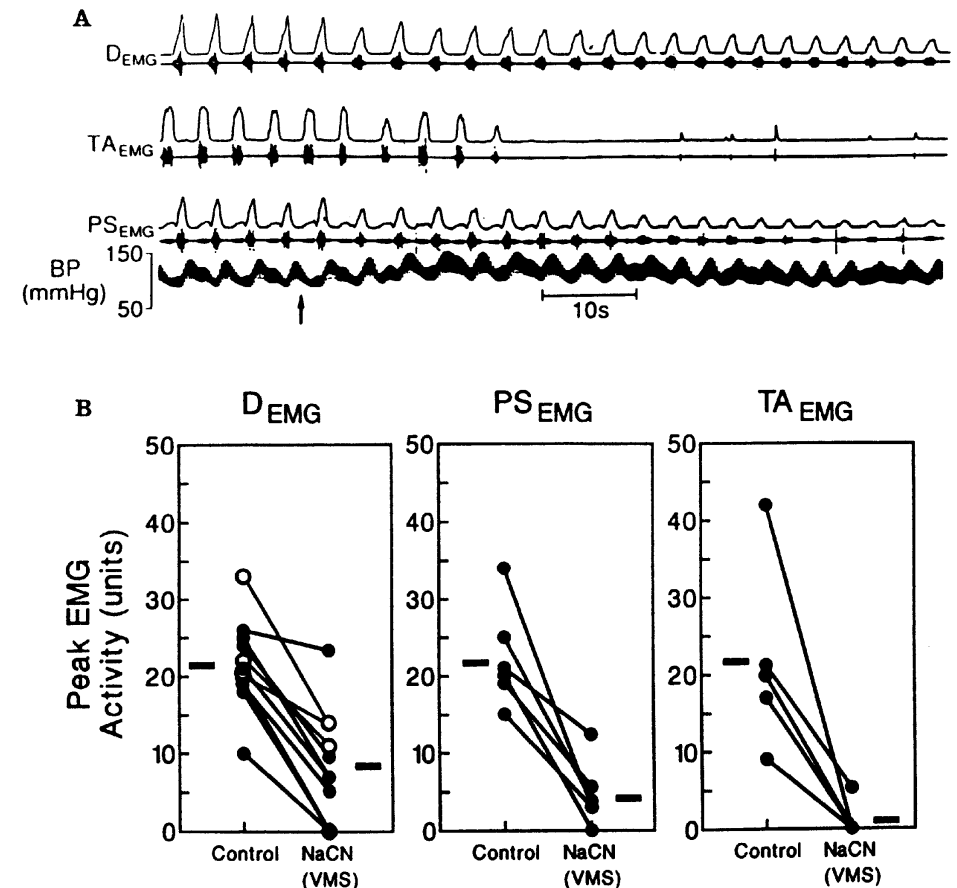


Figure 3 An example (panel A), and individual data (panel B) of responses of electrical activity of phrenic nerve (\circ) and electromyographic activity of diaphragm [(—),A], parasternal intercostal (B), and transversus abdominis muscle (C) to local application of 100 μ g of NaCN on intermediate area of ventral surface of medulla (panel B). Horizontal bars, mean values. (Modified from Ref. 12.)

chemoreceptors or whether a high density of capillaries, a high local blood flow, and the structure of the VMS facilitate the access of cyanide to deeper portions of the medulla.

To further study the role of these structures in hypoxia-induced respiratory depression and sympathetic excitation and to localize the site of action, NaCN was microinjected into the ventrolateral medulla in sites which were 1 to 12 mm caudal to the foramen cecum and 2 to 5 mm lateral to the midline (3). Injections were made unilaterally at a depth of 1 mm below the ventral surface. The central chemoreceptors have been reported to be located in the region explored. On the basis of focal cooling and the response to chemicals applied

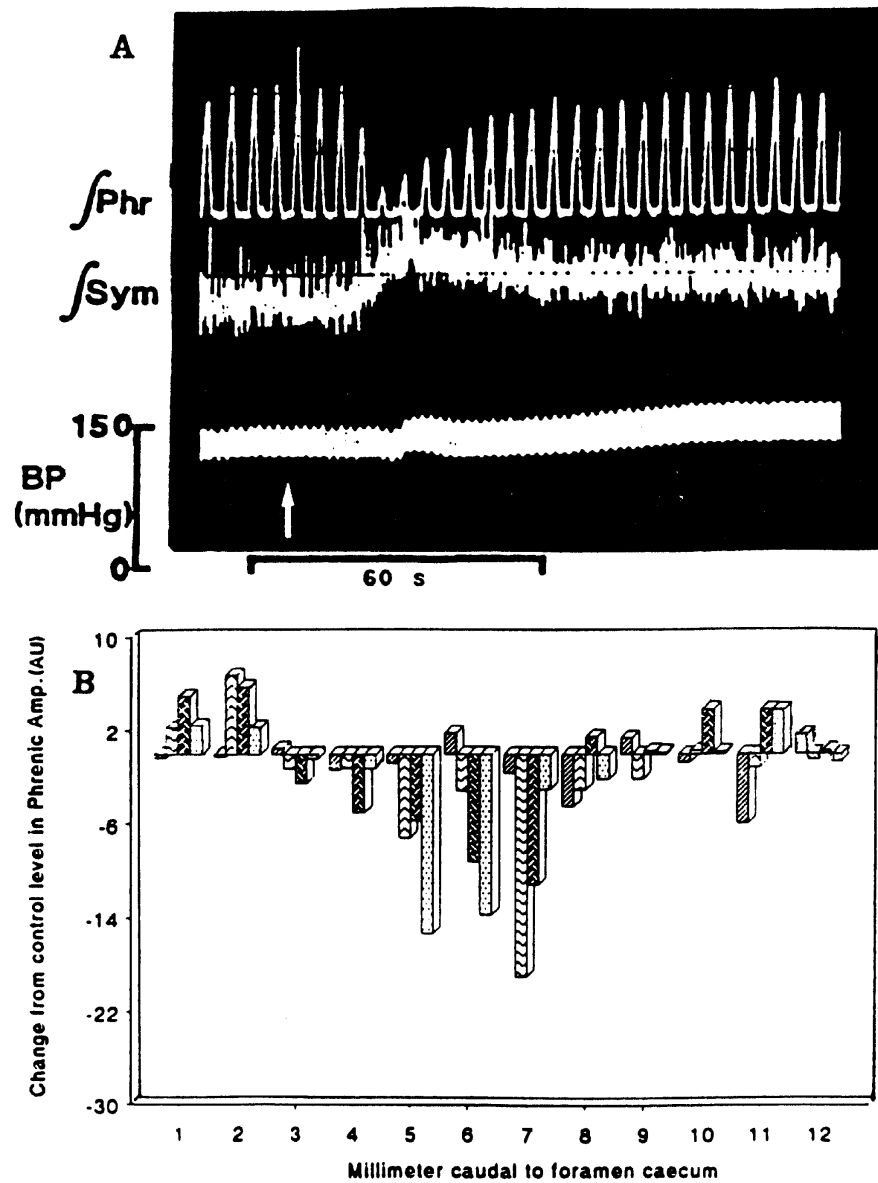
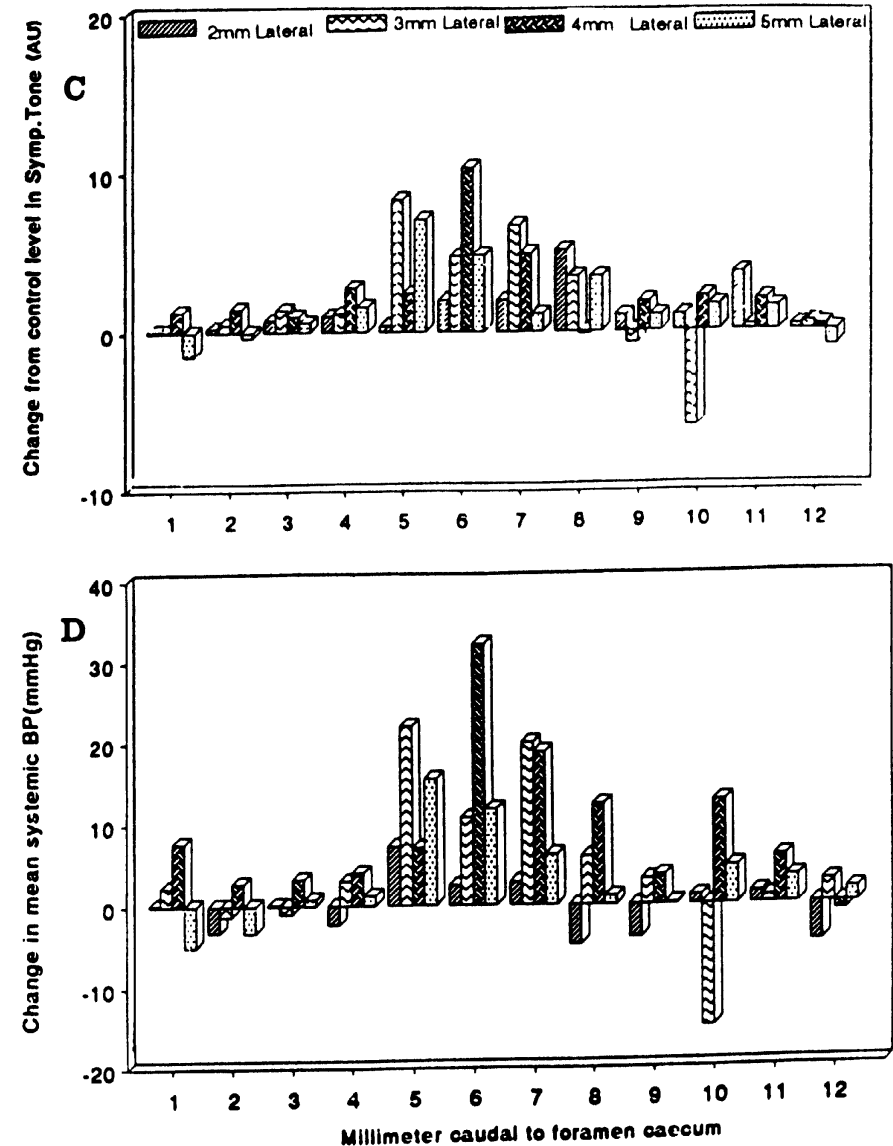


Figure 4 Panel A: Typical response to NaCN injection (100 ng/100 nl) into the ventrolateral medulla of integrated phrenic (Phr) and sympathetic (Sym) nerves, and arterial blood pressure (BP). Panels B, C, and D: Mean changes in phrenic amplitude, sympathetic tone, and blood pressure. \uparrow : time of injection. (Modified from Ref. 3.)

topically (using the foramen caecum as a reference), this area has been subdivided into three segments, i.e., rostral (R), intermediate (I), and caudal (C). In our experiment, the R segment roughly corresponds to 1.0 to 4.0 mm caudal; the I segment, 5.0 to 8.0 mm; and the C segment 9.0 to 12.0 mm caudal to FC, as shown in Figure 2.



Data were obtained from 269 injections at 48 sites near the ventral medullary surface in 17 adult cats. Statistical analysis showed that there were significant differences along the rostrocaudal axis in phrenic activity, sympathetic discharge, and blood pressure following injection. The responses at rostral and caudal sites were in general less than response in the intermediate area. The null hypothesis of no overall rostrocaudal difference with respect to all three measured parameters was strongly rejected at $P < 0.0001$, using Wilk's lambda statistic. However, the same analysis revealed only a marginal difference ($P \sim$

0.07) in vasomotor and respiratory responses to injections along the medial-to-lateral axis.

The predominant respiratory response to sodium cyanide microinjections into the VMS was depression of breathing. Usually the onset of depression was abrupt, occurring within 10 ± 5 s of the injection and recovered gradually over 45 ± 15 s. Figure 4, panel A, shows the effect of NaCN injection in one animal. Depression of breathing was obtained consistently from an area 5.0 to 7.0 mm caudal to the foramen cecum and 3.0 to 5.0 mm lateral to the midline ($P < 0.05$). This region corresponds to the intermediate area. Respiratory responses to the injection of NaCN more rostral and more caudal also caused depression, but to a lesser degree. The distribution of cyanide following microinjection was determined by using ^{14}C . As shown in Figure 5, cyanide was confined within 1 mm of the injection site after 7 min. Less than 10% of the injected cyanide was covalently bound to proteins.

As in phrenic nerve activity, there were no changes in sympathetic tone when NaCN was injected in either the most rostral or most caudal areas. Significant increases in sympathetic tone were obtained only between 5.0 to 8.0 mm caudal to the foramen cecum and 2.0 to 5.0 mm lateral to the midline ($P < 0.05$). In general, the onset latency and duration of response was similar to that of phrenic nerve activity. Figure 4 summarizes the mean phrenic (B) and the mean sympathetic (C) responses from the regions explored in these studies. Note that sympathetic activity increases most prominently in the intermediate area. Comparison of panels B and C show that there is considerable overlap in the regions involved in sympathetic excitation and respiratory depression. As in respiratory depression and sympathetic excitation, significant BP elevation was obtained between 5.0 to 8.0 mm caudal and 2.0 to 5.0 mm lateral (area I) with cyanide injection (D).

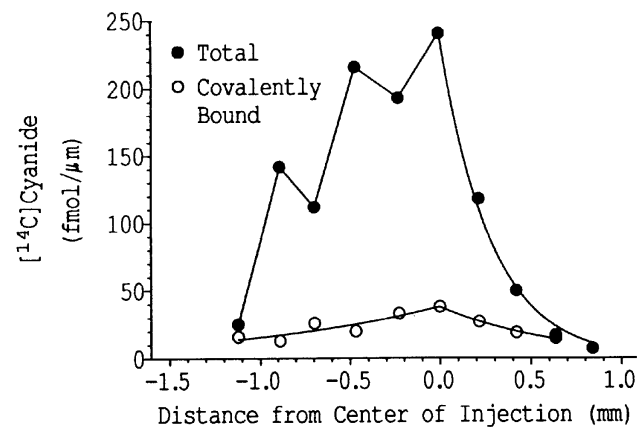


Figure 5 Distribution of ^{14}C cyanide following microinjection into the rostral ventrolateral medulla.

The major findings of these studies are that a significant depression of respiration and increases in sympathetic tone and blood pressure could be obtained by the topical application or injection of NaCN into the intermediate area of the ventral medulla. These responses are similar to those elicited by central hypoxia produced by intravertebral injections of hypoxic saline or NaCN (12) or topical application of NaCN on intermediate areas (11). Hence the origin of central hypoxic responses may be the ventrolateral medulla, particularly the intermediate region.

The excitotoxin kainic acid, when applied superficially on the rostral but not in the intermediate ventrolateral medulla, depressed phrenic activity irreversibly following an initial increase in BP (61). In contrast, our greatest phrenic depression and rise in BP with NaCN applications were obtained from the intermediate area, and both effects were reversible, indicating two different sites and mechanisms.

Although more direct actions of cyanide on nervous tissue have been proposed, it is generally believed that NaCN acts by blocking tissue oxidation, thereby causing histotoxic hypoxia (62). Aitken and Braitman (1989) studied the effects of NaCN on isolated hippocampal slice preparation and found that cyanide in lower doses, like hypoxia (63), reversibly blocks synaptic transmission (43).

The mechanisms through which hypoxia produces respiratory depression and sympathetic excitation are still speculative. Recently it has been shown that cyanide, like hypoxia, increases the release of several neurotransmitters, i.e., GABA (64), glutamate (65), and catecholamines (66). Whether the respiratory and vasomotor changes in response to NaCN injection are also mediated through neurotransmitter release is not known.

It has been shown in the past that respiratory and vasomotor control can be dissociated functionally under certain experimental conditions (67). Since maximum sympathetic response was obtained from the intermediate area and sympathoexcitatory neurons that project to the spinal cord can be found underlying the intermediate area (68), it is possible that these neurons are affected directly by hypoxia to excite sympathetic response.

Microinjection of NaCN into the RVLM in the rat raises systemic blood pressure (69). Morphological examination and study of cerebral ischemia have led some investigators to suggest that neurons in the RVLM may be sensitive to hypoxia (57). Our results support this hypothesis. However, a contribution from surrounding regions cannot be ruled out. Isolated regions scattered outside the intermediate area when injected with NaCN elicited only small, non-significant increases in sympathetic tone and blood pressure following NaCN injection. Both in the rostral and caudal medulla we also found small regions (1.0 to 2.0 mm and 10.10 to 12.0 mm caudal to FC, 3.0 to 5.0 mm lateral) where NaCN caused a slow augmentation of phrenic nerve activity with little or no change in systemic blood pressure. Also in the caudal medulla (10.0 mm caudal, 3.0 mm lateral) there was a region where NaCN lowered both sympathetic

tone and blood pressure. This site may be part of the vasodepressor area, since in this general region others have found a blood pressure-lowering response to electrical or chemical stimulation (70).

D. Response of Single Neurons in the Medulla to Systemic and Brainstem Hypoxia

To better characterize the responses of ventral-medullary neurons with different functional characteristics, we examined the behavior of respiratory modulated and nonmodulated neurons (including bulbo-sympathetic neurons) during hypoxia caused either by ventilation with 10% O₂ in N₂ or intravertebral infusion of deoxygenated saline. Experiments were performed in 10 anesthetized, vagotomized, sinus-denervated, paralyzed, and mechanically ventilated cats.

We found that the effects of systemic hypoxia on different types of neurons in the ventral medulla were varied and ranged from excitation in some cases to inhibition in others.

In our experiment, all inspiratory modulated neurons were inhibited by hypoxia but excited by hypercapnia. These neurons also increased their discharge frequency in response to proprio- or nociceptive stimuli. The findings of our study confirm the results of other investigators (71,72).

Expiratory modulated neurons behaved nonuniformly during hypoxia. One group became silent, as observed by others (73), while the other became tonic as phrenic nerve activity disappeared. To our knowledge, the tonic discharge we observed in some expiratory modulated neurons during hypoxia has not been described previously. It is possible that these tonic expiratory neurons were motoneurons supplying the muscles of the oropharyngeal region. Muscles of the oropharynx can discharge tonically during expiration (74).

There were no clear differences in the response to hypoxia of the neurons that were activated by peripheral sensory inputs versus those that were not. Some neurons were inhibited by hypoxia while others were excited. The excitation may have been caused by disinhibition rather than by direct excitation.

Neurons that decreased their activity or became silent during the apnea produced by hypoxia could still be excited as before, indicating that the polysynaptic pathways were functional. The amplitude of the evoked activity during the early part of respiratory arrest (10 s) was similar to that during the control period. However, in the later part of respiratory arrest (90 s), the evoked amplitude decreased to less than 50% of control values. Since these sensory neurons could still be activated during the early phase of central apnea, it seems likely that the decrease in discharge rate was caused by an active inhibition. Direct depression of neuronal excitability may occur in the later phase.

Similar observations on the neuronal excitability of phasic inspiratory modulated neurons during hypoxia have been reported by Richter et al. (75). They found that during hypoxia induced central apnea, "respiratory neurons

were still excitable by stimulus-evoked orthodromic and antidromic action potentials." This suggests that central apnea initially may be due to withdrawal of excitatory drive input and not to neuronal failure (75). This idea is further reinforced by observations that during central apnea, electrical stimulation of the sinus nerve or inhalation of high CO₂ initiates rhythmic phrenic nerve activity (76).

Twenty-nine percent of antidromically activated RVL neurons (bulbo-sympathetic) increased their discharge—an example is shown in Figure 6—while 71% decreased or became silent during apnea. However, intermediolateral stimulation could still excite the latter group during the early phase of apnea. Sympathetic nerve tonic activity during the later part of apnea could not be correlated with the discharge rate of the identified bulbosympathetic neurons. Moreover, sympathetic tone remained high even when the activity of these neurons decreased. This supports the idea that the rise in sympathetic tone during hypoxia does not depend exclusively on the medullary neurons and indicates that there are multiple sites for the hypoxic excitation of sympathetic activity, including spinal neurons. The spinal cord is known to be more resistant to hypoxia than the medulla (77). We have reported that sympathetic tone can be augmented in spinal animals by intrathecal administration of sodium cyanide (11). Moreover, we observed that central hypoxia produced by infusion of hypoxic saline into the vertebral artery and topical application of cyanide on the ventral surface of the medulla augments sympathetic activity.

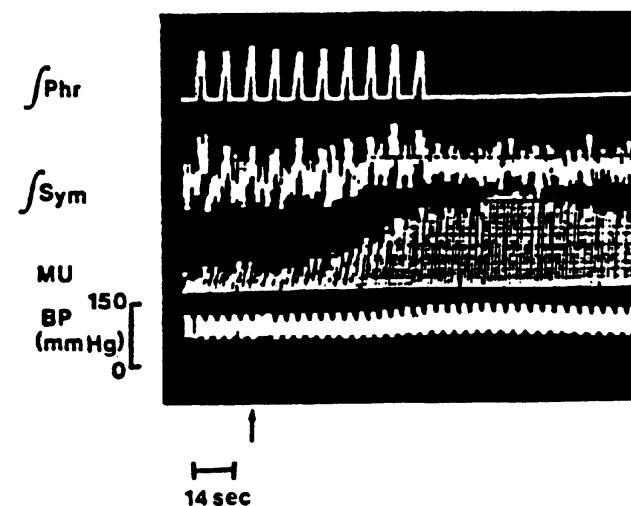


Figure 6 Response to hypoxia (6% O₂ in N₂) of ventral medullary sympathetic neuron activity of which increased by electrical stimulation of intermediolateral cell column. ↑: Beginning of hypoxia; Phr: integrated nerve activity; Sym: integrated sympathetic activity; Mu: raster display of medullary sympathetic unit discharge (per second); BP: arterial blood pressure.

This indicates that neurons in the medulla or supramedullary structure(s) are capable of contributing to hypoxic sympathoexcitation, and these tonic influences may affect sympathetic nerves differently (18).

Neurons that did not show respiratory modulation and were sensitive (78) or insensitive to hypercapnia (5% CO₂ in O₂) and sympathetic stimuli responded to hypoxia. However, responses were variable; some could be excited and some inhibited by hypoxic challenge (Fig. 7).

Our study shows that both respiratory bulbospinal sympathetic and non-respiratory neurons within the ventrolateral medulla that are depressed by hypoxia maintain their excitability during the early phase of respiratory depression to an adequate stimulus, as previously demonstrated for inspiratory neurons. Our results also suggest that the sympathetic activation that occurs with hypoxia probably arises from multiple sites, including the ventral medulla and spinal cord. These findings could be partially explained by an increase in H⁺ concentration, which is in agreement with earlier studies (79).

VI. In Vitro Studies on the Electrophysiological Effects of O₂ Deprivation in Ventrolateral Medullary Neurons

Expression of c-fos has been used to identify cells activated by specific stimuli such as CO₂. Data obtained from experiments in which the effect of CO₂ on c-fos expression have been assessed demonstrate that CO₂-responsive neurons are present in the ventrolateral medulla, including the parapyramidal region (27–29), and that the axonal targets of these cells include brainstem and spinal cord neurons. Conceivably these neurons are among the sites inhibited by hypoxia, leading to depression of respiratory activity. Recently we assessed the effects of O₂ reduction on the electrophysiological characteristics of parapyramidal

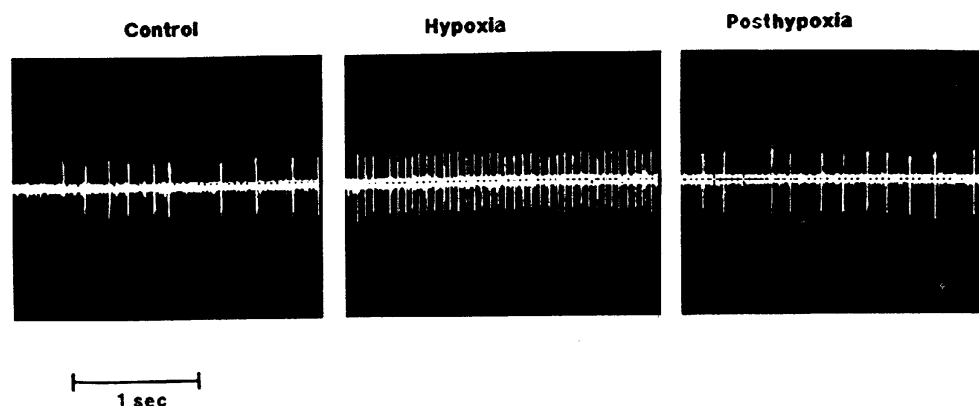


Figure 7 Response to hypoxia of ventral medullary neuron, activity of which did not change by hypercapnia (5% CO₂ in O₂) or electrical stimulation of intermediolateral cell column.

neurons in neonatal (7 to 20 days) and adult rats. Intracellular recordings were obtained in vitro from parapyramidal cells of the ventral half of medullary slices in control conditions (95% O₂ and 5% CO₂) and after exposure to anoxia (95% N₂ + 5% CO₂). Anoxia abolished all spontaneous activity of the cells, which were found to fire spontaneously at a rate of about 3 spikes/s. However, an electrical stimulus could generate action potentials, whose shape or size did not appear to differ significantly from the control. Anoxia decreased the somatic firing threshold. A transient decrease in the input resistance was also observed in anoxia. Complete recovery was obtained by switching to 95% O₂ and 5% CO₂. Subsequent anoxic insults did not produce a significant change in the resting potential. Neonatal cells survived in anoxia for >45 min, whereas survival was up to 30 min in adults. These results indicated that O₂ deprivation produces depolarization and abolishes spontaneous activity of ventromedullary cells. However, their excitability was not altered (37).

VII. Neurotransmitters Involved in Hypoxia-Induced Respiratory Depression and Sympathetic Excitation

An increasingly accepted idea is that moderately severe hypoxia affects the activity of neurotransmitters in the CNS, tipping the balance between inhibitory and excitatory influences on respiratory and sympathetic brainstem neurons (21,64–66). These effects may be local and/or global. Hypoxia may act on local areas of the ventrolateral medulla to inhibit respiration but excite sympathetic activity. Sympathetic excitation might then occur as a result of a disinhibition (a release of inhibitory effects) on vasomotor neurons. Alternatively, an overabundance of excitatory neuromodulations might also explain the CNS effects of hypoxia. Indeed, even with respect to breathing, a number of excitatory effects of central hypoxia, such as tachypnea, have been described during CNS hypoxia, particularly in unanesthetized animals.

Hypoxia may release inhibitory neurotransmitters, like GABA, opiates, dopamine, adenosine, and endothelin. GABA is the predominant inhibitory transmitter in the brain, and the depression of respiratory neuronal activity during hypoxia is a potentially adaptive response mediated principally by the effects of acidosis and increased GABA levels (3,12,21,66). Activation of GABAergic receptors has been specifically implicated in hypoxic depression in neonates (66).

Opiate receptors may participate in hypoxic depression in newborn neonates (80–82). Systemically administered, the opiate antagonist naloxone stimulates respiration in hypoxic neonates and can prevent apnea. Endorphin and enkephalin levels are sharply elevated in hypoxic neonates. The role of endogenous opiates in the cardiovascular responses to hypoxia remains largely unexplored, even though opiates have been implicated in vasomotor control, particularly in the RVLN and caudal VLM (CVLM) (17). However, the role of endogenous opiates in the response of adult organisms to hypoxia can be ques-

tioned. For example, naloxone only slightly attenuates respiratory depression in adult cats exposed to CO (22). Inconsistent results with naloxone in adult CNS hypoxia may be caused by the existence of multiple opiate receptor subtypes. The opiate receptor subtypes that mediate effects on respiration have not been identified with certainty, although μ -receptor subtypes appear to predominate.

Dopaminergic mechanisms also may play a role in hypoxic depression of respiration. It is known that dopamine is expressed by glomus cells in the carotid body and in several regions of brainstem. When it is released, it may cause respiration depression.

Adenosine has been implicated by a number of investigators in hypoxic depression of respiration, particularly in neonates (21,23,83,84). The adenosine antagonist theophylline is a respiratory stimulant used clinically to reverse hypoxic depression of respiration, while adenosine agonists depress respiration in normoxia. Brain levels of adenosine rise up to sevenfold during severe hypoxia (23). Brain adenosine interacts extensively with GABA, since nearly all putative adenosine neurons also synthesize GABA. Adenosine may be important as a neuromodulator regulating the activity of neurotransmitters during hypoxia.

Since theophylline (adenosine antagonist) reverses hypoxic depression and stimulates breathing (83), we investigated its effect on central hypoxia produced by vertebral artery injections of NaCN or saline equilibrated with N₂. Experiments were done in chloralose-anesthetized, paralyzed, vagotomized, and sinus-denervated cats ventilated with room air. Recordings were made from the N₂ phrenic and preganglionic cervical sympathetic nerves, along with blood pressure. The vertebral artery on one side was cannulated for injections of NaCN (10 and 20 μ g) and hypoxic saline (97% N₂ in 3% CO₂). Systemic administration of theophylline (5 mg/kg) either reduced the depression of phrenic amplitude (22 ± 5 AU %) or the duration of apnea (14 ± 7 s) caused either by NaCN or hypoxic saline but had little or no effect on the changes produced in sympathetic tone and blood pressure. We conclude that the central respiratory depression produced by NaCN or hypoxic saline may be in part due to the release of adenosine. However, the increase in BP and sympathetic tone seems not be due to inhibition of interneurons by adenosine.

Endothelin-1 (ET₁) is a member of a family of peptides found in endothelial and epithelial cells. This 21-amino acid peptide also is present in the brain, in a nonvascular distribution associated with specific nuclei (85). Recently, we have identified endothelin receptors in isolated cell membranes from the ventral medulla as the nonselective (ET) subtype which binds each of the endothelin isopeptides with high affinity (Fig. 8). In addition, we determined the effects of chronic hypobaric hypoxia (0.5 atm for 21 days) on medullary ET receptors and examined the possible role of ET in medullary cardiorespiratory control. For visualizing ET receptors, 15- μ m sections of rat medulla were preincubated 0.5 h, incubated 12 min with 50 pM [¹²⁵I]ET₁, washed 1 h, and either exposed to film or cross-linked in paraformaldehyde vapor and processed for

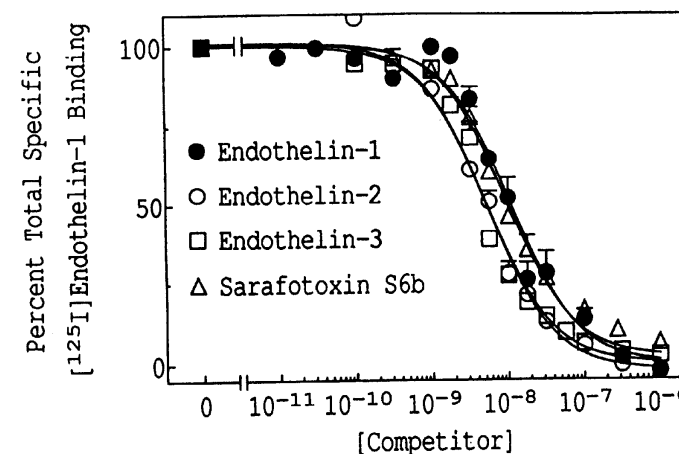


Figure 8 Dose-dependent inhibition of specific [¹²⁵I] endothelin-1 binding in ventrolateral medulla.

emulsion autoradiography. Nonspecific binding was defined with 1 μ M ET₁. Autoradiogram showed that ET receptors were enriched in VLM regions implicated in cardiorespiratory control: ventral surface, parapyramidal, retrotrapezoid, and rostroventrolateralis area. Autoradiographic grains were associated with neurons as well as glia and major vessels. In biochemical studies, intact

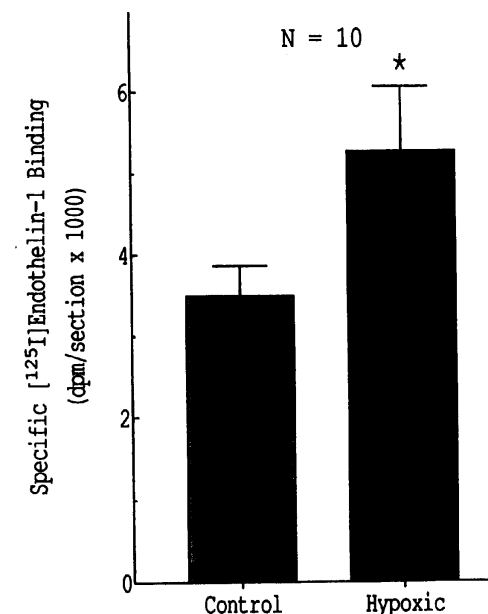


Figure 9 Effects of hypoxia on endothelin binding sites in rat medulla.

medullary sections from rats exposed to chronic hypoxia showed a higher density of ET binding sites than matched controls (Fig. 9; $150 \pm 18\%$ of control, $P < 0.05$). Regulation of ET receptors by hypoxia suggests a role in respiratory control. In vivo studies in anesthetized rats ($n = 15$) showed that bilateral micro-injections into superficial layers of ventral medulla with high ET receptor density, in doses of (1 to 10 pmol), produced expiratory apnea associated with an initial increase followed by a decrease in arterial blood pressure. Changes occurred within 30 s and lasted longer than 30 min. These findings are in agreement with recent studies (86). We conclude that ET receptors in the VLM (1) are upregulated by chronic hypoxia; (2) when activated, reduce respiratory activity; and (3) might be involved in hypoxic depression of respiration.

VIII. Conclusion

In conclusion, our studies suggest that structures within the ventrolateral medulla are involved in regulation of respiratory and sympathetic activity during hypoxia. Hypoxia acting on ventrolateral medullary neurons induces qualitatively different site-specific responses: generally it causes respiratory depression and sympathetic excitation. These responses may be of a protective nature and their value could be of particular importance to the in utero life of the fetus. During prolonged hypoxia, increase in activity of respiratory pumping muscles may lead to depletion of O_2 stores without increasing O_2 supply; decrease in their activity would conserve energy supplies necessary for maintenance of ionic gradients. In addition, increase in sympathetic activity may provide critical blood flow and redistribution of cardiac output during hypoxia.

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