Hypoxia-Induced Respiratory Patterned Activity in *Lymnaea* Originates at the Periphery

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Received 28 September 2000; accepted in final form 19 March 2001

**INTRODUCTION**

Respiration in most vertebrate species is a multi-component behavior, regulating oxygen and carbon dioxide levels. This is accomplished by the respiratory musculature whose activities are controlled by the CNS (Feldman and McCrimmon 1999). The CNS-derived patterned activity is further modulated by peripheral input from various chemoreceptors (Burleson and Smatresk 1989; Milsom and Brill 1986) and mechanoreceptors (Milsom 1990b), which play important roles in maintaining internal homeostasis. Chemical homeostasis involves a balance between carbon dioxide, oxygen, and pH levels, all of which vary among different species, depending on factors such as 1) whether the animal is a lung breather, 2) levels of cutaneous carbon dioxide excretion, and 3) the solubility of gases in the ventilatory system.

The respiratory rhythm underlying breathing behavior in most vertebrates can be generated in the absence of afferent fibers (von Euler 1986). However, peripheral input helps to ensure that the final motor pattern is behaviorally relevant. For example, both central and peripheral chemoreceptors in the decerebrated animals (von Euler 1986), central chemoreceptors in the isolated brain stem preparation (Harada et al. 1985), and other brain regions (such as cortex, hypothalamus, and cerebellum) alter ventilation to accommodate related motor functions such as speech, postural changes, and locomotion (Aritav et al. 1995; Eldridge et al. 1981; Mitchell 1993; Waldrop and Porter 1995). Together, central and peripheral elements allow an animal to modulate its breathing behavior in accordance with its metabolic demands (Feldman and McCrimmon 1999).

In contrast to our vast knowledge of central and peripheral components of respiratory rhythm generation in vertebrates, little is known about the chemosensory basis of respiratory behavior in invertebrates. In our laboratory, we have thus utilized the fresh water mollusk *Lymnaea stagnalis* to investigate the neuronal basis of respiratory behavior. *Lymnaea* is a bimodal breather and thus can use cutaneous gas exchange under water (skin respiration), or lung exchange (aspirational lung breathing) in the air with the atmosphere (Jones 1961). To exchange gas with the atmosphere, the hypoxic animal surfaces in the isolated ganglionic preparation (Harada et al. 1985), and other brain regions (such as cortex, hypothalamus, and cerebellum) alter ventilation to accommodate related motor functions such as speech, postural changes, and locomotion (Aritav et al. 1995; Eldridge et al. 1981; Mitchell 1993; Waldrop and Porter 1995). Together, central and peripheral elements allow an animal to modulate its breathing behavior in accordance with its metabolic demands (Feldman and McCrimmon 1999).

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The central pattern generating neurons (CPG) and the motor neurons controlling the pneumostome musculature have been identified, and their synaptic connections are well characterized (Syed et al. 1990). Specifically, the identified neurons right pedal dorsal 1 (RPeD1), input 3 interneuron (IP3I), and visceral dorsal 4 (VD4) are the key components of the central respiratory rhythm generating network. The visceral ganglia H, I, J, K cells comprise the motoneuronal pool that regulates the activities of the pneumostome opening and closing muscles (Syed and Winlow 1991; Syed et al. 1991). In isolated brain preparations, the CPG neurons are sufficient to generate the basic respiratory rhythm, as was demonstrated unequivocally by the in vitro reconstruction of the circuit. In cell culture, the three-cell CPG network generated fictive respiratory rhythmic activity identical to that observed in isolated ganglion preparations (Syed et al. 1990). However, both in isolated brain preparations (where spontaneous respiratory activity was absent) and in the in vitro reconstructed circuit, the respiratory rhythm was initiated only after the electrical stimulation of RPeD1; the “normal” source for this excitatory drive to RPeD1 in the intact animals remained unidentified.

In this study, we provide evidence that the hypoxia-induced chemosensory drive in Lymnaea originates at the periphery and is most likely conveyed to the central respiratory CPG neurons via RPeD1. We also demonstrate that the periphery exerts a suppressive, regulatory control on the frequency of patterned respiratory activity in semi-intact preparations, suggesting that the spontaneously occurring respiratory bursts recorded from the isolated brain preparation resulted from the removal of peripheral input. Taken together, this study underscores the importance of peripheral, chemosensory input in the initiation and regulation of respiratory behavior in Lymnaea.

METHODS

Animals

Laboratory-raised stocks of the freshwater snail Lymnaea stagnalis were maintained in well-aerated pond water and were fed lettuce. Animals with a shell length of 25–30 mm (5–6 mo old) were used in all experiments.

Semi-intact preparations

Semi-intact preparations were made as described earlier (Inoue et al. 1996a; Syed et al. 1991). Briefly, the animals were anesthetized either with 10% benzethonium chloride or 2% halothane, and their shell and the foot musculature were removed using scissors. The central ring ganglia and all attached visceral organs (heart, kidney, lung and pneumostome and mantle) were left intact. The resulting central ring ganglia and all attached visceral organs (heart, kidney, lung and pneumostome and mantle) were removed using scissors. The saline was buffered with 10.0 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and the pH was adjusted to 7.9–8.0 with 1N NaOH (Syed and Winlow 1991). The central ring ganglion was physically separated from the periphery by insertion of a plastic partition. Nerves were allowed to pass through grooves in partition, and the openings were subsequently sealed by petroleum jelly (Vaseline). This approach allowed us to completely separate the central compartment from the periphery, thus enabling the superfusion of either one or the other chamber with various experimental solutions. Saline with various gas mixtures was prepared and delivered directly to the preparations with a fast perfusion system (10 ml/min). Specifically, normoxic solution was prepared by bubbling air directly into normal saline (ambient PO2 in Calgary = 135 mmHg), whereas hypoxic and hypercapnic conditions were produced by bubbling 90% N2 + 10% O2 and 10% CO2, respectively. To prevent air contamination of the experimental solutions, the dissection dish was sealed off by parafilm (except to allow cell penetration). In addition, the solution was delivered directly to the preparation under a fast perfusion system (10 ml/min). The PO2 and PCO2 in the experimental chamber were continuously monitored (Hudson Ventronics; model 5584EC). Moreover, pH, CO2, and O2 tensions of experimental solutions were analyzed at room temperature (22°C) using IL-1301 (Instrumentation Laboratories, Lexington, MA).

Isolated brain preparations

To prepare isolated brain preparations, the central ring ganglia were dissected from intact animals as described earlier (Syed et al. 1999). The isolated ganglia were pinned down in a dissection dish containing normal Lymnaea saline.

Surgical procedures

To produce nerve crushes in the intact animals, anesthetized snails were placed in a dissection dish, the shell was pulled back, and a dorsal midline incision was made. The body wall was pulled gently to expose the right internal and external parietal nerves and the anal nerve. These nerves innervate the pneumostome and the mantle cavity area. A pair of fine forceps was used to crush the above three nerves in intact animals. A crush to the left parietal nerve (which innervates the left body wall) served as a control. Following crushes, no suture was necessary, and the animals were allowed to recover overnight in well-aerated pond water.

Behavioral analysis

Either time-lapse video recordings or visual inspections were made to monitor the respiratory behavior of the operated animals. All experiments involving behavioral analysis were performed “blind.”

Electrophysiology

Neurons from both isolated and semi-intact animals were recorded intracellularly using glass microelectrodes filled with a saturated solution of K2 SO4 (resistance 25–30 MΩ) and Leitz micromanipulators. The intracellular signals were amplified by Neurodata amplifier, displayed on Textronix oscilloscopes, and printed on Gould chart recorder.

Statistical analysis

The mean ± SE of the respiratory discharges/10 min was plotted. Statistical analysis of the data was performed using a one-way ANOVA followed by a post hoc Tukey test. Data were considered to be significant if P < 0.05. All chemicals were purchased from Sigma.

RESULTS

Hypoxia-induced respiratory drive in Lymnaea originates at the periphery

Respiration in Lymnaea is a hypoxia-driven behavior; however, the origin (central or peripheral) of this chemosensory drive is unknown. To define the locus at which the hypoxia-induced respiratory activity originates, we recorded from either isolated central ring ganglia or semi-intact preparations, both prior (normoxic) to and during a hypoxic challenge. It is
important to note that as compared with 50–75% N₂, maximum respiratory activity was induced by 90% N₂ + 10% O₂ with higher O₂ (e.g., only 50–70% N₂) resulting in less activity. Thus the above gas mixture was used to provide hypoxic challenge throughout this study.

In semi-intact preparations (central ring ganglia and attached peripheral organs), simultaneous intracellular recordings were made from RPeD1 and a visceral J (VJ) pneumostome opener motor neuron. Under normal (normoxic) air conditions, no spontaneously occurring patterned respiratory activity was present (n = 11, Fig. 1A). However, superfusion of the peripheral compartment with hypoxic saline induced the concurrent bursting discharges in RPeD1 and the VJ cell are characteristics of the pneumostome opening, or expiratory phase of the respiratory pattern in Lymnaea (Fig. 1B) (Syed and Winlow 1991). At the same time pneumostome opening and closing movements were recorded. These discharges are driven by excitatory input from Input 3 (IP3), the only input known to excite the RPeD1 neuron. In this case, RPeD1 is activated first with the VJ motor neurons subsequently being driven by the IP3 input (Fig. 1). It is important to note that the only excitatory input known to excite RPeD1 in the intact brain is produced by IP3I (pneumostome opening-expiration) (see Syed and Winlow 1991). RPeD1 is the first cell to receive IP3 activity, which subsequently drives VJ motor neurons (Fig. 1).

The patterned respiratory discharges and the resultant behavior disappeared on return to normoxic saline (Fig. 1C). Superfusion of the central compartment (in a semi-intact animal) with hypoxic saline did not induce respiratory discharges in RPeD1 and the motor neuron, nor was the respiratory behavior triggered (not shown, but see Fig. 7).

To test further the sensitivities of respiratory neurons within the central ring ganglia to a hypoxic stimulus, the isolated central ring ganglia (CRG) were exposed to normoxic, hypoxic, or anoxic (0% O₂) saline (Fig. 2A). Respiratory activity was monitored intracellularly from RPeD1 and a VJ neuron. In these isolated ganglionic preparations, robust rhythmical, fictive respiratory discharges were recorded from both neurons under normoxic conditions (n = 9, 21.4 ± 1.8 discharges/10 min, mean ± SE). Superfusion of the CNS preparation with hypoxic saline did not significantly alter the patterned respiratory discharges in the respiratory neurons (20.7 ± 1.6 discharges/10 min, P > 0.05). Bathing the CNS preparation with anoxic saline brought about a significant decrease (P < 0.01) in respiratory activity in both RPeD1 and the VJ neuron (15.5 ± 1.4 discharges/10 min). Thus the isolated CRG alone did not respond to the hypoxic challenge.

In sharp contrast to the isolated brain preparation, however, patterned respiratory activity was affected dramatically by the hypoxic challenge in semi-intact preparations (Fig. 2B). These changes were, however, observed only when the peripheral, but not the central compartment, was bathed with hypoxic saline. Specifically, under normal (normoxic) conditions, the observed respiratory discharges averaged 1.1 ± 0.5/10 min. However, superfusion of the peripheral compartment with the hypoxic saline resulted in a significant increase in respiratory activity (n = 8, 4.1 ± 0.9/10 min; P < 0.01), which on wash out with normoxic saline showed a recovery trend toward the baseline. Superfusion of anoxic saline, on the other hand, completely abolished the respiratory activity in semi-intact preparations, which remained suppressed for prolonged periods of time (30–60 min, n = 8), even after wash out with normoxic saline.

To test for the sensitivity of respiratory CPG neurons to CO₂, both isolated (n = 10) and semi-intact preparations (n = 9) were superfused with hypercapnic saline while the central respiratory neurons were recorded intracellularly (Fig. 3). One to 5% CO₂ did not affect respiratory activity in either isolated or semi-intact preparations (data not shown). However, superfusion of either isolated brain (Fig. 3A) or semi-intact preparation (CNS and peripheral organs; Fig. 3B), with 10% CO₂ saline significantly (P < 0.01) reduced spontaneously occurring respiratory discharges. Because the pH of the perfusate

**CRG and semi-intact preparations are insensitive to hypercapnia**

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**FIG. 1.** Hypoxia-induced respiratory drive in Lymnaea originates at the periphery. To test for the origin of chemosensory driven, respiratory activity in Lymnaea, right pedal dorsal neuron 1 (RPeD1), and the pneumostome opener motor neuron visceral J (VJ) cell were recorded simultaneously in a semi-intact preparation. The compartment containing the central ring ganglia was physically separated from the periphery (pneumostome, mantle, lung, kidney, and heart) and sealed. A: under normoxic conditions, the central pattern generator (CPG) neuron (RPeD1) and a VJ cell did not show spontaneous respiratory discharges (top panel), nor were pneumostome opening and closing movements observed (not shown). B: within minutes of perfusing hypoxic saline (2nd panel), characteristic rhythmic respiratory discharges appeared in both RPeD1 and VJ cell (2nd and 3rd panels). C: spontaneous respiratory activity returned to baseline levels after wash out with normoxic saline. All traces represent continuous recordings.
also changed to 7.4–7.5 (within 5 min) under these hypercapnic (10% CO₂) saline conditions, we asked whether the observed CO₂-induced changes in the respiratory activity were the result of this pH change rather than the increased CO₂ itself. To test this possibility, both isolated and semi-intact preparations were perfused with salines of various pH. In both preparations, respiratory activity in saline with a pH of 7.5 was significantly lower than that observed at a normal pH of 8 (Fig. 4; n = 8, P < 0.01). These data suggest that the observed changes in the respiratory activity in 10% CO₂ saline may have resulted from changes in the pH of the perfusate.

Chronic hypoxia treatment of intact animals alters respiratory activity in semi-intact but not in isolated brain preparations

We next asked whether chronic (6–12 h) hypoxic exposure of intact animals, prior to dissection and intracellular recording, altered respiratory activity in either isolated (Fig. 5A) or semi-intact preparations (Fig. 5B). After 6–12 h of hypoxic exposure, either isolated ganglionic, or semi-intact preparations were made, and spontaneously occurring respiratory discharges (IP3 activity) were recorded. Semi-intact, but not isolated brain preparations (P > 0.05) from chronic hypoxia treated animals, exhibited enhanced spontaneous respiratory activity under normoxic recording conditions. Although semi-intact preparations obtained from animals that were exposed to hypoxia for 6 h exhibited significantly (P < 0.01) higher respiratory episodes as compared with their control counterparts (n = 10), there was no further increase in respiratory activity with an additional 6 h of hypoxia (Fig. 5B). These data demonstrate that prior environmental history of an animal is an
important determinant of its motor output recorded in a reduced preparation. Moreover, the locus for these modulatory changes in *Lymnaea* respiratory patterned activity most likely involves peripheral elements. Our data also demonstrate that, like mammalian preparations, respiratory behavior in *Lymnaea* exhibits long-term modulatory changes in response to an hypoxic challenge.

Axotomy of the internal and external parietal nerves disrupted normal respiratory behavior

To demonstrate further that chemosensory input to and from the periphery is indeed required for normal respiratory behavior in the intact animal, the right internal and external parietal nerves, as well as the anal nerve (these nerves innervate the pneumostome and mantle cavity area) were axotomized, and the respiratory behavior of freely moving animals was examined. Because the above nerves carry both afferent and efferent projections to and from the CNS (including RPeD1), we predicted that severing these projections would disrupt normal respiratory behavior. Axotomized animals exhibited significantly fewer respiratory movements (pneumostome openings; 3.0 ± 0.9; n = 16) 1 day after axotomy than experimental animals (8.1 ± 1.6; n = 16; P < 0.05; Fig. 6). By day 7, however, the respiratory behavior exhibited by the experimental animals was similar to the control animals.

*Peripheral induced hypoxia drive is conveyed to the central CPG neurons via RPeD1*

The above data suggested to us that the respiratory drive in *Lymnaea* originates in the periphery. Because RPeD1 is the only respiratory CPG neuron with peripheral projections (projects via right internal, external, and anal nerves), we hypothesized that the hypoxic information is either carried by RPeD1 itself from the periphery, or is driven indirectly by peripherally located chemosensory elements. To distinguish...
between these possibilities, the peripheral compartment was superfused with hypoxic saline containing either normal or 0 Ca\(^{2+}\) (0 Ca\(^{2+}\) and high Mg\(^{2+}\) saline) (Syed and Winlow 1991). Within a few minutes of hypoxic exposure of the peripheral compartment, a pattern of bursting activity was triggered in RPeD1 (\(n = 7\), Fig. 7A). These hypoxia-induced effects on patterned activity in RPeD1 were abolished when the hypoxic saline did not contain Ca\(^{2+}\) (i.e., zero Ca\(^{2+}\) and high Mg\(^{2+}\) saline; Fig. 7B). These data suggest that RPeD1 receives a chemical, excitatory input from the peripherally located chemosensory elements. Conversely, the superfusion of only the central ring ganglia (containing RPeD1’s somata) with hypoxic saline did not trigger patterned respiratory activity (Fig. 7C). Together, the above data are consistent with our hypothesis that the hypoxia-sensitive chemosensory drive in Lymnaea originates at the periphery and is conveyed to the central CPG neurons indirectly via RPeD1.

**DISCUSSION**

In isolated ganglionic preparations, as well as in the in vitro reconstructed network, RPeD1 stimulation via current injection can trigger fictive respiratory activity in Lymnaea (Inoue et al. 1996b; Syed and Winlow 1991; Syed et al. 1990). However, the origin of this excitatory drive to RPeD1, which would normally initiate respiratory activity in intact and semi-intact preparations, remained unknown. The data presented here demonstrate that hypoxia-induced chemosensory input from the periphery provides the necessary excitatory input to RPeD1 via chemical synaptic connections. Moreover, in isolated brain preparations, the frequency of spontaneously occurring respiratory discharges was much higher than in isolated preparations, as well as higher than normal respiration in intact animals. Taken together, our data emphasize the importance of peripheral sensory input in the initiation and regulation of the respiratory rhythm. This study does not, however, undermine the importance of the CPG in rhythm generation per se.

In addition to hypoxia, freely behaving Lymnaea is also responsive to hypercapnic water (5% CO\(_2\), 21% O\(_2\), 84% N\(_2\)), which increases their overall respiratory drive (L. L. Moroz, N. I. Syed, K. Lukowiak, A.G.M. Bulloch, and S. U. Hasan, unpublished observations). However, hypoxia and hypercapnia-driven respiratory activity was found to differ qualitatively. Specifically, hypoxia challenge increased the frequency of spontaneous pneumostome opening and closing movements, while hypercapnic water prolonged only the duration of pneumostome openings (Moroz et al., unpublished observations). These data thus demonstrate that intact animals can respond to both hypoxic and hypercapnic environments; however, their behavioral responses under these conditions are qualitatively different. These behavioral observations in the intact animals do not, however, agree with our data on semi-intact preparations. One likely explanation for this discrepancy may reside in the fact that hypercapnia-sensitive chemosensory cells may be located either on the foot and/or body wall musculature, which

![Figure 6](http://jn.physiology.org/)

**FIG. 6.** Feedback to and/or from the periphery is required for normal respiratory behavior. Intact animals were anesthetized and the internal parietal, external parietal, and anal nerves were crushed. In control animals, the left parietal nerve, which does not innervate the pneumostome area, was crushed. The respiratory rate of experimental animals on day 1 after surgery was significantly lower than that of control animals (\(P < 0.005, n = 16\), but not on day 7.

![Figure 7](http://jn.physiology.org/)

**FIG. 7.** Peripherally induced respiratory activity in Lymnaea is eliminated by zero Ca\(^{2+}\) saline. While recording from RPeD1 in a semi-intact preparation, the peripheral compartment (\(n = 7\)) was superfused with hypoxic (90% N\(_2\) + 10% O\(_2\)) saline. Within seconds of arrival of hypoxic saline (arrow), rhythmic discharges were observed in a previously quiescent RPeD1 (A). These were completely abolished if the hypoxia solution did not contain Ca\(^{2+}\) (zero Ca\(^{2+}\) and high Mg\(^{2+}\) saline; B). Superfusion of the central compartment, containing the RPeD1 somata with hypoxic saline did not result in bursting activity in RPeD1 (C).
in the study was surgically removed to prepare semi-intact preparations. Thus under these experimental conditions, only hypoxia-sensitive elements located adjacent to the pneumostome and mantle area remained intact. If CO₂-sensitive chemoreceptors were indeed surgically removed, then how did our semi-intact preparations exhibit reduced respiratory activity in response to hypercapnia (10% CO₂)? We believe that even though Lymnaea’s natural habitat is stagnant water, where CO₂ levels may reach 5% (normal pond water = approximately 1–2% CO₂), the hypercapnic challenge delivered to semi-intact preparations in the present study is likely unphysiological. Thus CO₂ either directly or indirectly (via pH changes) may have exerted toxic (acidosis) effects on the respiratory CPG (as well as peripheral) neurons. This possibility, however, remains to be tested experimentally.

In contrast with the land snail Helix, in which neurons within the CRG exhibited respiratory sensitivity to PCO₂ (Erlichman and Leiter 1993, 1994; Erlichman et al. 1994), the Lymnaea CRG were insensitive to hypercapnia. Although the possibility of peripherally located CO₂-sensitive receptors in Lymnaea remains to be determined, the data from the above two species do nevertheless suggest that land and freshwater snails may have adopted different evolutionary strategies (central vs. peripheral) to meet their respective respiratory needs.

In contrast to hypoxia, an anoxic challenge suppressed the respiratory activity in semi-intact preparation. Likewise, previous studies on intact animals have demonstrated that snails kept in an anoxic environment rest motionless at the bottom of the tank and death follows within 6–12 h (Moroz et al., unpublished observations). An obvious explanation for this might be that the lack of O₂ shuts down most metabolic activity, thus rendering the animal incapable of various cellular functions.

In vertebrates, respiratory chemoreceptors are located both centrally in the medulla (Bruce and Cherniak 1987; Fitzgerald and Deyahi 1982; Gonzalez et al. 1992; Hitzig and Jackson 1978; Loescheke 1982; Nattie 1991; Smatresk 1990; Smatresk and Smits 1991) and peripherally in the carotid bodies and aortic arteries (Benchetrit et al. 1977; Hitzig and Jackson 1978; Ishii et al. 1985; Smatresk 1990). Central chemoreceptors are sensitive to PCO₂ and pH of the extracellular fluid bathing the ventral surface of the medulla (Wilding et al. 1992). Although the existence of medullary chemoreceptors has been demonstrated in turtles (Benchetrit et al. 1977), toads (Hitzig and Jackson 1978), and lampreys (Rovainen 1977), the exact location, the stimuli (i.e., PCO₂ or pH), and the mechanisms by which these receptors are activated are not fully understood (Bruce and Cherniak 1987; Gonzalez et al. 1992).

Consistent with peripherally located chemosensors in Lymnaea, mammalian peripheral chemoreceptors in the carotid body and the aorta are sensitive to changes in PO₂ and pH. In other vertebrates, such as amphibians, PO₂-sensitive receptors are located in the carotid labyrinth (Ishii and Ishii 1976), whereas in reptiles they are distributed throughout the carotid and pulmonary arteries (Coates and Ballani 1987). In fish and lungfish, PO₂-sensitive receptors are found in gill arteries (Burleson and Smatresk 1989; Johansen and Lenfant 1968; Milson and Brill 1986). Thus peripheral chemosensory cells in reptiles, fish, lungfish, and crayfish (Ishii et al. 1989) are intimately associated with blood vessels either at or near the heart and/or the respiratory organs. Consistent with the above studies, the hypoxia-sensitive chemoreceptors in Lymnaea also appear to be located either at or near the heart, lung, and pneumostome area.

The best-studied mammalian respiratory chemoreceptors are located in the carotid bodies (Duchen and Biscoe 1991; Osani et al. 1997; Stea and Nurse 1991). The biophysical properties as well as a putative mechanism by which these cells convey the hypoxia signal to the CNS have been investigated in culture (Duchen and Biscoe 1991; Stea and Nurse 1991a; Youngson et al. 1993). The transmitter released from the chemoreceptors is hypothesized to activate neuronal endings, which in turn conveys the hypoxia signal to the CPG. In the present study, we have demonstrated that the chemosensory cells located in the pneumostome area (ospheradial ganglia) may exhibit properties similar to those of carotid body chemoreceptors. When activated by the hypoxic stimulus, they excite RPeD1, which in turn triggers respiratory activity in the CPG. Specifically, if RPeD1’s nerve endings were serving as a chemosensor, then perfusing the peripheral compartment with hypoxic saline in the presence of 0 Ca²⁺ saline would also have excited this cell. This, however, was not the case. Although the precise location of the hypoxia-sensitive chemoreceptors remains to be elucidated, one possibility is the ospheradial ganglion.

Although nerve crushes adjacent to the pneumostome area suppressed respiratory activity in intact animals, this perturbation was transient (observed only up to day 3). Normal respiratory behavior was restored within 1 wk of axotomy. This suggests that either as yet unidentified elements compensated for the loss of function, or that functional regeneration occurred. Other data from our laboratory support the latter. Specifically, we have demonstrated that, following axotomy, successful regeneration not only restores functional synaptic connections within the CNS but also leads to restoration of normal respiratory behavior in intact animals (Z. Haque, K. Lukowiak, and N. I. Syed, unpublished observations). These data thus suggest that the restoration of normal behavior after the nerve crush most likely involved regeneration of central and/or peripheral projections.

The data presented in this study also demonstrate that the pretreatment of animals with chronic hypoxia can modulate their respiratory output for a longer period of time. The semi-intact preparations made from animals exposed to hypoxia for 6–12 h exhibited a higher frequency of spontaneously occurring respiratory movements, even though the neuronal activity was recorded in the presence of normoxic saline. However, exposure of these semi-intact animals to hypoxic saline did not further alter the frequency of respiratory episodes (data not shown), suggesting that the chronic hypoxia treatment of intact animals may have already exerted a maximal effect. Moreover, because chronic hypoxic treatment of the intact animals enhanced respiratory activity in semi-intact but not isolated ganglionic preparations, these data further suggest that the modulation of respiratory behavior by chronic hypoxia, like acute hypoxia, involves peripheral chemoreceptors. Changes induced by chronic hypoxia in this study are consistent with those described earlier on mammalian preparations (see Powell et al. 1998), where animals exposed to chronic hypoxia were found to hyperventilate (Okubo and Mortola 1988). Similarly, prolonged hypoxic exposure at birth was also found to increase the overall firing frequency of the carotid body chemoreceptors (Hertzberg et al. 1992; Soulier et al. 1997).
In summary, this study demonstrated that hypoxia-induced respiratory activity in *Lymnaea* originates at the periphery and is likely conveyed to the central CPG neurons indirectly via RPeD1’s peripheral projections. Nevertheless, our data do not, however, rule out the involvement of other, as yet unidentified, cellular elements in the perception of chemosensitivity. The importance of peripheral sensory input in the control of respiratory rhythmogenesis in this model system provides us with an excellent opportunity to examine the cellular and synaptic mechanisms underlying hypoxia-induced regulation of breathing behavior.

N. I. Syed is an AHFMR Senior Scholar.

This work was supported by CIHR Canada.

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