Effect of nitric oxide in the nucleus isthmi on the hypoxic and hypercarbic drive to breathing of toads

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Effect of nitric oxide in the nucleus isthmi on the hypoxic and hypercarbic drive to breathing of toads. Am J Physiol Regulatory Integrative Comp Physiol. 281: R338–R345, 2001.—Nucleus isthmi (NI) is a mesencephalic structure of the amphibian brain that has been reported to participate in CO2 chemoreception and in the ventilatory response to hypoxia. However, no information exists about the modulators and/or mediators involved. In the present study, we assessed the participation of nitric oxide (NO) in the hypoxic and hypercarbic drive to breathing, specifically in the NI. We compared the ventilatory and cardiovascular responses with hypoxia and hypercarbia after microinjecting 100 nmol/0.5 μl of Nω-nitro-l-arginine methyl ester (L-NAME; an NO synthase blocker) into the NI of toads (Bufo paracnemis). L-NAME had no effect under resting conditions. Hypoxia elicited an increase in ventilation in control and vehicle toads by elevating tidal volume (VT). Hypercarbia caused hyperventilation in all groups due to an increase in both VT and frequency. The microinjection of L-NAME into the NI elicited an increase in ventilatory response to hypoxia and hypercarbia due to a higher VT. We conclude that NO in the NI has an inhibitory effect when the respiratory drive is high, acting on VT.

bufo; nitric oxide synthase

In amphibian species, the breathing pattern is episodic. In such pattern, lung ventilation occurs in single events or is grouped into episodes of many breaths separated by nonventilatory periods (apnea) of variable duration (23).

Few studies have investigated the mechanisms of neurorespiratory control in amphibians. Early studies by Oka (28, 29) reported that in bullfrogs the area where brain stem transections affect the breathing pattern includes the nucleus isthmi (NI). This nucleus is a mesencephalic structure of the amphibian brain located between the roof of the midbrain and the cerebellum (22). More recently, it has been shown that NI plays an important role in either CO2 chemodetection or more likely in integration of CO2 chemoreceptor information in bullfrogs (20). We have recently shown that NI lesion causes an augmented ventilatory response to hypoxia, a result that suggests that NI exercises an inhibitory modulation of pulmonary ventilation in toads (10). Therefore, NI is an important site for the control of breathing, although the mediators and/or modulators involved have not been identified.

Nitric oxide (NO) is a free radical gas that can mediate important physiological regulatory events in cell regulation, cell-to-cell communication and signaling, functioning as an intracellular messenger, neurotransmitter, and hormone (26). It is synthesized from L-arginine by a family of enzymes, the NO synthases (NOS) (11), of which three types have been identified: the neuronal, the endothelial, and the inducible forms (7). In mammals, NO has been shown to play an important role in mediating central hypoxic ventilatory reflexes (14, 15).

In view of these considerations, the goals of the present study were to examine the role of the NO pathway in the hypoxic and hypercarbic ventilatory responses occurring specifically in the NI of toads (Bufo paracnemis) by microinjecting a nonselective NOS blocker [Nω-nitro-l-arginine methyl ester (L-NAME)]. In addition, we intended to investigate whether the intracerebral microinjection of L-NAME affects the cardiovascular system, because it is well-known that central NO plays a role in the regulation of blood pressure (see Ref. 25), but its site of action is unclear.

MATERIALS AND METHODS

Animals. Toads (Bufo paracnemis Lutz) of either sex weighing 150–200 g were collected in the vicinity of Ribeirão Preto, SP, Brazil, during the rainy summer months. The toads were maintained in containers with free access to water and basking area. Food was withheld during the 2 wk before the experiment. Each animal was used only once, and all experiments were performed between 8:00 AM and 12:00 PM.

Surgery. Animals were anesthetized in an aqueous solution of 3-aminobenzoic acid ethyl ester (MS-222; 0.3% Sigma) and implanted with a Silastic tube segment, 1.5 mm in outer diameter, into the right femoral artery. The animal’s head was then fixed to a Kopf stereotaxic apparatus, and the skin was then fixed to a Kopf stereotaxic apparatus, and the skin
covering the skull was removed with the aid of a bone scraper. An opening was made in the skull above the mid-brain region using a small drill (Foredom Electric; Bethel, CT). A guide cannula prepared from a hypodermic needle segment 14 mm in length and 0.6 mm in outer diameter was attached to the tower of the stereotaxic apparatus and placed unilaterally in contact with the exposed surface of the mid-brain inside the NI region (according to the coordinates of the stereotaxic atlas for Bufo paracembris; Ref. 18). The orifice around the cannula was filled with a paste consisting of a mixture of equal parts of paraffin and glycerine. The cannula was attached to the bone with stainless steel screws and acrylic cement. A tight-fitting stylet was kept inside to prevent occlusion. The experiments were initiated 48 h after brain surgery.

**Measurements of ventilation.** Measurements of pulmonary ventilation (V̇) were performed by the pneumotachograph method (13), which is based on the Poiseuille principle that a laminar flow of a gas is proportional to the pressure gradient across a tube. A lightweight transparent face mask provided an air-tight connection between the nostrils and a Fleisch tube. Inspiratory and expiratory gas flows were monitored by means of a differential pressure transducer (Validyne, Northridge, CA) connected to the same physiograph.

**Measurements of mean arterial blood pressure and heart rate.** Mean arterial blood pressure (MAP) was measured by connecting the arterial catheter to a Gould pressure transducer (Gould Instrument Systems, Valley View, OH), and the signals from the transducers were recorded on paper (Gould). Heart rate (HR) was determined by counting pressure pulses.

**Determination of the effect of L-NAME microinjection into the NI on ventilatory and cardiovascular responses to hypoxia.** Two days after surgery, the animal was placed in a plastic chamber kept at the experimental temperature of 25°C. The animal chamber was continuously flushed with humidified air (1.5 l/min). In one group (n = 7), experimental animals received one microinjection of L-NAME (Sigma; 100 nmol/0.5 µl) dissolved in mock cerebrospinal fluid (mCSF) of a composition described in previous studies (5, 6, 9). The vehicle group (n = 7) was treated with intracerebral microinjections of mCSF of the same volume. Intracerebral injections were performed with a thin dental needle introduced until its tip was 2 mm below the cannula end. A volume of 0.5 µl was injected over a period of 30 s using a Hamilton microsyringe. The movement of an air bubble inside the PE-10 polyethylene tubing connecting the microsyringe to a dental needle confirmed drug flow. The control group (n = 10) was not subjected to brain surgery. Doses and methods of L-NAME administration were chosen on the basis of previous studies (9, 16) and because, when preliminary doses were tested, the ventilatory responses to the dose of 100 nmol/0.5 µl were the most consistent and repeatable.

Once conditions were stable under normoxia (around 30 min), MAP and V̇ were recorded. Hypoxic gas mixtures (7 and 5% inspired O₂, AGA, Sertaozinho, SP, Brazil) were then applied in random order for 30 min each at the same flow. No time interval was allowed between hypoxic exposures. Hypoxic levels and the period of time for hypoxic exposure before measurements were chosen on the basis of pilot experiments and previous studies (10, 31).

**Determination of the effect of L-NAME microinjection into the NI on ventilatory and cardiovascular responses to hypercarbia.** The same procedure was used in this experimental protocol. In the experimental group (n = 10), animals received one microinjection of L-NAME (Sigma; 100 nmol/0.5 µl) dissolved in mCSF. The vehicle group (n = 6) was treated with intracerebral microinjections of vehicle of the same volume, and the control group (n = 9) was not subjected to brain surgery.

Once conditions were stable under normoxia (30 min), MAP and V̇ were recorded. A hypercarbic gas mixture (3% inspired CO₂, AGA) was then applied for 30 min at the same flow (1.5 ml/min).

**Histological procedures.** At the end of the experiments, the animals were killed with MS-222 (0.3% Sigma) and perfused through the heart with Ringer followed by 10% formalin solution. A dental needle was inserted through the guide cannula, a 0.5-µl microinjection of Evan’s blue was performed, and the animals’ heads were removed and fixed in 10% formalin solution. On the following day, the brains were removed from the skull, immersed in paraffin, sectioned on a microtome, and stained with hematoxylin-eosin for light microscopy determination of the region reached by the microinjection needle.

**Calculations and statistical analysis.** All values are reported as means ± SE. MAP, HR, and V̇ were calculated on the basis of 10-min recording periods. Respiratory frequency (f) was quantified by analyzing the number of respiratory events (lung breaths) per minute. Tidal volume (VT) was obtained from the integrated area of the inspiratory flow signal. Bucal ventilations were identified by small positive and negative pressures and lung ventilations by higher positive pressures as previously described by Jones (19). V̇ = VT × f was expressed as milliliters BTPS per kilogram per minute. Duration of each nonventilatory period (apnea) and number of breaths per burst were measured. A breathing burst consists of inhalations that are not separated by a nonventilatory period longer than the length of two ventilation cycles between them. Instantaneous breathing frequency (the frequency of breathing while actually breathing) was determined according to the criteria of Kinkead and Milsom (21) by calculating the inverse of the period between them and multiplying by 60.

The effects of hypoxia and hypercarbia on ventilatory and cardiovascular variables were evaluated by two-way ANOVA followed by a point-by-point one-way ANOVA and paired t-test, respectively, to assess differences between groups. The Tukey-Kramer multiple comparisons test was applied as a post hoc test. Paired Student’s t-test was used to compare the effects of 5% inspired O₂ before or after 7% O₂, as well as 7% O₂ before or after 5% O₂. A P value of less than 0.05 was considered significant.

**RESULTS**

**Effect of hypoxia on the ventilatory response of the control and vehicle groups.** Figure 1 shows the effect of hypoxia on ventilation. In the control and vehicle groups, hypoxia caused an increase in pulmonary ventilation that was due to an elevation in VT. Statistical analysis of the data obtained under 5% inspired O₂ before or after 7% O₂, as well as 7% O₂ before or after 5% O₂ revealed no carryover effect (data not shown).

**Effects of L-NAME microinjected into the NI on the ventilatory response to normoxia and hypoxia.** Figure 1 also shows the effects of L-NAME microinjection into the NI on ventilation. During normoxia and 7% O₂, V̇ did not significantly differ between groups. The ventilatory response to 5% inspired O₂ was significantly increased in L-NAME-treated toads (P < 0.05 for control and vehicle groups, one-way ANOVA), which was also caused by an elevation in VT. The increase of V̇ was significantly higher compared with normoxia and
Effects of L-NAME microinjected into the NI on instantaneous breathing frequency, number of breaths per burst, and duration of the nonventilatory period in Bufo paracnemis in control, vehicle, and L-NAME groups submitted to hypoxia.

Table 1. Values of instantaneous breathing frequency, number of breaths per burst, and duration of the nonventilatory period of Bufo paracnemis in control, vehicle, and L-NAME groups subjected to hypoxia.

<table>
<thead>
<tr>
<th>Inspired O₂ %</th>
<th>Control (n = 10)</th>
<th>Vehicle (n = 7)</th>
<th>L-NAME (n = 7)</th>
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<tr>
<td>Instantaneous breathing frequency, min⁻¹</td>
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<tr>
<td>21</td>
<td>57.7 ± 6.9</td>
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<td>7</td>
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<td>5</td>
<td>63.2 ± 3.3</td>
<td>71.9 ± 5.9</td>
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<td>Number of breaths per burst</td>
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<tr>
<td>21</td>
<td>6.1 ± 0.6</td>
<td>4.1 ± 0.4</td>
<td>5.1 ± 0.7</td>
</tr>
<tr>
<td>7</td>
<td>12 ± 1.2†</td>
<td>10.1 ± 1.6</td>
<td>12.5 ± 1†</td>
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<tr>
<td>5</td>
<td>9.1 ± 0.5*†</td>
<td>9.3 ± 0.9</td>
<td>9.4 ± 0.6</td>
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<td>Duration of nonventilatory period, s</td>
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<tr>
<td>21</td>
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<td>35.8 ± 5.5*†</td>
<td>44.6 ± 7.3*</td>
<td>47.5 ± 7.3*</td>
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<tr>
<td>5</td>
<td>24.9 ± 3.1*</td>
<td>33.1 ± 4.3*</td>
<td>32.3 ± 3.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE. L-NAME, N⁵-nitro-L-arginine ester. *Significant effect of hypoxia (7 and 5% O₂) compared with normoxic value (1-way ANOVA); †significant effect of 7% inspired O₂ compared with 5% inspired O₂ (1-way ANOVA); ±significant effect of hypoxia compared with normoxia and hypoxia (7 and 5% O₂). 

7% inspired O₂ (P < 0.01 for normoxia and P < 0.05 for 7% inspired O₂, one-way ANOVA).

Effects of L-NAME microinjected into the NI on the breathing pattern under conditions of normoxia and hypoxia. Table 1 shows the effects of L-NAME microinjection into the NI on instantaneous breathing frequency, number of breaths per burst, and duration of the nonventilatory period. Under normoxia and hypoxia, no difference in instantaneous breathing frequency, number of breaths per burst, or duration of the nonventilatory period was observed between groups. Hypoxia had no effect on instantaneous breathing frequency for any of the groups. The number of breaths per burst increased significantly in all groups during 7% O₂ (P < 0.001 for control and L-NAME groups, P < 0.05 for the vehicle group, one-way ANOVA), Hypoxia (7% inspired O₂) caused an increase in the duration of the nonventilatory period in all groups (P < 0.001 for all groups, one-way ANOVA). Hypoxia (5% O₂) also caused an increase in the duration of the nonventilatory period in all groups (P < 0.01 for the L-NAME group and P < 0.05 for the control and vehicle groups, one-way ANOVA).

Figure 2 shows the pulmonary ventilation recordings obtained for the control, vehicle, and L-NAME groups during normoxia and hypoxia (7 and 5% O₂).

Effects of L-NAME microinjected into the NI on MAP and HR response to normoxia and hypoxia. Table 2 shows the effects of L-NAME microinjection into the NI on MAP and HR. None of the experimental conditions had any significant effect on MAP or HR.

Effect of hypercarbia on the ventilatory response of the control and vehicle groups. Figure 3 shows the effect of hypercarbia on ventilation. In the control and vehicle groups, hypercarbia caused an increase in VT and frequency that resulted in an elevation in V̇₁ (P = 0.0023 for the control and P = 0.0030 for the vehicle group, paired t-test).

Effects of L-NAME microinjected into the NI on the ventilatory response to normocarbia and hypercarbia. Figure 3 also shows the effects of L-NAME microinjection into the NI on ventilation. Under normocarbia, V̇₁ did not significantly differ between groups. Hypercarbia also caused an increase in VT in L-NAME-treated toads due to an elevation of V̇₁ and frequency (P = 0.0015, paired t-test). The hypercarbic ventilatory response to 3% CO₂ was significantly increased in the L-NAME group compared with control and vehicle animals (P < 0.05 for the control and vehicle groups, one-way ANOVA).
Effects of L-NAME microinjected into the NI on the breathing pattern under normocarbia and hypercarbia.

Table 3 shows the effects of L-NAME microinjection into the NI on instantaneous breathing frequency, number of breaths per burst, and duration of the non-ventilatory period. Under normocarbia, none of the experimental conditions had any significant effect on breathing pattern in any group. Hypercarbia had no effect on instantaneous breathing frequency in any group. The number of breaths per burst increased significantly in all groups during 3% CO₂ (P = 0.0038 for the control group, P = 0.0039 for the vehicle group, and P = 0.015 for the L-NAME group, paired t-test). Hypercarbia caused a decrease in duration of the non-ventilatory period in all groups (P = 0.038 for the control group, P = 0.0003 for the vehicle group, and P = 0.034 for the L-NAME group, paired t-test).

Figure 4 shows the pulmonary ventilation recordings obtained for control, vehicle, and L-NAME groups during normoxia and hypoxia (7 and 5% O₂). Insp., inspiratory gas flow.

\[ \text{Effects of } L\text{-NAME microinjected into the NI on the breathing pattern under normocarbia and hypercarbia.} \]

\[ \text{Table 3 shows the effects of L-NAME microinjection into the NI on instantaneous breathing frequency, number of breaths per burst, and duration of the non-ventilatory period. Under normocarbia, none of the experimental conditions had any significant effect on breathing pattern in any group. Hypercarbia had no effect on instantaneous breathing frequency in any group. The number of breaths per burst increased significantly in all groups during 3% CO}_2 \text{ (P = 0.0038 for the control group, P = 0.0039 for the vehicle group, and P = 0.015 for the L-NAME group, paired t-test). Hypercarbia caused a decrease in duration of the non-ventilatory period in all groups (P = 0.038 for the control group, P = 0.0003 for the vehicle group, and P = 0.034 for the L-NAME group, paired t-test).} \]

\[ \text{Figure 4 shows the pulmonary ventilation recordings obtained for control, vehicle, and L-NAME groups during normoxia and hypoxia (7 and 5% O}_2 \text{). Insp., inspiratory gas flow.} \]

Fig. 2. Pulmonary ventilation recordings obtained for control, vehicle, and L-NAME groups during normoxia and hypoxia (7 and 5% O₂). Insp., inspiratory gas flow.
DISCUSSION

We have recently reported that NI electrolytic lesions increase ventilatory responses to hypoxia (10), but the mechanisms and/or mediators involved were not assessed. In the present study, we provide evidence that the NO pathway in the NI participates in hypoxic drive to breathing because microinjection of a nonselective NOS blocker (L-NAME) into the NI of toads caused an increased ventilatory response to hypoxia. Thus the elimination of NO in the NI may be responsible for the increased sensitivity of the respiratory system to hypoxia observed after NI lesion. Additionally, we confirm that NO in the NI plays an inhibitory role during hypercarbia-induced hyperventilation (preliminary data from these experiments were presented at the meeting NO BRAZIL, Basic and Clinical Aspects of Nitric Oxide; Ref. 9).

The respiratory control of ectotherms resembles the mammalian control system; the rhythmogenic and pattern-forming elements are adapted to meet the demands determined by the environment, behavior, metabolic needs, and breathing mechanisms. In amphibians, studies have evaluated the chemical drive to breathing and the receptors involved. Hypoxia and hypercarbia increase ventilation through different sets of receptors. Hypoxic ventilatory responses are elicited by peripheral arterial chemoreceptors (35). However, under hypercarbia, the ventilatory response is mainly mediated by central chemoreceptors that are sensitive to increasing H⁺ concentrations in the extracellular fluid surrounding this site (5, 6, 33) backed up by a peripheral component (5, 33).

In the present study, hypoxia (7 and 5% inspired O₂) produced VT elevations in control and vehicle groups. In all groups, the hypoxia-induced hyperventilation resulted from an increase in VT, whereas respiratory frequency remained unchanged (Fig. 1). Accordingly, the duration of the nonventilatory period increased under hypoxia (Table 1). In agreement with our re-

Table 2. Values of mean arterial blood pressure and heart rate of Bufo paracnemis in control, vehicle, and L-NAME groups submitted to hypoxia

<table>
<thead>
<tr>
<th>Inspired O₂ %</th>
<th>Control (n = 10)</th>
<th>Vehicle (n = 7)</th>
<th>L-NAME (n = 7)</th>
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<td>Mean blood pressure, mmHg</td>
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<td>42.7 ± 2.3</td>
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<td>41.4 ± 1.5</td>
<td>32.6 ± 2.0</td>
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<td>5</td>
<td>44.5 ± 4.0</td>
<td>38.6 ± 1.9</td>
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<td>Heart rate, beats/min</td>
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<td>33.9 ± 5.3</td>
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Values are means ± SE.

mental conditions had any significant effect on MAP or HR.

Microinjection sites in the NI. A diagrammatic representation of the microinjection sites in the NI of the toads used in this study is shown in Fig. 5.

Table 3. Values of instantaneous breathing frequency, number of breaths per burst, and duration of nonventilatory period of Bufo paracnemis in control, vehicle, and L-NAME groups submitted to hypercarbia

<table>
<thead>
<tr>
<th>Inspired CO₂ %</th>
<th>Control (n = 9)</th>
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<td>Number of breaths per burst</td>
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<td>3</td>
<td>4 ± 0.9*</td>
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Values are means ± SE. *Significant effect of hypercarbia (3% CO₂) compared with normocarbic value (paired t-test).
results, previous studies with anuran species (10, 21) reported that the hypoxia elicited hyperventilation by increasing the VT. In our experiments, we observed that hypoxia had no effect on instantaneous breathing frequency, which has been used as a reliable indicator of the endogenous respiratory rhythm in intermittent breathers (see Ref. 21).

In a recent study from our laboratory, we described an increase in ventilatory response to hypoxia after electrolytic lesion of the NI, indicating that this structure exercises an inhibitory modulation of pulmonary ventilation by acting on the number of breaths per burst (10). The NI in anurans is made up of a cortex and a medulla, the cortex being rich in cells, whereas the medulla has scattered cells and numerous nerve fibers (22). NI has been described in all vertebrates except cyclostomes and mammals (18). According to Larsell (22), this nucleus appears from its connections and relationships to clearly correspond, in the frog, to the medial geniculate body in mammals. More recently, it has been shown that NOS is expressed in the isthmic region (24). Although NOS was originally identified in mammalian tissues, there is evidence that NO might be a neuronal messenger of early phylogenetic origin and conserved through the evolution (24). Accordingly, a substantial number of studies has indicated that NO is involved in a wide variety of central and peripheral processes in vertebrates and invertebrates (see Ref. 12), such as mediating central hypoxic ventilatory reflexes (14, 15). Moreover, a recent study (17) has suggested that endogenous NO is important for the neurotransmission and/or neuromodulation of respiratory drive to breathing in the bullfrog brain stem.

The importance of NO can be demonstrated by inhibition of its effect (30) using L-arginine analogs such as L-NAME. In the present study, we have chosen L-NAME because it is a nonselective inhibitor of NOS and acts on both the constitutive and inducible isoforms of the enzymes. As to the duration of action of L-NAME, Ayers et al. (1) showed that brain NOS activities returned to baseline level 48 h after the intra-

![Fig. 4. Pulmonary ventilation recordings obtained for control, vehicle, and L-NAME groups during normocarbia and hypercarbia (3% CO₂).](http://ajpregu.physiology.org/Downloadedfrom)

<table>
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<th>Inspired CO₂, %</th>
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<td>Heart rate, beats/min</td>
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Values are means ± SE.
cerebroventricular injection of 5 mg of L-NAME in the rat brain. Additionally, Salter et al. (32) reported that maximal inhibition of NOS was sustained for ~6 h after lateral ventricular administration of 30 and 100 
\( \mu \text{g} \) of L-NAME. This relatively long-lasting effect of L-NAME indicates that NOS inhibition was maximal and constant during our measurements.

Under normoxia, we found no change in pulmonary ventilation in L-NAME-treated toads, indicating that NO has no role under resting conditions. Hypoxia induced hyperventilation under 5% but not 7% inspired \( \text{O}_2 \) in the L-NAME group (Fig. 1). The ventilatory response to 7% inspired \( \text{O}_2 \) tended to be higher, but the increase was not significant. Perhaps only severe hypoxia is able to fully activate NOS. Microinjection of L-NAME into the NI elicited an increased response to 5% inspired \( \text{O}_2 \), indicating that the inhibitory effect of NI on the ventilatory response to hypoxia might be mediated by NO. Current findings suggest that NO can mediate both excitatory and inhibitory components of the hypoxic ventilatory response (27). Our data indicate that NO in the NI acts as an inhibitory mediator and/or modulator.

As to the response to \( \text{CO}_2 \), we observed that hypercarbia caused hyperventilation in all groups due to an increase in both \( V_T \) and respiratory frequency (Fig. 3). The increase in hypercarbia-induced tachypnea was due to a reduced nonventilatory period and increased number of breaths per burst (Table 3).

Compared with hypoxia, the effects of NO on the ventilatory response to \( \text{CO}_2 \) are poorly understood. In our experiments, the microinjection of L-NAME into the NI caused a significant increase in ventilatory response to hypercarbia but not under normocarbia.

The increased ventilation observed in L-NAME-treated toads was due to a higher \( V_T \) (Fig. 3). Therefore, the available data support the notion that NO in the NI may exert an inhibitory influence on the integration of the \( \text{CO}_2 \) drive to breathing. In this regard, a previous study (34) reported that the ventilatory \( \text{CO}_2 \) sensitivities of the peripheral and central chemoreflex loops are depressed after intravenous administration of a NOS inhibitor (\( \text{N}^\text{w}-\text{nitro-L-arginine} \)) in anesthetized cats. More recently, Barros and Branco (2) demonstrated that the ventilatory response to hypercapnia does not change by \( \text{N}^\text{w}-\text{nitro-L-arginine} \) in rats. In addition, these authors (2) observed an unusual ventilatory pattern during air breathing 2 h after NOS inhibition. This pattern consisted of episodes of many breaths separated by episodes of few breaths, similar to the pattern of some ectothermic vertebrates. The presence of such pattern in that study indicates that respiratory control is highly conserved in vertebrates and that NO plays a role in normal respiratory function in rats.

In agreement with previous studies on anuran species, hypoxia (10, 31) and hypercarbia (3) had no effect on cardiovascular responses. Moreover, the present results are consistent with previous studies that have observed that NI does not modulate cardiovascular control (10, 20). Accordingly, microinjection of L-NAME into the NI caused no significant changes in MAP or HR.

In conclusion, our data indicate that NO in the NI has an inhibitory influence on the hypoxic and hypercarbic drive to breathing, acting on \( V_T \). The present observations, taken together with other studies on the presence of NOS in amphibians (4, 8), indicate a con-
considerable degree of phylogenetic conservation of the NO pathway amongst vertebrates.

**Perspectives**

Despite recent advances, the mechanisms of neuro-respiratory control in amphibians are far from understood. Recently, Kinkead et al. (20) found that NI plays an important role in the integration of CO2 drive to breathing. Later we reported (10) that this nucleus is also involved in the ventilatory response to hypoxia, exercising an inhibitory influence on pulmonary ventilation. The present study demonstrates that NO in the NI exerts an inhibitory modulation of the hypoxic and hypercarbic drive to breathing. Future research will be necessary to establish the mechanisms by which NO regulates the hypoxic and hypercarbic drive to breathing inside the NI.

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