Developmental Aspects and Mechanisms of Rat Caudal Hypothalamic Neuronal Responses to Hypoxia

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Horn, Eric M., Glenn H. Dillon, Yi-Ping Fan, and Tony G. Waldrop. Developmental aspects and mechanisms of rat caudal hypothalamic neuronal responses to hypoxia. J. Neurophysiol. 81: 1949–1959, 1999. Previous reports from this laboratory have shown that a high percentage of neurons in the caudal hypothalamus are stimulated by hypoxia both in vivo and in vitro. This stimulation is in the form of an increase in firing frequency and significant membrane depolarization. The goal of the present study was to determine if this hypoxia-induced excitation is influenced by development. In addition, we sought to determine the mechanism by which hypoxia stimulates caudal hypothalamic neurons. Caudal hypothalamic neurons from neonatal (4–16 days) or juvenile (20–40 days) rats were patch-clamped, and the whole cell voltage and current responses to moderate (10% \( {O_2} \)) or severe (0% \( {O_2} \)) hypoxia were recorded in the brain slice preparation. Analysis of tissue oxygen levels demonstrated no significant difference in the levels of tissue oxygen in brain slices between the different age groups. A significantly larger input resistance, time constant and half-time to spike height was observed for neonatal neurons compared with juvenile neurons. Both moderate and severe hypoxia elicited a net inward current in a significantly larger percentage of caudal hypothalamic neurons from rats aged 20–40 days (juvenile) as compared with rats aged 4–16 days (neonatal). In contrast, there was no difference in the magnitude of the inward current response to moderate or severe hypoxia between the two age groups. Those cells that were stimulated by hypoxia demonstrated a significant decrease in input resistance during hypoxic stimulation that was not observed in those cells unaffected by hypoxia. A subset of neurons were tested independent of age for the ability to maintain the inward current response to hypoxia during synaptic blockade (11.4 mM Mg\(^{2+}\)/0.2 mM Ca\(^{2+}\)). Most of the neurons tested (88.9%) maintained a hypoxic excitation during synaptic blockade, and this inward current response was unaffected by addition of 2 mM cobalt chloride to the bathing medium. In contrast, perfusion with the Na\(^+\) channel blocker, tetrodotoxin (1–2 \( \mu \)M) or Na\(^+\) replacement with N-methyl-D-glucamine (NMDG) significantly reduced the inward current response to hypoxia. Furthermore, the input resistance decrease observed during hypoxia was attenuated significantly during perfusion with NMDG. These results indicate the excitation elicited by hypoxia in hypothalamic neurons is age dependent. In addition, the inward current response of caudal hypothalamic neurons is not dependent on synaptic input but results from a sodium-dependent conductance.

INTRODUCTION

The caudal portion of the mammalian hypothalamus has long been known to play a modulatory role in cardiovascular and respiratory regulation (Kabat 1936; Kabat et al. 1935; Spencer 1894). Several decerebration studies have shown the caudal hypothalamus to be facilitatory to the cardiorespiratory responses to hypoxia and hypercapnia (Hayashi and Sinclair 1991; Nielsen et al. 1986; Tenney and Ou 1977). Neurons recorded in the caudal hypothalamus of rats, cats, and rabbits are stimulated by hypoxia and/or hypercapnia, and a significant portion of these neurons have cardiorespiratory related rhythms (Cross and Silver 1963; Dillon and Waldrop 1993; Ryan and Waldrop 1995). When recorded in vitro, these neurons are stimulated by hypoxia and hypercapnia, extending the possibility that these neurons may be central chemoreceptors (Dillon and Waldrop 1992). Moreover, a significant number of neurons stimulated by hypoxia in the rat caudal hypothalamus that are responsive to hypoxia project to the periaqueductal gray in the midbrain; an area also known to modulate cardiorespiratory function (Ryan and Waldrop 1995).

During early mammalian development, hypoxia elicits a transient increase in ventilation followed by an overall depression. This response contrasts with the sustained ventilatory increase observed in adults (Rigatto 1984). Although the reasons for this depression observed in early development are unknown, many developmental studies examining the role of various brain regions during hypoxia have been performed to address this question. Decerebration at the midbrain/pontine junction in rabbit pups abolished the ventilatory depression during hypoxia, implicating more rostral brain areas in this response (Martin-Body and Johnston 1988). In contrast, studies in the lamb, rabbit, and rat provided evidence that areas in the pons and medulla were all that were needed for the ventilatory depression during hypoxia in neonates (Fung et al. 1996; Gluckman and Johnston 1987; Waite et al. 1996). Given these differences, the exact site(s) in the brain causing the ventilatory depression in the newborn is still not completely known, nor is the role of caudal hypothalamic neurons in this response.

The aforementioned studies implicate the caudal hypothalamus as a potential region that may influence the developmental changes in the response to hypoxia. Thus the purpose of the present study was to elucidate any differences in oxygen sensitivity of caudal hypothalamic neurons during development. In addition, the involvement of sodium and calcium channels in the hypoxia response of these neurons was studied. The results of this work have determined that there are significant differences in the hypoxic responses of neonatal and juvenile caudal hypothalamic neurons and these responses are due to a sodium-dependent conductance mediated through tetrodotoxin-sensitive sodium channels.
METHODS

Oxygen tension in caudal hypothalamic tissue during development

All animal protocols and procedures described were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Guidelines of the Laboratory Animal Care Advisory Committee at the University of Illinois. All chemicals listed were obtained from Sigma (St. Louis, MO). Sprague-Dawley rats obtained from a regulated colony were classified into two age groups: neonatal (4–16 days old) and juvenile (20–40 days old). The animals were decapitated and the brains carefully removed and placed in ice-cold artificial cerebrospinal fluid bubbled with 95% O₂-5% CO₂ [which contained (in mM) 116 NaCl, 10 glucose, 5.4 KCl, 1.8 CaCl₂, 0.9 NaHPO₄, 26.2 NaHCO₃, and 15 HEPES, pH 7.35, 295 mOsm]. The caudal hypothalamus then was blocked off according to visual cues on the ventral portion of the brain and sectioned into 400- to 600-μm coronal slices on a tissue chopper. The slices then were rapidly transferred into a brain slice chamber and perfused with 5% CO₂-95% O₂ equilibrated artificial cerebrospinal fluid at a flow rate of ~1 ml/min. The slices had a humidified 5% CO₂-95% O₂ gas mixture flowing over their surface, and the temperature of the chamber was stabilized at 35°C using a servo-controlled water circulator. After the tissue had stabilized for ~1 h, a fine-tipped (5- to 10-μm diam, 0.1-torr resolution) Clark style polarographic oxygen electrode (Diamond General, Ann Arbor, MI) was passed at increasing depths through the posterior hypothalamic area, as identified from stereotaxic atlases (Paxinos 1991; Paxinos and Watson 1986). At each depth (0–150 μm, 50-μm increments) the oxygen tension was measured during control (95% O₂), moderate hypoxic (10% O₂-95% CO₂-85% N₂) and severe hypoxic (0% O₂-5% CO₂-95% N₂) conditions for both neonatal and juvenile tissue.

Whole cell current responses to hypoxia during development

Caudal hypothalamic slices of both neonatal and juvenile rats were prepared as in the preceding section. Electodes with a tip impedance of 5–7 MΩ were pulled on a vertical, two-stage pipette puller (Narishige, PP-83, Tokyo, Japan), fire-polished and filled with an intracellular solution [which contained (in mM) 130 K⁺ gluconate, 10 EGTA, 10 HEPES, 1 CaCl₂, 2 MgCl₂, and 1 ATP, pH 7.25, 275 mOsm]. Whole cell recordings of caudal hypothalamic neurons were obtained using the “blind-patch” technique (Blanton et al. 1989). Membrane currents were recorded in voltage-clamp mode using an AxoClamp 1D patch-clamp amplifier (Axon Instruments, Foster City, CA) with the signal filtered at 5 kHz using a four-pole low-pass Bessel filter. While in the voltage-clamp mode, the series resistance and whole cell capacitance were determined using the circuitry of the amplifier. Because the average whole cell current recorded was <200 pA, the series resistance was not compensated. All of the cells also were recorded briefly in current-clamp mode to assess basal and active membrane potential characteristics. Basal electrophysiological and hypoxic response data were obtained from only those cells that had a resting membrane potential of at most ~40 mV and could be held at ~60 mV with no more that 150 pA of current. Once the basal electrophysiological characteristics of each cell had been analyzed, the membrane current responses to moderate and/or severe hypoxia (2–5 min) were determined. Cells that displayed an inward current during hypoxia of >20 pA were considered stimulated, whereas those that had a response <20 pA were considered unstimulated. This level was chosen based on minor baseline shifts in the recordings and the resolution limits of the amplifier (10 pA). Additionally, some cells were also tested for a membrane response to hypercapnia (7% CO₂-93% O₂).

Ionic properties of the cellular response to hypoxia

Some cells displaying inward current responses to hypoxia subsequently were perfused with a low-calcium (0.2 mM), high-magnesium (11.4 mM) medium (synaptic blockade) to block all classical chemical synaptic transmission (Dean and Boulant 1989). This medium has been shown in this laboratory to reversibly abolish evoked synaptic potentials recorded in the CA1 region of the hippocampus on stimulation of the Schaffer collaterals (Dillon and Waldrop 1992; Nolan and Waldrop 1993). A cell responsive to hypoxia during synaptic blockade indicated the cell had an innate ability to respond to hypoxia that was not due to input from other cells. After testing for the response to hypoxia during synaptic blockade, the current component of this response was analyzed further using ion channel modifying agents including tetrodotoxin (TTX, 1–2 μM), cobalt chloride (2 mM), or after replacing the extracellular sodium with N-methyl-d-glucamine (NMDG). To assess for possible changes in input resistance during hypoxia, the current responses to step voltage commands were digitized at 10–20 kHz and analyzed using PClamp 5.5 software in a subset of cells during control and hypoxic conditions.

Current-clamp experiments also were performed on a separate set of neurons. For these experiments, the membrane voltage responses to hypoxia (10% O₂ and 0% O₂) were recorded with an Axoclamp 2A amplifier (Axon Instruments). Slice preparation, seal formation, and hypoxic stimulation were performed identically as in the voltage-clamp recordings. In a subset of these neurons, the fluorescent dye Lucifer yellow (0.1–0.2%) was included in the pipette for later morphological characterization of the cells studied.

Correction of junction potential

Separate experiments were performed to correct for the slight junction potential that develops at the tip of the electrode caused by the different ion components between the bathing media and the intracellular pipette solution (Fenwick et al. 1982). The junction potential experiments consisted of placing the microelectrode containing the intracellular solution into the normal bathing solution, adjusting the DC offset to zero and then placing the tip into intracellular media. The potential that developed on switching between the two media was recorded using the patch-clamp amplifier and used to correct the resting membrane potentials recorded from the cells studied. In all of the cells tested, the membrane potential was adjusted by ~11.0 mV to correct for the observed junction potential (actual value: −11.3 ± 0.3 mV, n = 27).

Data and statistical analysis

Tissue oxygen recordings were reported as means ± SE and compared between neonatal and juvenile tissue using Student’s t-test (P < 0.05). All electrophysiological data were recorded on a Guild chart recorder and digitized and stored on video cassette tape using data-acquisition software (P-Clamp, Axon Instruments), and a modified four-channel video cassette recorder (A. R. Vetter, Rebersburg, PA). Data were reported as means ± SE (except percentages). Comparisons were made between: neonatal and juvenile cell characteristics and responses to hypoxia, stimulated and unstimulated neuronal characteristics, and the cellular response to hypoxia during perfusion with various pharmacological agents. χ², Student’s paired and unpaired t-tests (with Bonferroni correction when necessary) and repeated measures ANOVA (with Tukey’s post hoc analysis) tests were used with the significance level set at P < 0.05.

RESULTS

Oxygen tension in caudal hypothalamic tissue during development

The oxygen tension measured during control conditions (95% O₂) was significantly higher in neonatal (10.0 ± 0.4 days
old, \(n = 6\) when compared with juvenile (23.8 \(\pm\) 0.7 days old, \(n = 6\)) tissue at depths equal to and \(>100\) \(\mu\)m (Fig. 1A). During the moderate or severe hypoxic stimulus, there was no significant difference in oxygen tension between the two ages when tested at 50, 100, and \(150\) \(\mu\)m (Fig. 1B). Depths \(>150\) \(\mu\)m were not tested for differences in oxygen tension because the average depth of all the cellular recordings was less than this level for both the neonates (115.9 \(\pm\) 16.0 \(\mu\)m, \(n = 26\)) and juveniles (128.8 \(\pm\) 8.9 \(\mu\)m, \(n = 76\)). On the basis of these findings, we are confident that the stimuli received by neurons at the different ages were identical and cannot account for the differences in the observed responses.

**Basal neuronal characteristics during development**

Basal electrophysiological characteristics were examined in 49 caudal hypothalamic neurons from 24 neonatal rats and 96

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**TABLE 1. Basal electrophysiological characteristics of caudal hypothalamic neurons**

<table>
<thead>
<tr>
<th></th>
<th>Neonate (11.1 (\pm) 0.5 days old)</th>
<th>Juvenile (26.5 (\pm) 0.5 days old)</th>
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<tbody>
<tr>
<td>Membrane potential, mV</td>
<td>(-50.6 \pm 1.3) (49)</td>
<td>(-49.4 \pm 0.8) (96)</td>
</tr>
<tr>
<td>Input resistance, M(\Omega)</td>
<td>348.8 (\pm) 16.5 (49)</td>
<td>294.8 (\pm) 8.4 (96)*</td>
</tr>
<tr>
<td>Whole cell capacitance, pF</td>
<td>38.3 (\pm) 2.0 (49)</td>
<td>38.9 (\pm) 1.8 (96)*</td>
</tr>
<tr>
<td>Tau, ms</td>
<td>13.3 (\pm) 1.0 (49)</td>
<td>11.3 (\pm) 0.6 (96)*</td>
</tr>
<tr>
<td>Action potential amplitude, mV</td>
<td>57.9 (\pm) 2.9 (18)</td>
<td>53.7 (\pm) 1.7 (43)</td>
</tr>
<tr>
<td>Half spike height time, ms</td>
<td>0.49 (\pm) 0.02 (18)</td>
<td>0.40 (\pm) 0.01 (43)*</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE, \(n\) in parentheses. Neonates were 11.1 \(\pm\) 0.5 days old, juveniles were 26.5 \(\pm\) 0.5 days old. * Significantly different from neonate \((P < 0.05, \text{Student’s unpaired } t\)-test).
neurons from 49 juvenile rats (Table 1). There were no significant differences in the average number of cells recorded per animal between neonates and juveniles (2.04 ± 0.26 vs. 1.96 ± 0.17; \( P > 0.05 \)). No significant differences were observed in resting membrane potential or whole cell capacitance between neonatal and juvenile caudal hypothalamic neurons (Table 1). In contrast, the average resting input resistance, time constant and half-time to spike height was significantly larger in the neonatal neurons as compared with the juvenile neurons, signifying a greater amount of resting membrane current and faster action potential generation in the juvenile neurons (Table 1).

**Whole cell current responses to hypoxia during development**

The most striking difference found between the two age groups was in the ability to respond to both a moderate or severe hypoxic stimulus. In the juvenile caudal hypothalamus, a significantly larger percentage (55.6%; 20/36) of the tested neurons were stimulated by moderate hypoxia as compared with neonatal (27.3%; 9/33) neurons (Fig. 2A). In addition, caudal hypothalamic neurons of juvenile rats (65.1%; 56/86) were significantly more likely than neonates (42.9%; 18/42) to be stimulated by the severe hypoxic stimulus (Fig. 2A). An example of the current response to severe hypoxia for both a neonatal and juvenile caudal hypothalamic neuron is shown in Fig. 3. In contrast to the differences observed in the proportions of caudal hypothalamic neurons stimulated by both moderate and severe hypoxia, there were no differences between neonates and juveniles in the magnitude of the inward current response of stimulated cells (Fig. 2B).

As compared with the responses to hypoxia, the caudal hypothalamic responses to hypercapnia between both the neonates and juveniles were less robust. Only 22.2% (4/18) of neonatal and 10.5% (2/19) of juvenile caudal hypothalamic neurons tested displayed an inward current response to hypercapnia, and these neurons generally did not respond to hypoxia...
Voltage-clamped neurons stimulated by moderate hypoxia had a significantly lower resting input resistance compared with unstimulated neurons (306.2 ± 15.3 vs. 354.6 ± 19.4 MΩ). In addition, neurons stimulated by severe hypoxia had a significantly lower resting input resistance (292.1 ± 9.4 vs. 318.5 ± 12.2 MΩ), higher resting cell capacitance (41.6 ± 2.1 vs. 36.3 ± 1.9 pF) and shorter half-time to spike height (400 ± 20 vs. 450 ± 20 μs) compared with unstimulated cells. However, when these analyses were corrected for age, all significant differences were eliminated. Therefore the main differences between stimulated and unstimulated cells appear to be related to the developmental stage of the neuron.

Morphological characterization of 23 caudal hypothalamic neurons was performed. Neurons filled with Lucifer yellow showed no differences in basal characteristics or hypoxic depolarization compared with cells not filled (average hypoxic membrane response for filled = 4.8 ± 0.5 mV and unfilled = 6.2 ± 1.0 mV, P > 0.05). Neurons stimulated by hypoxia (10 and 0% O₂, n = 16) were mostly bipolar and had dendritic fields extending to or toward the third ventricle or ventral surface of the brain. The average size of the soma of these neurons was 18.3 ± 1.7 × 28.8 ± 3.2 μm. The morphological attributes of a hypoxia-stimulated neuron are shown in Fig. 4, C and D. Neurons unstimulated by hypoxia were either bipolar (n = 3) or tripolar (n = 2); none showed the numerous dendritic extensions from the cell soma that frequently were observed in hypoxia-stimulated neurons. The bipolar unstimulated neurons were quite long and relatively narrow (53.0 ± 5.8 × 15.7 ± 0.8 μm), whereas the tripolar neurons in this category were similar in size to hypoxia-stimulated neurons. An example of a bipolar neuron that was inhibited by hypoxia is shown in Fig. 5, A–C.

Current and ionic properties of the cellular response to hypoxia

Not every cell was tested with both moderate and severe hypoxia, but in those cells (n = 32) that were tested for both, the magnitude of the inward current response to hypoxia increased with decreasing levels of oxygen (10% O₂: 90.3 ± 25.1 pA vs. 0% O₂: 183.9 ± 49.5 pA, P < 0.05). Because the magnitude of the response was greater during severe hypoxia, the ionic properties of the inward current response to hypoxia were tested during the more severe stimulus only. Current-voltage relationships were compared during normoxia and hypoxia to determine input resistance changes. In those cells that displayed an inward current response to hypoxia of >20 pA (stimulated), a significant decrease in input resistance was observed compared with cells with a response between 0 and 20 pA (unstimulated), indicating an inward cationic current is active during hypoxia (Table 2). Furthermore, the current-to-voltage relationships during hypoxia of stimulated neurons displayed a significant increase in the reversal potential of whole cell current during hypoxia as compared with unstimulated neurons (Fig. 6).

In 36 of the cells responding to hypoxia with an inward current, the response to hypoxia was tested in a low-Ca²⁺/high-Mg²⁺ medium to inhibit chemical synaptic transmission. In 32 of these cells tested (88.9%), the response to hypoxia was maintained. The hypoxic inward current response of these cells during perfusion with control media was 196.9 ± 34.4 and...
128.4 ± 25.4 pA during synaptic blockade (P > 0.05, Student’s unpaired t-test).

Twenty-one neurons that maintained the response to hypoxia in the synaptic blockade media were tested for the inward current response to hypoxia during perfusion with either CoCl₂, TTX, or both as shown in Fig. 7. In eight of these cells, addition of CoCl₂ (2 mM) alone had no effect on the response to hypoxia (Fig. 8A). In contrast, addition of TTX (1–2 µM) after synaptic blockade in a group of eight separate cells virtually abolished the response to hypoxia (Fig. 8B). The differing effects of CoCl₂ and TTX were tested together in five neurons with similar results as when tested separately, namely TTX abolishing the response and CoCl₂ having no effect (Fig. 8C). To verify further the influence of sodium current on this response, extracellular sodium was replaced with NMDG in six separate neurons. To a similar magnitude as perfusion with TTX, NMDG significantly attenuated the inward current response to hypoxia (TTX attenuation: 78.7 ± 4.5% and NMDG attenuation: 63.5 ± 12.9%, P > 0.05). Furthermore, in three of the neurons perfused with NMDG, the large decrease in input resistance observed during hypoxia was abolished (Control Rᵢ decrease: 56.1 ± 5.9% and NMDG Rᵢ decrease: 3.1 ± 11.9%, P < 0.05).

**DISCUSSION**

A major finding in this study was the disparity in the ability of neonatal caudal hypothalamic neurons to respond to moderate or severe hypoxia as compared with juveniles. Our results indicate that fewer neonatal than juvenile neurons respond to hypoxia; however, the magnitude of the response to hypoxia does not differ between stimulated neonatal and juvenile neurons. Other investigators have demonstrated differential responses to hypoxia between neonatal and juvenile/adult neurons in the rat brain stem (Haddad et al. 1990). A possible explanation for the differences observed is a disparity in the actual oxygen tensions between the neonatal and juvenile tissues during hypoxia. A difference in the tissue oxygen concentration between neonatal and juvenile/adult neurons in the rat brain stem was demonstrated previously in the hypoglossal nucleus at all levels >21%, but there was no significant difference when the oxygen concentration delivered to the tissue was <21% (Jiang et al. 1991). The results of the present study clearly show that the oxygen tension during both levels of hypoxia between neonatal and juvenile caudal hypothalamic tissue is not significantly different. There is, however, a significant difference in the oxygen tension between neonatal and juvenile tissue at depths ≥100.
μM when perfused with 95% O₂. This most likely is the result
of the neonatal brain being less dependent on aerobic metab-
olism for energy (Clark et al. 1993; Xia et al. 1992). Therefore
because almost all of the oxygen available to the tissue comes
from the top of the chamber, the neonatal tissue is less of an
oxygen sink compared with the juvenile tissue at greater
depths. Therefore we believe that the decrease in the propor-
tion of neonatal caudal hypothalamic neurons responding to
hypoxia is due to a lack in the oxygen sensing ability of these
neurons early in development.

In this study, no significant differences were found in the
magnitude of the inward current responses to moderate or
severe hypoxia between neonatal and juvenile caudal hypotha-
lamic neurons. There is, however, a trend showing a greater
inward current response to moderate and severe hypoxia in the
juveniles as compared with neonate. One cannot rule out the
possibility of a difference in the response between the ages
because the heterogeneity of cell types in this area adds to the
high variance observed in the responses that was not overcome
even by the large sample size in this study. This is in contrast
to results in ventrolateral medullary neurons from this labora-
tory and hypoglossal neurons from another laboratory that have
shown larger membrane potential responses to hypoxia in adult
neurons as compared with neonates (Haddad and Donnelly
1990; Nolan and Waldrop 1996). This difference may be due to
the relatively smaller range of membrane voltage responses
(~1 orders of magnitude) compared with the inward current
response (~3 orders of magnitude), leading to a smaller vari-
ance in the membrane-voltage analyses.

This study also demonstrated that the input resistance, time
constant and half-time to spike peak in neonatal caudal hypo-
thalamic neurons were all significantly larger as compared with
the juvenile. These findings suggest that neonatal cells have a

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**Table 2.** Input resistance ($R_i$) changes during severe hypoxia between stimulated and unstimulated caudal hypothalamic neurons

<table>
<thead>
<tr>
<th></th>
<th>Unstimulated</th>
<th>Stimulated</th>
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<tr>
<td>$n$</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Inward current response, pA</td>
<td>$-8.8 \pm 2.0$</td>
<td>$-185.6 \pm 39.5^*$</td>
</tr>
<tr>
<td>$R_i$ during 95% O₂, MΩ</td>
<td>$308.8 \pm 20.1$</td>
<td>$264.8 \pm 15.0$</td>
</tr>
<tr>
<td>$R_i$ during 0% O₂, MΩ</td>
<td>$301.4 \pm 23.1$</td>
<td>$165.5 \pm 17.8^+$</td>
</tr>
<tr>
<td>Decrease from control, %</td>
<td>$3.3 \pm 1.6$</td>
<td>$36.1 \pm 5.0^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE. * Significant difference from unstimulated cells ($P < 0.05$, Student’s unpaired $t$-test with Bonferroni correction where necessary). † Significant difference from $R_i$ during 95% O₂ ($P < 0.05$, Student’s paired $t$-test with Bonferroni correction).
lower channel density and therefore are less excitable. Similar results previously have been shown in neurons of the genio-glossal nucleus of the rat brain stem (Nunez-Abades et al. 1993). In contrast, there was no significant difference found in the average resting membrane potential between neonatal and juvenile caudal hypothalamic neurons. These differences in channel density appear not to affect those channels responsible for the resting membrane potential, such as potassium leak channels, but may be involved in initiating spiking behavior. Thus compared with neonates, the juvenile neurons may be more able to demonstrate increases in firing during periods of hypoxia even if the specific mechanism of oxygen sensitivity is fully developed in neonatal neurons.

Prior work from this laboratory has established the ability of the caudal hypothalamus to modulate the respiratory responses to hypoxia (Horn and Waldrop 1997). Microinjection of the excitatory amino acid antagonist, kynurenatic acid, into the caudal hypothalamus significantly attenuates the respiratory response to hypoxia in anesthetized rats (Horn and Waldrop 1994). In addition, caudal hypothalamic neurons recorded in vivo are excited by hypoxia in the absence of peripheral chemoreceptors and a large percentage of these neurons have firing rhythms related to respiration (Dillon and Waldrop 1993; Ryan and Waldrop 1995). Furthermore, a large number of neurons in the rat caudal hypothalamus that are excited by hypoxia project to the midbrain periaqueductal gray, another area known to modulate respiratory output (Ryan and Waldrop 1995). These results suggest that most caudal hypothalamic neurons are responsive to changes in arterial O2 concentration and this responsiveness helps modulate the respiratory responses to hypoxia. Unfortunately the present study was unable to identify the precise characteristics of hypoxia-responsive caudal hypothalamic neurons that make them different from nonresponsive cells. When electrophysiological parameters were compared between responsive and nonresponsive neurons, no differences were found that could not be explained by the age of the neuron. There were, however, slight morphological differences between the responsive and nonresponsive neurons. The hypoxia-responsive neurons were mostly multipolar, whereas the nonresponsive neurons were usually bipolar with less extensive projections; but the significance of these morphological differences needs further investigation.

This study demonstrates that neurons in the rat caudal hypothalamus develop the ability to respond to hypoxia during
the first 3 wk of postnatal development. The biphasic respiratory response to hypoxia (initial increase then sustained decrease in ventilation) in rats early in development has been well characterized, although the exact mechanism for this response is unknown (Eden and Hanson 1987). The favored hypothesis for the depression stage in the biphasic response to hypoxia is an underdeveloped facilitatory area in the CNS of neonates that usually counteracts inhibitory respiratory drive during hypoxia (Waites et al. 1996). The caudal hypothalamus is one such facilitatory area that counteracts inhibitory ventilatory drive from other areas of the brain during hypoxia (Tenney and Ou 1977). Previous findings have shown that decerebration at the mid-
brain/pontine junction abolishes the depression of respiration during hypoxia in neonatal rabbits (Martin-Body and Johnston 1988). Furthermore, the biphasic response to hypoxia is present when the brain is sectioned rostral to the caudal hypothalamus but is absent after specific bilateral lesions in the upper lateral pons or red nucleus of lambs and rabbits (Dawes et al. 1983; Gluckman and Johnston 1987; Waites et al. 1996). Because the studies implicating discrete nuclei in the pons and midbrain in the biphasic response used electrolytic instead of chemical lesions, the results observed could be due to destruction of caudal hypothalamic projections through these areas (Vertes and Crane 1996). The present findings suggest that the lack of excitation from caudal hypothalamic neurons may be involved in the depression stage of the biphasic response to hypoxia in neonates. The overall oxygen sensitivity of neurons in this area may not be fully developed in neonates leading to an inability to counteract cortical inhibitory drive during hypoxia. However, a recent study counteracted this hypothesis by demonstrating the maintenance of the depression stage of the biphasic response to hypoxia after a midcollicular decerebration (Fung et al. 1996). The investigators in this study, however, still could not rule out the involvement of diencephalic areas in the biphasic response to hypoxia because such a large area was eliminated (Fung et al. 1996).

Previous reports looking at the mechanism of hypoxic sensitivity in central neurons demonstrated the importance of sodium currents in the membrane response to hypoxia in the hypoglossal motor nucleus and hippocampus (Cummins et al. 1991; Haddad and Jiang 1993). The present results further support the involvement of sodium channels in the cellular response of hypoxia in central neurons. Because the characteristics of this response in caudal hypothalamic neurons is a slow, sustained inward sodium current, the involvement of fast-inactivating sodium currents is unlikely even though the present current was inhibited by TTX. One sodium current that could mediate this type of response, however, is the persistent sodium current, which is a noninactivating TTX-sensitive sodium current dictated by differential gating of the same protein channel as the fast-inactivating current (Alzheimer et al. 1993). The persistent sodium current is involved mainly in modulating neuronal and muscle excitability and recently has been shown to display increased activity during hypoxia in cardiac myocytes (Crill 1996; Ju et al. 1996). Thus the persistent sodium current is a likely candidate causing the observed response in these neurons, although direct evidence is needed.

In contrast to sodium current, the inward current response to hypoxia of caudal hypothalamic neurons was shown to be unaffected by calcium current, although there was a tendency for a decrease in the current response after perfusion with low-calcium solution. However, this tendency to decrease was most likely due to blockade of excitatory synaptic input onto these cells that has been shown in prior reports to facilitate the response to hypoxia in vivo (Horn and Waldrop 1994). Moreover, the lack of influence by calcium channels was supported further by the observation that perfusion with CoCl2 had no effect on the inward current response to hypoxia. These findings are in contrast to results showing the involvement of calcium channels in the cellular response to hypoxia in ventrolateral medullary neurons and myocardial cells (Fearon et al. 1997; Sun and Reis 1994). Furthermore, several types of oxygen-sensitive potassium channels have been described in peripheral (carotid body glomus, arterial smooth muscle cells) and central (neocortical, substantia nigra neurons) cell types that display both increases and decreases in conductance during anoxia (Ganfornina and Lopez-Barneo 1991; Jiang and Haddad 1994; Weir and Archer 1995). Because a decrease in potassium conductance causes membrane depolarization, these channels were not analyzed in the present study due to the inward current response arising from an increase in membrane conductance. Thus there appears to be a panoply of oxygen-sensitive channels in a variety of cell types involved with cardiorespiratory control. More work is needed to determine the exact role of these channels in both the cellular and respiratory responses to hypoxia.

In conclusion, a smaller proportion of neurons in the caudal hypothalamus of neonatal rats become stimulated during hypoxia as compared with neurons in more mature rats. In addition, the magnitude of the inward current response to hypoxia of caudal hypothalamic neurons is similar during rat development. Furthermore, the inward current response to hypoxia in these neurons appears to be due to TTX-sensitive sodium channels. The sum of these results implies that caudal hypothalamic neurons act as central chemoreceptors; this lack of oxygen sensitivity in many of the neurons during development may be a contributing factor to the respiratory depression observed in neonatal rats during hypoxia.

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