Effect of long-term intermittent and sustained hypoxia on hypoxic ventilatory and metabolic responses in the adult rat

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Reeves, Stephen R., Evelyne Gozal, Shang Z. Guo, Leroy R. Sachleben, Jr., Kenneth R. Brittian, Andrew J. Lipton, and David Gozal. Effect of long-term intermittent and sustained hypoxia on hypoxic ventilatory and metabolic responses in the adult rat. J Appl Physiol 95: 1767–1774, 2003; 10.1152/japplphysiol.00759.2002.—The effects of chronic sustained hypoxia (SH) on ventilation have been thoroughly studied. However, the effects of intermittent hypoxia (IH), a more prevalent condition in health and disease are currently unknown. We hypothesized that the ventilatory consequences of SH and IH may differ and be related to changes in N-methyl-D-aspartate (NMDA) glutamate receptor subunit expression. To examine these issues, Sprague-Dawley adult male rats were exposed to 30 days of either SH (10% O2) or IH (21% and 10% O2 alternations every 90 s) or to normoxia (RA), at the end of which ventilatory and O2 consumption responses to a 20-min acute hypoxic challenge (10% O2) were conducted. In addition, dorso-caudal brain stem tissue lysates were harvested at 1 h, 6 h, 1 day, 3 days, 7 days, 14 days, and 30 days of SH and IH and analyzed for NR1, NR2A, and NR2B NMDA glutamate receptor expression by immunoblotting. Normoxic ventilation was higher after both SH and IH (P < 0.001). Peak hypoxic ventilatory response was higher after IH but not after SH compared with RA. However, hypoxic ventilatory decline was more prominent after SH than IH (P < 0.001). NR1 expression showed a biphasic pattern of expression over time that was essentially identical after IH and SH (P value not significant). However, NR2A and NR2B expression was higher in IH compared with SH and RA (P < 0.01). We conclude that long-lasting exposures to SH and IH enhance normoxic ventilation but are associated with different time domains of ventilation during acute hypoxia that may be accounted in part by changes in NMDA glutamate receptor subunit expression.

intermittent hypoxia; chronic sustained hypoxia; hypoxic ventilatory response; glutamate receptors; N-methyl-D-aspartate

THE ACUTE VENTILATORY RESPONSE to hypoxia in adult mammalian species is biphasic. After an initial ventilatory enhancement, there is a subsequent decrease in ventilation to levels that are lower than the peak early ventilatory increase (41). This later decrease in ventilatory output has been termed hypoxic ventilatory decline (HVD) and appears to be the result of complex interactions between excitatory and inhibitory influences on peripheral chemoreceptors, central respiratory neurons, and metabolic pathways (41). In contrast to the dynamic changes associated with acute hypoxia, sustained exposure to environmental hypoxia (SH) is associated with a progressive time-dependent increase in ventilation, i.e., ventilatory acclimatization to hypoxia, a phenomenon that has been well documented in a variety of mammalian species including humans (10, 14, 19, 28, 36). The process of adaptation to hypoxia that results in ventilatory acclimatization appears to involve both ends of the primary synaptic pathways underlying the ventilatory response, such that development of increased sensitivity of the peripheral chemoreceptors occurs in parallel with integration of afferent input and amplification of centrally generated efferent output (2, 12).

It is noteworthy that, in addition to long-term sojourns at high altitude and chronic lung disease, SH occurs in burrowing mammals as well as in other species. Intermittent hypoxia (IH) is also a frequent occurrence as exemplified by the high prevalence of lung diseases and sleep-disordered breathing. However, the effects of IH on respiratory control in general, and on the hypoxic ventilatory response (HVR) in particular, have not been extensively investigated. We therefore conducted the present study to examine the differential effects of long-term exposure to IH and SH on normoxic ventilation and on the HVR characteristics. Furthermore, because activation of N-methyl-D-aspartate (NMDA) glutamate receptors within the dorso-caudal brain stem is critical to HVR (34, 35), we assessed changes in NMDA receptor subtype expression within this region.

METHODS

Adult Sprague-Dawley male rats were purchased from Charles River and used for all experiments (weights: 250–275 g). The experimental protocols were approved by the Institutional Animal Use and Care Committee and are in close agreement with the National Institutes of Health Guide in the Care and Use of Animals. All efforts were made to...
IH and SH protocols. Animals were placed in four identical commercially designed chambers (30 × 20 × 20 in.; Oxycycler model A440X, Reming Bioinstruments, Redfield, NY), which were operated under a 12:12 light-dark cycle (6:00 AM–6:00 PM). Gas was circulated around each of the chambers, attached tubing, and other units at 60 l/min (i.e., one complete change per 10 s). The O2 concentration was continuously measured by an O2 analyzer and was changed by a computerized system controlling the gas valve outlets, such that the moment-to-moment desired O2 concentration of the chamber was programmed and adjusted automatically. Deviations from the desired concentration were met by addition of N2 or O2 through solenoid valves. Ambient CO2 in the chamber was periodically monitored and maintained at <0.01% by adjusting overall chamber basal ventilation. The gas was also circulated through a molecular sieve (type 3A, Pisons) so as to remove ammonia. Humidity was measured and maintained at 40–50% by circulating the gas through a freezer and silica gel. Ambient temperature was kept at 22–24°C. The IH profile consisted of alternating air-sampling room air and 10% O2 every 90 s. The SH profile consisted of exposure to 10% O2. Control animals were exposed to circulating normoxic gas in one of the chambers.

Ventilatory and metabolic recordings. Respiratory measures were continuously acquired in the freely behaving animals via the barometric method (Buxco Electronics, Troy, NY) (3, 38). To minimize the long-term effect of signal drift due to temperature and pressure changes outside the chamber, a reference chamber of equal size in which temperature and pressure changes outside the chamber were routine to account for inspiratory and expiratory barometric asymmetries (15). Environmental temperature was maintained slightly below the thermoneutral range (24–26°C). At least 60 min before the start of each protocol, animals were allowed to acclimatize to the chamber, in which humidified air (70–90% relative humidity) was passed through at a rate of 5 l/min, by use of a precision flow pump-reservoir system. Pressure changes in the chamber due to the inspiratory and expiratory temperature changes were measured by using a high-gain differential pressure transducer (Validyne, model MP45-1) (11). Signal analogs were continuously digitized and analyzed on-line by a microcomputer software program (Buxco Electronics). A rejection algorithm was included in the breath-by-breath analysis routine and allowed for accurate rejection of motion-induced artifacts. Tidal volume (VT), respiratory frequency (f), Ve, mean inspiratory flow (V(1/T)), and inspiratory duty cycle (Tr/Ttot) were computed and stored for subsequent off-line analysis.

Metabolic rate was measured by using an indirect calorimetry system similar to that described by Jensen and colleagues (27), consisting of the two plethysmographs, pumps, flow controllers, valves, and analyzers. The system was computer controlled to sequentially measure the O2 and CO2 concentrations in the two plethysmographic chambers at sampling intervals of 15 s. The system operated as follows: air taken from a common air source was pulled through the plethysmographic chambers, a blank chamber, and a reference line connected via separate but identical air-sampling pathway. The blank chamber was used as reference to monitor ambient O2 and CO2 concentrations periodically. The condensation in the air exiting the chambers was removed with electronic sample coolers (Universal Analyzers, Carson City, NV). Respired gas was pushed with pumps (model 107CAB18, Thomas Industries, Sheboygan, WI) through mass flow controllers (Teledyne Hastings-Raydist, Hampton, VA), which maintained constant but adjustable airflow (0.25–5.0 l/min). The air then traveled to a manifold containing three valves. At predefined intervals, the computer sent a 5-V signal to close a specified valve, corresponding to a particular chamber, thereby shunting air through the O2 and CO2 analyzers (Ametek, S-5A/III, AET Technologies, Pittsburgh, PA, and Gascard I Edinburgh Sensors, Edinburgh, UK, respectively). Because each analyzer is differential and compared the O2 and CO2 levels in the chamber airstream with levels in a reference line, air was used as the calibration gas to zero the analyzers. The span calibrations for the O2 and CO2 analyzer were set by using O2 (1% balance N2) and CO2 (0.8%), respectively, as primary gas standards (Air Liquide, Louisville, KY). The zero and span calibrations were checked several times until each analyzer read precisely at the given concentration. The time of measurement, differential O2 concentrations, flow rate, and O2 consumption (V0), were calculated and stored in the computer configured with data-acquisition hardware and software (Buxco Electronics). O2 and CO2 values corresponding to 10 consecutive samples were averaged at time points representing normoxic baseline, peak HVR (pHVR), and HVD.

Acute hypoxic ventilatory challenges. Hypoxic ventilatory challenges were conducted at day 30 of IH or SH exposures and were repeated 30 days after discontinuation of the exposures (animals were returned to room air for that period). Animals were left in room air for 3 h during which they were weighed and placed in the barometric recording chamber. After stable baseline normoxic values were obtained for at least 5 min, rats were switched to 10% O2-balance N2 by using a premixed gas mixture. The hypoxic challenge lasted for 20 min, after which room air was reintroduced in the recording chamber, and recovery was recorded for 10 min. Ventilatory measures were averaged in 1-min intervals and plotted. ANOVAs (one-way or two-way) were employed to compare normoxic ventilation, pHVR over 1-min, and HVD. Significant comparisons were followed by Newman-Keuls tests. A P value <0.05 was considered to achieve statistical significance.

Immunoblot analysis. Rats were exposed to room air or to 1 h, 6 h, 1 day, 3 days, 7 days, 14 days, and 30 days of either IH or SH and were euthanized with a pentobarbital overdose. The skull was rapidly opened, and the brain was extracted, immediately placed on dry ice, and dissected under surgical microscopy. The obex was visually identified, and a coronal section 1.5 mm caudal to 1.5 mm rostral to the obex was performed. The dorsal half of this brain stem section was carefully removed, snap frozen in liquid nitrogen, and kept at −80°C until analysis. Tissues were homogenized at 0°C with a tissue blender in 20 mM Tris-HCl buffer (pH 7.5), containing 2 mM EDTA, 0.5 mM EGTA, 25 µg/ml leupeptin, 25 µg/ml apronitin, and 1 mM PMSF. The homogenate was centrifuged at 10,000 g at 4°C to remove cell debris. Protein content was measured in each soluble fraction by using the Bradford method (DC-Bio-Rad protein assay), and samples were frozen at −80°C until analysis. Proteins (75 µg/sample) were subjected to SDS-PAGE (8% acrylamide gel) and transferred on a 0.2 µm nitrocellulose membrane. Membranes were blocked for 1 h in a 5% non-fat dry milk solution in TBS-Tween. After overnight incubations with antibodies to NR1, NR2A, and NR2B, membranes were washed and incubated for 1 h with a horseradish peroxidase-labeled goat anti-mouse antibody (1:20,000; Kirkegard & Perry Laboratorios, Gaithersburg, MD). In initial experiments, a control
lysate provided for each NMDA receptor subunit was included. The concentrations of the NMDA receptor antibodies were as follows: NR1 (Chemicon, Temecula, CA, cat. no. AB1516, 1:500); NR2A (Upstate Biotechnology, Waltham, MA, cat. no. 06-313, 1:250); NR2B (Upstate Biotechnology, cat. no. 06-600, 1:250). Proteins were visualized by enhanced chemiluminescence (ECL, Amersham), and semiquantitative analysis of NR subunit bands was performed by scanning densitometry. In addition, blots were reprobed with a monoclonal antibody against β-actin for further standardization of loading inequalities. At least five different experiments were conducted for each time point. To normalize data across experiments, densitometric values for each time point were expressed as a ratio in which either the β-actin or the NR1 densitometric readings served as the denominator.

Data analysis. All values are shown as means ± SE unless indicated otherwise. Differences in data among the three experimental groups were compared by two-way ANOVA for repeated measures and the Newman-Keuls test, or by paired t-tests as appropriate. A P value of <0.05 was considered to achieve statistical significance.

RESULTS

Normoxic ventilation. SH-exposed rats had lower body weights than rats exposed to IH and normoxic room air (RA). Therefore, all ventilatory measurements were corrected for body weight as appropriate. Normoxic ventilation was significantly greater in IH- and SH-exposed rats compared with RA rats at the end of the 30-day exposure (Fig. 1; P < 0.001). The mean values of VT, f, VE, VE/TI, VE/Ttot, VO2, and VE/O2 are shown in Table 1 for all three groups. Significant differences emerged in respiratory strategies between hypoxia-exposed animals and controls, as well as between IH and SH. Indeed, VT was not affected by IH, such that the increases in VE were exclusively due to increased f. This is in contrast with the similar increase in ventilation in SH-exposed rats that was due to balanced contributions from both VT and f. There were no significant differences in VO2 among the three groups; however, both IH and SH-exposed rats displayed significantly increased ventilatory equivalents (Table 1; P < 0.001).

After 30 days of recovery, changes in normoxic ventilation resolved in both IH and SH, with VT and f values also becoming similar to those measured in RA [P value not significant (NS)]. There were no differences in VO2 in all three groups after recovery.

Hypoxic ventilatory responses. All animals in the three experimental groups displayed ventilatory increases on application of the hypoxic gas (Fig. 1; Table 2). However, pHVR was significantly higher in SH-exposed rats compared with either IH or RA (Table 2; Fig. 1; P < 0.001). RA rats increased ventilation by ~100% and SH-exposed rats by ~170% compared with their respective baseline normoxic ventilation. However, even though IH-exposed raised their ventilatory output in response to acute hypoxia, the magnitude of this increase was only 55% (P < 0.001 ANOVA). The relative contributions of VT and f to these overall ventilatory changes is shown in Table 2. Briefly, SH-exposed rats demonstrated a preferential increase in f that accounted for the markedly greater overall pHVR in these animals. Oxygen consumption increased to similar levels in all three groups during pHVR (Tables 1 and 2), but the ventilatory equivalents were preferentially increased in SH-exposed rats (Table 2).

HVD was markedly attenuated after IH exposures (P < 0.001 vs. RA), whereas SH did not modify the magnitude of HVD (Table 3, Fig. 1; P value NS vs. RA). During HVD, the ventilatory decline in SH-exposed

Table 1. Ventilatory measurements during normoxia in rats exposed to IH, SH, or RA

<table>
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<tr>
<th></th>
<th>RA</th>
<th>IH</th>
<th>SH</th>
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<tbody>
<tr>
<td>VT,m/g body wt</td>
<td>0.0052 ± 0.00030</td>
<td>0.0051 ± 0.00026</td>
<td>0.0066 ± 0.0004*</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>101.2 ± 4.6</td>
<td>155.7 ± 13.7†</td>
<td>121.5 ± 3.1*</td>
</tr>
<tr>
<td>VE, m/min ¹-g body wt ⁻¹</td>
<td>0.52 ± 0.01</td>
<td>0.79 ± 0.04†</td>
<td>0.89 ± 0.05†</td>
</tr>
<tr>
<td>VE/TI, m/min ¹-s ⁻¹</td>
<td>0.022 ± 0.001</td>
<td>0.035 ± 0.002†</td>
<td>0.038 ± 0.003†</td>
</tr>
<tr>
<td>VE/Ttot</td>
<td>0.35 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>VO₂,m/min ¹-kg⁻¹</td>
<td>17.6 ± 2.26</td>
<td>18.1 ± 1.06</td>
<td>18.8 ± 1.41</td>
</tr>
<tr>
<td>VO₂/O₂</td>
<td>29.5 ± 2.5</td>
<td>43.6 ± 3.1†</td>
<td>42.5 ± 2.3‡</td>
</tr>
<tr>
<td>Weight, g</td>
<td>415.8 ± 11.7</td>
<td>411.1 ± 11.8</td>
<td>357.8 ± 18.3‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15 rats/group. When appropriate, values have been corrected for body weight. IH, intermittent hypoxia; SH, sustained hypoxia; RA, normoxic room air; VT, tidal volume; f, respiratory frequency; VE, minute ventilation; VE/TI, mean inspiratory flow; VE/Ttot, inspiratory duty cycle; VO₂, O₂ uptake. *P < 0.01 vs. RA; †P < 0.001 vs. RA.
rats was again primarily ascribable to decreases in respiratory rate. In IH-exposed animals, HVD was not readily apparent (pHVR vs. HVD: P value NS) because of sustained and even incremental increases in f (Table 3; Fig. 1). When ventilatory changes were normalized for preceding pHVR, HVD was greatest in SH- and smallest in IH-exposed animals (P < 0.001, ANOVA). Interestingly, V\textsubscript{O\textsubscript{2}} was similar in SH and RA but not reduced in IH (P < 0.001). Thus ventilatory equivalents during HVD were higher in IH and similar in RA and SH (P < 0.01; Table 3).

**NMDA glutamate receptor expression.** IH and SH induced mild, albeit significant, early changes in the expression of NMDA receptors (NR1 subunit) (Fig. 2). However, such changes had resolved by 30 days of exposure in both groups. For both NR2A and NR2B subunits of the NMDA receptor, IH induced significant increases in expression that did not occur in SH-exposed rats (Fig. 2).

**DISCUSSION**

In this study we have shown that IH and SH induce fundamentally different alterations in the characteristics of the HVR and that some of these differences may be related to the divergent changes in NMDA receptor subtype expression within the dorsocaudal brain stem. Before we address the major issues revealed by these experiments, some technical issues need to be addressed. First, on the basis of prior experiments using similar exposures, it is unlikely that the experimental hypoxic profiles were associated with significant disruption of the sleep-wake cycle (21) or that such disruption, even if minimal, may have substantially modified the intrinsic properties of respiratory controllers (6). Indeed, the effects of sleep deprivation on control of breathing appear to be modest and clearly not of the magnitude uncovered herein (47, 48). Second, it is important to emphasize that, by virtue of the nature of the hypoxic profiles, animals exposed to SH spent twice as much time in hypoxia compared with IH. Notwithstanding this issue, the long period of exposure (30 days) should have allowed for full expression of any adaptive mechanisms that may have been triggered by either one of the two experimental paradigms. In addition, because SH was associated with significant weight reductions compared with either IH or RA, ventilatory measures were corrected for body weight, and metabolic measurements using indirect calorimetric approaches were also incorporated to distinguish between ventilatory and metabolic adaptations potentially triggered by the acute hypoxic challenge. Finally, it needs to be emphasized that our tissue-sampling approach may be associated with relative variability in sample homogeneity and that the NMDA glutamate receptor expression findings do not differentiate among the various nuclei and cell types that populate the dorsocaudal brain stem. Indeed, astrocytes, glial cells, microglia, and various types of neurons will all be represented in the protein lysates.

Increased normoxic ventilation occurred in both IH and SH. However, there were significant differences in the ventilatory strategy adopted by these animals. Indeed, although IH and SH exhibited similarly enhanced overall ventilation, IH-exposed rats preferentially increased their respiratory rate, whereas both VT and frequency contributed to the heightened ventila-

<p>| Table 2. Ventilatory measurements during peak hypoxic ventilatory response in the course of a 20-min acute hypoxic challenge in rats exposed to IH, SH, or RA |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>IH</th>
<th>SH</th>
</tr>
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<tbody>
<tr>
<td>VT, ml/g body wt</td>
<td>0.0058 ± 0.0003</td>
<td>0.0070 ± 0.0003*</td>
<td>0.0057 ± 0.0005†</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>177.1 ± 22.7</td>
<td>176.1 ± 22.3</td>
<td>297.4 ± 43.7†</td>
</tr>
<tr>
<td>V\textsubscript{E}, ml/min/S·g body wt</td>
<td>1.03 ± 0.08</td>
<td>1.23 ± 0.15*</td>
<td>2.22 ± 0.35†</td>
</tr>
<tr>
<td>VT/Ti, ml/g body wt S·1·s</td>
<td>0.038 ± 0.004</td>
<td>0.061 ± 0.005†</td>
<td>0.077 ± 0.011†</td>
</tr>
<tr>
<td>Ti/Ttot</td>
<td>0.39 ± 0.01</td>
<td>0.34 ± 0.01*</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>V\textsubscript{O\textsubscript{2}}, ml/min/S·kg</td>
<td>24.7 ± 2.1</td>
<td>27.4 ± 2.5</td>
<td>28.6 ± 2.3</td>
</tr>
<tr>
<td>VT/V\textsubscript{O\textsubscript{2}}</td>
<td>41.7 ± 5.8</td>
<td>44.8 ± 3.9</td>
<td>77.8 ± 5.7†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15 rats/group. When appropriate, values have been corrected for body weight. *P < 0.01 vs. RA; †P < 0.001 vs. RA.

<p>| Table 3. Ventilatory measurements during hypoxic ventilatory decline in the course of a 20-min acute hypoxic challenge in rats exposed to IH, SH, or RA |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>IH</th>
<th>SH</th>
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<tbody>
<tr>
<td>VT, ml/g body wt</td>
<td>0.0048 ± 0.0002</td>
<td>0.0048 ± 0.0003</td>
<td>0.0058 ± 0.0003†</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>134.6 ± 9.6</td>
<td>221.4 ± 28.2†</td>
<td>128.8 ± 7.7</td>
</tr>
<tr>
<td>V\textsubscript{E}, ml/min/S·g body wt</td>
<td>0.65 ± 0.09</td>
<td>1.07 ± 0.15†</td>
<td>0.74 ± 0.04*</td>
</tr>
<tr>
<td>VT/Ti, ml/g S·1·s</td>
<td>0.021 ± 0.001</td>
<td>0.046 ± 0.006†</td>
<td>0.035 ± 0.002*</td>
</tr>
<tr>
<td>Ti/Ttot</td>
<td>0.30 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>V\textsubscript{O\textsubscript{2}}, ml/min/S·kg</td>
<td>20.2 ± 2.7</td>
<td>28.9 ± 3.4†</td>
<td>21.7 ± 2.4</td>
</tr>
<tr>
<td>VT/V\textsubscript{O\textsubscript{2}}</td>
<td>32.2 ± 3.3</td>
<td>37.0 ± 4.1*</td>
<td>34.1 ± 3.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15 rats/group. When appropriate, values have been corrected for body weight. *P < 0.01 vs. RA; †P < 0.001 vs. RA.
tion measured in SH-exposed rats. These findings, which were completely reversed by a month of normoxic recovery, would suggest that the adaptive mechanisms recruited by IH and SH leading to enhanced ventilatory drives are intrinsically different and would primarily involve neural regions underlying respiratory rhythm in IH. As further confirmatory evidence of the relative hyperventilation induced by the hypoxic exposures independent of the type of hypoxic exposure, $\dot{V}E/\dot{V}O_2$ ratios were markedly higher in these animals during normoxia. Our findings are in close agreement with those previously reported for SH in the rat (1, 16, 36, 43) and further indicate that chronic intermittent hypoxia, although inducing similar overall hyperventilation to those elicited by long-term exposures to SH, differs from the latter by recruiting different VT-f relationships, thereby suggesting that different neurotransmitters may be involved. Thus the putative role of tyrosine hydroxylase and norepinephrine (44), dopamine D2 receptors (13, 25), and platelet-derived growth factor-β receptors (2) in the ventilatory acclimatization to IH will need to be explored in future studies.

Acute exposure to hypoxia resulted in significant increases in ventilation in all three experimental groups. However, the magnitude of the absolute ventilatory increase in SH-exposed rats was markedly greater and was achieved through frequency increases, leading to substantial hyperventilation as shown by $\dot{V}E/\dot{V}O_2$. This is further reinforced by the possibility that the ventilatory increases occurring in SH would reflect an even greater hypoxic drive, considering that for an assumed constant dead space, the $PCO_2$ would have been lower after SH compared with IH. This greatly contrasts with the absence of any increase in $\dot{V}E/\dot{V}O_2$ among the IH-exposed rats, suggesting that although long-term exposures to IH lead to increased ventilatory drive during normoxia, and even further increase during acute hypoxia (as evidenced from VT/Ti increases), they are not accompanied by commensurate changes in metabolic expenditure, suggesting that early adaptations in metabolic regulation during an acute hypoxic challenge are less well adaptively regulated after IH exposures compared with SH exposures. The mechanisms underlying the differences in the ventilatory and metabolic adaptations are currently unknown and raise intriguing possibilities. Indeed, the current conceptual framework assigns to hypoxia the induction of a balanced suppression of ATP-demand and ATP-supply pathways. For survival, these pathways will be maintained at critical levels during prolonged hypoxia by preferential regulation of the expression of several proteins in a context of decreased protein translation. The initiation of this process requires $O_2$ sensing and downstream activation of signal-transduction pathways, leading to significant gene-protein induction and metabolic reprogramming, i.e., functional adaptation (5, 23). It is therefore possible that either the same adaptive mechanisms are activated by IH and SH but at different magnitude and time course, that different adaptive mechanisms are recruited, or that both possibilities may coexist and depend on the functional component being examined. One of these possibilities, i.e., altered expression of...
NMDA glutamatergic transmission, was examined in this study, and the findings suggest that changes in NMDA receptor subtypes 2A and 2B may contribute to aforementioned differences between IH and SH.

It has been postulated that SH leads to blunting of the HVR (31). However, this has not been consistently observed across mammalian species. Indeed, Aaron and Powell (1) showed that chronic exposures to 7 wk of SH induced increased pHVR in rats, and more recently the same laboratory showed that the increased HVR was mediated, at least in part, by changes in dopamine receptor expression (12, 13). Our findings in the SH group are in close agreement with these findings. Interestingly, however, IH-exposed rats showed relatively blunted pHVR responses compared with SH. This was somewhat unexpected, because previous work using short-term IH exposures revealed serotonin-dependent long-term facilitation of central respiratory output (18, 29) and also evidence for SH-induced potentiation of HVR in the developing rat through increased neuronal nitric oxide synthase enzyme activity (20). However, both of these two IH paradigms were short lasting. It is therefore possible that long-lasting IH may result in biphasic modulation of pHVR, whereby pHVR will be enhanced during the early stages of IH, and that this enhancement will be followed by progressive attenuation of pHVR compared with SH exposures.

In this study we found significant differences in the ability to sustain ventilation during the late phase of the acute ventilatory response among the three experimental groups. On average, HVD corresponded to VE values that were reduced by 35, 12, and 66% compared with corresponding preceding pHVR in RA-, IH-, and SH-exposed rats, respectively (P < 0.001, ANOVA). Thus IH reduced HVD, whereas SH enhanced HVD during an acute poikilocapnic hypoxic challenge. The effects of brain hypoxia on the control of breathing have been the object of many previous studies and are believed primarily to promote ventilatory decline (4). Such depressant effects, which are consistently observed in anesthetized preparations in the absence of peripheral chemoreceptor stimulation, have been advanced to explain the biphasic nature of the ventilatory response to hypoxia observed in intact animals. However, other studies have found that, in unanesthetized goats, isolated brain hypoxia induced either by environmental hypoxia after peripheral chemodenervation or by systemic hypoxia and selective carotid perfusion with normoxic blood characteristically results in hyperventilation (46). Furthermore, when the carotid bodies are concomitantly stimulated by hypoxia, the resulting increase in metabolic rate is similar, independent of whether the brain is hypoxic or normoxic (9, 43). Although extrapolation from large animals, such as goats, to smaller mammals, such as rats, is not always possible, because response patterns to hypoxia greatly differ among these two species, our VE/V02 findings during HVD in the normoxic control rats would support the presence of a relative hyperventilation during HVD. However, CO2 levels were not measured, and this precludes definitive conclusions. Notwithstanding such limitation, V02/V02 was slightly, albeit significantly, higher during HVD compared with baseline in normoxic rats. However, in IH-exposed rats, V02 failed to decrease over time after peak increases, suggesting that acute SH ultimately leads to differential metabolic responses and consequently ventilatory recruitments in rats subjected to long-term exposures to either IH or SH. It could be further postulated that the ability to induce reductions in metabolic costs is advantageous for survival and confers tolerance to hypoxia (5, 22–24). This is best exemplified by the increased hypoxic tolerance of most neonatal mammalian species as well as that of nonmammalian species, because an important common denominator to their increased tolerance to hypoxia consists in the effective downregulation of metabolism during hypoxia (7, 22, 24, 26). In this scenario, SH and RA animals behaved similarly, whereas IH-exposed rats did not reduce their V02 during the late phase of acute hypoxia. On the other hand, the relative upregulation of ventilation relative to V02 in SH-exposed rats during pHVR may indicate that the adaptive mechanisms to hypoxia triggered by this stimulus are less efficient during early phases of acute hypoxic exposure and could lead substantial morbidity under selected circumstances (5, 8).

We are unaware of similar studies on NMDA receptor changes with hypoxia in the literature, and the mechanistic implications of such expression changes in the dorsocaudal brain stem were obviously not elucidated in the present study. Studies employing in situ hybridization and immunocytochemical techniques have shown that NR1 subunits are ubiquitously distributed throughout the central nervous system whereas the four NR2 subunits display differential expression patterns in several mammalian species including humans (33, 39, 42). In general, within the cerebral cortex, hippocampus, and cerebellum, NMDA receptors are mainly distributed in neuronal cell bodies and dendrites. NR1 and NR2 are also found intracellular membranes such as mitochondria and rough endoplasmic reticulum whereas in synapses they appear to be limited to the postsynaptic membrane and density (33, 45). Our laboratory has previously shown that NMDA glutamate receptors are critical for HVR in the adult rat (34) and that these receptors are densely expressed in the dorsocaudal brain stem, where they undergo substantial expression changes during postnatal development (35). However, the effect of hypoxia on these receptors has only been summarily examined. Indeed, Pichiu and colleagues (40) showed that mild hypobaric hypoxia resulted in decreased [3H]MK-801 binding and affinity to the NMDA receptor in membranes prepared from cerebral cortex, hippocampus, and corpus striatum of juvenile (3-wk-old) rats, and, in fact, exposure of young neonatal rats to a short severe hypoxic challenge was associated with long-lasting alterations in the modulation of NMDA receptors to glycine and glutamate (37). Furthermore, repeated brief hypoxic exposures of hippocampal slices were associated with the generation of a hyperexcitabile
state that was blocked by administration of the NMDA receptor antagonist (+/−)-2-amino-5-phosphonopentanoic acid (17). We postulate that the differences in NR2A and NR2B NMDA receptor subtype expression following IH and SH may underlie some of the functional differences discussed above.

In summary, long-term IH and SH are associated with similar increases in normoxic ventilation in the adult male rat, albeit mediated by different VT-f relationships. Furthermore, they induce discrepant alterations in the temporal and magnitude characteristics of ventilatory and metabolic responses to an acute hypoxic challenge that coincide with differential relationships. Furthermore, they induce discrepant alterations in the expression of NMDA glutamate receptor subunits within the dorsocaudal brain stem. We postulate that long-term IH is associated with differential induction of survival mechanisms compared with SH, and in selected circumstances these phenomena could lead to increased vulnerability.

DISCLOSURES

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