Adult carotid chemoafferent responses to hypoxia after 1, 2, and 4 wk of postnatal hyperoxia

G. E. Bisgard,1 E. B. Olson Jr.,2 Z.-Y. Wang,3 R. W. Bavis,4 D. D. Fuller,1 and G. S. Mitchell1
Departments of 1Comparative Biosciences, 2Population Health Sciences, and 3Surgical Sciences, University of Wisconsin, Madison, Wisconsin 53706

Submitted 24 October 2002; accepted in final form 12 May 2003

Bisgard, G. E., E. B. Olson Jr., Z.-Y. Wang, R. W. Bavis, D. D. Fuller, and G. S. Mitchell. Adult carotid chemoafferent responses to hypoxia after 1, 2, and 4 wk of postnatal hyperoxia. J Appl Physiol 95: 946–952, 2003; 10.1152/japplphysiol.00985.2002.—Exposing newborn rats to postnatal hyperoxia (60% O2) for 1–4 wk attenuates the ventilatory and phrenic nerve responses to acute hypoxia in adult rats. The goal of this research was to increase our understanding of the carotid chemoreceptor afferent neural input in this depressed response with different durations of postnatal hyperoxic exposure. Rats were exposed from a few days before birth to 1, 2, or 4 wk of 60% O2 and studied after 3–5 mo in normoxia. The rats were anesthetized with urethane. Whole carotid sinus nerve (CSN) responses to NaCN (40 μg/kg iv), 10 s of asphyxia and acute isocapnic hypoxia (arterial PO2 45 Torr) were determined. Mean CSN responses to stimuli after postnatal hyperoxia were reduced compared with controls. Responses in rats exposed to 1 wk of postnatal hyperoxia were less affected than those exposed to 2 and 4 wk of hyperoxia, which were equivalent to each other. These studies illustrate the importance of normoxia during the first 2 wk of life in development of carotid chemoreceptor afferent function.

asphyxia; sodium cyanide; carotid body development

HYPOXIA (60% O2) FOR THE FIRST 1–4 WK OF LIFE SIGNIFICANTLY ATTENUATES VENTILATORY AND PHRENIC NERVE RESPONSES TO ACUTE HYPOXIA IN ADULT RATS (2, 7, 14). FUNCTIONAL IMPAIRMENT IS PERMANENT IN RATS EXPOSED TO 4 WK OF POSTNATAL HYPOXIA (7). THE IMPAIRED RESPONSE IS ASSOCIATED WITH DEPRESSED DEVELOPMENT OF THE CAROTID BODY (CB) (7, 14) AND ATTENUATED CAROTID CHEMOAFFERENT FUNCTION, AS ASSESS BY WHOLE CAROTID SINUS NERVE (CSN) RECORDING. FOR EXAMPLE, CSN NEURAL DISCHARGES IN RESPONSES TO INTRAVENOUS NaCN AND TO ASPHYXIA (ACUTE HYPERCAPNIC HYPOXIA) ARE GREATLY REDUCED IN ADULT RATS SUBJECTED TO POSTNATAL HYPOXIA (7, 14). IMPAIRED DEVELOPMENT IS SUGGESTED BY THE OBSERVATION THAT CB VOLUME IS ONLY ~30–35% OF NORMAL IN ADULT RATS AFTER POSTNATAL HYPOXIA (7, 8). IN ADDITION, AFFERENT INNERVATION OF THE CB IS REDUCED IN NEONATAL RATS AFTER POSTNATAL HYPOXIA (E.G., THE NUMBER OF UNMYELINATED CSN AFFERENT AXONS IS 41% LESS THAN NORMAL IMMEDIATELY AFTER 4 WK OF POSTNATAL HYPOXIA) (6). REDUCED NUMBERS OF CSN AXONS HAVE NOT BEEN CONFIRMED IN ADULT RATS AFTER POSTNATAL HYPOXIA, BUT THE EFFECT IS LIKELY TO PERSIST BECAUSE AFFERENT NEURAL DEVELOPMENT IS NOT EXPECTED AFTER 4 WK OF AGE. THESE FUNCTIONAL AND MORPHOLOGICAL CHANGES ARE NOT OBSERVED IN ADULT RATS SUBJECTED TO 4 WK OF SIMILAR HYPOXIA (14).

CB DEVELOPMENT PROMINENTLY TAKES PLACE OVER THE FIRST 2 WK OF LIFE IN RATS (1, 5, 12, 19). IN ACCORDANCE, POSTNATAL HYPOXIA IN THE FIRST OR SECOND WEEK OF LIFE ATTENUATES PHRENIC NERVE RESPONSES TO ACUTE HYPOXIA IN ADULT RATS TO AN EXTENT EQUIVALENT TO 4 WK OF POSTNATAL HYPOXIA (2). STUDIES OF THE CAROTID CHEMOAFFERENT NEURAL RESPONSES (RECORDED FROM THE CSN) IN ADULT RATS SUBJECTED TO POSTNATAL HYPOXIA HAVE BEEN LIMITED TO RATS EXPOSED TO 4 WK OF POSTNATAL HYPOXIA, AND ONLY RESPONSES TO NaCN (20 μg iv) AND ASPHYXIA HAVE BEEN DETERMINED (7, 14). TO BETTER UNDERSTAND THE CONSEQUENCES OF POSTNATAL HYPOXIA ON CAROTID CHEMOAFFERENT FUNCTION, WE EXAMINED CSN RESPONSES TO NaCN, ASPHYXIA, AND ACUTE ISOCAPNIC HYPOXIA IN ADULT RATS AFTER EXPOSURE TO 1, 2, AND 4 WK OF POSTNATAL HYPOXIA. THE RESULTS INDICATE THAT THE DURATION OF HYPOXIA FROM BIRTH DETERMINES ADULT CAROTID CHEMOAFFERENT RESPONSES TO ACUTE HYPOXIC STIMULI.

METHODS

Experimental procedures used were approved by the Animal Care Committee of the School of Veterinary Medicine of the University of Wisconsin-Madison.

Animal model. Pregnant female Harlan Sprague-Dawley rats (strain 236b, Harlan Sprague Dawley, Madison, WI) were placed in 60% O2-balance N2 in the last 2–4 days of pregnancy. The females were maintained in the hyperoxic chamber through parturition with their litters and for 1, 2, or 4 wk postpartum. After hyperoxic exposures, the rats were returned to air until adulthood (3–5 mo of age). Acute neurophysiological studies were then carried out on these animals. Only males were retained for study to avoid potential female hormonal effects on carotid chemoafferent function (15). Age-matched control, normoxic rats were obtained from parallel groups of pregnant females kept under similar conditions but not exposed to hyperoxia. Body weights at the time of study were 436 ± 21 (SE) g (n = 17) for normoxic

946

8750-7587/03 $5.00 Copyright © 2003 the American Physiological Society http://www.jap.org

Address for reprint requests and other correspondence: G. E. Bisgard, Dept. of Comparative Biosciences, Univ. of Wisconsin-Madison, 2015 Linden Dr., Madison, WI 53706 (E-mail: bigardg@svm.vetmed.wisc.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
controls vs. 396 ± 21 g (n = 28) for postnatal hyperoxia-treated rats (not significantly different, P = 0.21).

**Animal preparation.** Rats were placed in a chamber containing isoflurane (~3% in air) for rapid induction of anesthesia. When the animals were unconscious, 0.33 M urethane was administered intraperitoneally (1 g/kg). Supplementary anesthetic (2 ml 0.33 M urethane) was given as needed if surgical anesthesia had not been reached after 5 min. Depth of anesthesia throughout the surgical procedures was determined by toe pinch reflex assessment. After surgical preparation and during paralysis for neural recording, anesthesia was assessed by arterial blood pressure response to toe pinch. An increase in arterial blood pressure during toe pinch indicated the need for additional anesthetic. When surgical anesthesia was achieved, the femoral artery and vein were cannulated for arterial pressure recording, arterial blood sampling, and intravenous administration of drugs and solutions. Body temperature was monitored by rectal thermometer, and body temperature was maintained near 37°C with a warm water-circulated blanket. An endotracheal cannula was inserted, and the animals were artificially ventilated with a Harvard Apparatus, Wallingford, CT) and maintained near 40 Torr. Throughout surgical preparation, the animals were ventilated with 25% FiO2/H11011 ushane (2.5 mg/kg iv). Raw nerve activity was amplified with pancuronium (2.5 mg/kg iv). Raw nerve activity, discharge frequency, and arterial blood pressure were continuously recorded with a rapid-responding flow-through analyzer (Noavametrics, Wallingford, CT) and maintained near 40 Torr. Throughout surgical preparation, the animals were ventilated with 25–30% O2 to maintain slightly hyperoxic arterial blood [arterial PO2 (PaO2) = 110–130 Torr].

**CSN recording.** The CSN was exposed via ventral cervical midline incision. It was dissected to its junction with the glossopharyngeal nerve and cut at the junction. The nerve was carefully cleaned and placed on tungsten electrodes for bipolar whole nerve recording. The animal was then paralysed with pancuronium (2.5 mg/kg iv). Raw nerve activity was amplified (>10,000), monitored, and recorded on a personal computer. A window discriminator (model WD-1000) and ratemeter (model RIC-1000, CWE, Ardmore, PA) were used to obtain the discharge rate in 1-s bins [allowing determination of peak and average discharge rate (Hz)]. The data were recorded on a personal computer using commercial software (WINDAQ, Dataq, Akron, OH) and included raw nerve activity, discharge frequency, and arterial blood pressure. The investigator carrying out experiments and measuring records was blinded as to the treatment group (control vs. hyperoxia treatment groups).

Baseline discharge rate at a PaO2 of ~100 Torr was arbitrarily set near 300 Hz by using the window discriminator as described by Ling et al. (14). This was done realizing that this baseline activity contains chemoreceptor activity, baroreceptor activity (in some cases), and electrical noise. There was no baseline activity contains chemoreceptor activity, baroreceptor activity (in some cases), and electrical noise. There was no baseline activity was amplified (>10,000), monitored, and recorded on a personal computer. A window discriminator (model WD-1000) and ratemeter (model RIC-1000, CWE, Ardmore, PA) were used to obtain the discharge rate in 1-s bins [allowing determination of peak and average discharge rate (Hz)]. The data were recorded on a personal computer using commercial software (WINDAQ, Dataq, Akron, OH) and included raw nerve activity, discharge frequency, and arterial blood pressure. The investigator carrying out experiments and measuring records was blinded as to the treatment group (control vs. hyperoxia treatment groups).

Baseline discharge rate at a PaO2 of ~100 Torr was arbitrarily set near 300 Hz by using the window discriminator as described by Ling et al. (14). This was done realizing that this baseline activity contains chemoreceptor activity, baroreceptor activity (in some cases), and electrical noise. There was no reduction in discharge frequency when PaO2 was raised from near 100 Torr with 100% O2 inhalation; thus we could not use this maneuver to define the baseline normoxic discharge frequency. Therefore, we elected to examine the increase in CSN discharge frequency with acute stimuli as a measure of carotid chemaofaferent function. Retrospectively, we have reexamined our data to determine whether our selected baseline (300 Hz) would be appropriate for obtaining the maximal change in CSN discharge frequency with acute hypoxic stimuli. This was done by inserting digital data (WINDAQ Program) representing the same recorded control baseline and acute responses previously measured into an Excel program. This enabled us to set baseline discharge rate to any level and determine which baseline discharge rate produces the maximum value of the response in question. We discovered that the use of baseline values other than 300 Hz changed neither any statistical differences we found between treat-
The tissues were embedded on dry ice with OCT compound and sectioned with a cryostat at 10 μm. Approximately 150 sections were cut. To identify the sections containing CB, every fifteenth section was selected and stained with hematoxylin and eosin and examined under the microscope. For those serial sections containing CB, every fourth section was processed with hematoxylin and eosin staining, and digital photoimages (×200) were imported into the computer by using a Spot digital camera. The CB area was then outlined and measured by using the Image-Pro Plus computer software (Media Cybernetics, Silver Spring, MD). Calibration was made by using a micrometer. CB volume was estimated on the basis of the CB area in each section, section thickness, and the total number of sections containing CB.

Data analysis. Means ± SE were obtained for each treatment. Mean responses between groups (normoxic and 1, 2, and 4 wk of postnatal hyperoxia treatment) were subjected to one-way ANOVA with the Tukey-Kramer post hoc test. A value of $P < 0.05$ was considered significant.

RESULTS

**NaCN response.** CSN responses to intravenous NaCN in control rats were brisk, reaching a mean maximal increase in frequency of $363 ± 25$ Hz ($n = 17$) (Fig. 1). The response to NaCN was not significantly depressed by previous treatment with 1 wk of perinatal hyperoxia. However, mean responses of the 2- and 4-wk postnatal hyperoxia groups were significantly lower than the control group but were not different from each other (Fig. 2). Arterial blood gases, acid-base, and blood pressure values were similar for each experimental group (Table 1).

**Asphyxia response.** Responses to asphyxia are shown in Fig. 3. A similar pattern as with responses to NaCN was found; i.e., the response was attenuated more with longer durations of postnatal hyperoxia (Fig. 4). The

![Fig. 1](http://jap.physiology.org/)

**Fig. 1.** A: example of the response of the carotid sinus nerve (CSN) to intravenous NaCN (40 μg/kg) in a normal control animal. B: effects of a saline flush in the same animal as shown in A with the same total volume as when NaCN was injected. C: response in an adult rat that had been treated with 2 wk of postnatal hyperoxia. Top trace, raw whole nerve activity; middle trace, arterial blood pressure (Art BP); bottom trace, ratemeter output in 1-s bins. Note that the injection of NaCN caused an initial increase in BP with minimal effect on frequency of discharge.

![Fig. 2](http://jap.physiology.org/)

**Fig. 2.** Change in CSN activity to intravenous injection of NaCN (40 μg/kg) in adult animals. Values are means ± SE. *Mean significantly different from control, $P < 0.001$. † Mean significantly different from 1-wk group mean, $P < 0.05$.  

---

498 POSTNATAL HYPOXIA AND ADULT ARTERIAL CHEMORECEPTION  

J Appl Physiol • VOL 95 • SEPTEMBER 2003 • www.jap.org
mean discharge frequency increase with asphyxia was 225 ± 14 Hz (n = 17) in control rats. All values in hyperoxia-treated rats were significantly below control rats, and all groups studied were significantly different from each other except the 2- and 4-wk postnatal hyperoxia groups. Arterial blood gas, acid-base, and blood pressure values were similar for each experimental group (Table 1).

**Acute hypoxia response.** Again the responses were similar to those found with asphyxia and NaCN (Fig. 5). The mean change in discharge frequency with acute hypoxia was 168 ± 14 Hz (n = 14) in control rats. Mean values for all postnatal hyperoxia groups were significantly lower than the control group; however, none of the postnatal hyperoxia groups were significantly different from each other. Arterial blood gas, acid-base, and blood pressure data are shown in Table 2.

**CB volume.** Four weeks of postnatal hyperoxia causes hypoplasia of the CB (7, 8). The morphology of the resulting small CB appears to be otherwise normal (8). We now show that 1 wk of postnatal hyperoxia also causes carotid body hypoplasia (Table 3). CB volume

### Table 1. Arterial blood-gas, pH, and blood pressure values during NaCN and asphyxia responses

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 wk Hyperoxia</th>
<th>2 wk Hyperoxia</th>
<th>4 wk Hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NaCN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pao2, Torr</td>
<td>108 ± 3</td>
<td>110 ± 3</td>
<td>108 ± 5</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>Paco2, Torr</td>
<td>38.5 ± 0.5</td>
<td>39.4 ± 0.9</td>
<td>39.3 ± 0.7</td>
<td>39.1 ± 0.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.01</td>
<td>7.40 ± 0.01</td>
<td>7.40 ± 0.01</td>
<td>7.39 ± 0.01</td>
</tr>
<tr>
<td>Systolic BP, Torr</td>
<td>158 ± 6</td>
<td>162 ± 7</td>
<td>155 ± 23</td>
<td>149 ± 8</td>
</tr>
<tr>
<td>Diastolic BP, Torr</td>
<td>103 ± 4</td>
<td>96 ± 5</td>
<td>105 ± 6</td>
<td>99 ± 6</td>
</tr>
<tr>
<td><strong>Asphyxia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pao2, Torr</td>
<td>103 ± 3</td>
<td>101 ± 3</td>
<td>109 ± 4</td>
<td>107 ± 4</td>
</tr>
<tr>
<td>Paco2, Torr</td>
<td>37.9 ± 0.6</td>
<td>39.4 ± 0.9</td>
<td>38.8 ± 0.7</td>
<td>39.1 ± 0.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.01</td>
<td>7.40 ± 0.01</td>
<td>7.41 ± 0.01</td>
<td>7.39 ± 0.01</td>
</tr>
<tr>
<td>Systolic BP, Torr</td>
<td>153 ± 6</td>
<td>158 ± 9</td>
<td>161 ± 9</td>
<td>148 ± 11</td>
</tr>
<tr>
<td>Diastolic BP, Torr</td>
<td>103 ± 5</td>
<td>91 ± 5</td>
<td>117 ± 10</td>
<td>106 ± 11</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are means ± SE; n; no. of animals. Pao2, arterial Po2; Paco2, arterial PCO2; BP, blood pressure.

Fig. 3. Examples of response of the CSN to 10 s of asphyxia in a normal control adult rat (A), an adult rat that had been treated with 2 wk of postnatal hyperoxia (B), and an adult rat that had been treated with 4 wk of postnatal hyperoxia (C). Top trace, raw whole nerve activity; middle trace, arterial blood pressure; bottom trace, ratemeter output in 1-s bins.

Fig. 4. Change in CSN activity to 10 s of asphyxia in adult animals. Values are means ± SE. *Mean significantly different from control, P < 0.01. **Mean significantly different from 1-wk group mean, P < 0.01.
was similar after either 1 or 4 wk of postnatal hyperoxia (30–35% of control).

**DISCUSSION**

Carotid chemosensory responses to acute stimuli are diminished in adult rats after the first 1, 2, or 4 wk of postnatal hyperoxia. The impaired responses appear to depend on the duration of postnatal hyperoxia from birth, but in no case were the responses of the 2- and 4-wk hyperoxia exposures statistically different, suggesting that 2-wk exposure is as effective as 4-wk exposure in attenuating the carotid chemosensory response to stimuli in adult rats. We hypothesize that hyperoxia causes chemosensory inhibition, thus removing critical simulation required by the CB and CSN to grow and develop normally within a critical developmental window (the first 2 wk of life).

**Critique of methods.** Limitations of whole CSN recording include lack of certainty that the same fibers are being recorded at all times during a study, something that can be determined only from single-fiber studies. Baroreceptor neural discharge, fiber death, electrical noise, and nerve shifting on the recording electrode are also possible problems that could confound data interpretation. We used short-term responses (seconds with NaCN and asphyxia, minutes with acute hypoxia) and the window discriminator to minimize these problems. Arbitrarily setting the baseline CSN to near 300 Hz during normoxia (set by window discriminator) has little influence on the magnitude of response (see below). However, it is not possible to know precisely what the normoxic baseline activity is because one cannot fully distinguish between baseline noise and action potentials. As indicated in METHODS, we retrospectively tested all responses across many baselines and found that a floating baseline did not alter our conclusions.

The duration of maternal hyperoxia before birth is consistent between treatment groups. Thus, although it might have some effects, prenatal hyperoxia does not alter the fundamental conclusion that different durations of hyperoxia from birth have different effects on responses in the adult. Hyperoxia confined only to the second week of life significantly depresses phrenic nerve response to acute hypoxia in adult rats (2), indicating that prenatal hyperoxia is not necessary to produce the effects we have seen.

**Relationship to previous studies.** CSN responses to NaCN and asphyxia obtained in these studies were similar to data previously obtained in adult rats treated with 4 wk of postnatal hyperoxia (7, 14). These earlier investigations did not examine responses in rats treated with shorter periods of postnatal hyperoxia nor did they examine CSN responses to acute hypoxia. Hanson et al. (10) examined single- or few-fiber CSN responses to acute hypoxia in newborn kittens after exposure to 30% O_2 from just before birth to 12–23 days of age. They found a nearly complete lack of response to hypoxia compared with normoxic controls. The same group obtained similar results from whole CSN recording in rats that had been maintained in 30% O_2 for the first 5–10 wk of life (4). Carroll et al. (3) have recently shown that resting discharge frequency and the response to acute hypoxia are depressed in single CSN chemosensory fibers immediately after 11–16 days of postnatal hyperoxia (60% O_2) in rat pups. In none of the above studies were adult

---

**Table 2. Arterial blood-gas, pH, and blood pressure values during normoxia control and acute hypoxia.**

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1 wk</td>
<td>2 wk</td>
<td>4 wk</td>
</tr>
<tr>
<td>PaO_2, Torr</td>
<td>107 ± 4</td>
<td>98 ± 2</td>
<td>110 ± 4</td>
<td>104 ± 4</td>
</tr>
<tr>
<td>PaCO_2, Torr</td>
<td>38.4 ± 0.6</td>
<td>39.1 ± 0.8</td>
<td>40.2 ± 0.2</td>
<td>39.8 ± 1.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.01</td>
<td>7.40 ± 0.01</td>
<td>7.41 ± 0.01</td>
<td>7.38 ± 0.01</td>
</tr>
<tr>
<td>Systolic BP, Torr</td>
<td>158 ± 6</td>
<td>156 ± 8</td>
<td>156 ± 8</td>
<td>155 ± 5</td>
</tr>
<tr>
<td>Diastolic BP, Torr</td>
<td>108 ± 4</td>
<td>92 ± 5</td>
<td>105 ± 9</td>
<td>99 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals.

**Table 3. Carotid body size in adult (3–4 mo old) rats after postnatal hyperoxia**

<table>
<thead>
<tr>
<th>Duration of Postnatal Hyperoxia</th>
<th>Carotid Body Size, ×10⁶ µm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk</td>
<td>5.7 ± 1.3*</td>
</tr>
<tr>
<td>Control</td>
<td>16.2 ± 1.6*</td>
</tr>
<tr>
<td>4 wk</td>
<td>3.3 ± 0.2†</td>
</tr>
<tr>
<td>Control</td>
<td>11.5 ± 0.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Data obtained from frozen tissues. †Previously published data (7, 8) from paraffin-embedded tissues that shrink ~30% compared with frozen tissue.
animals tested after growing up in normoxia as we have done in the present study.

Recently, Bavis et al. (2) demonstrated that 1 wk of hyperoxia (60% O_2) either in the first or second week of life significantly impaired phrenic nerve responses to acute hypoxia in adult rats to an extent similar to 4-wk hyperoxia exposures. These phrenic responses parallel our CSN responses to acute hypoxia; i.e., our CSN responses were not different between rats treated with 1, 2, and 4 wk of postnatal hyperoxia. However, Bavis et al. did not examine responses to the more extreme stimulus of intravenous NaCN. The observation that NaCN induced a brisk CSN response in 1-wk hyperoxia-treated rats does not necessarily invalidate conclusions about the response to acute hypoxia and likely reflects the more intense, possibly maximal response, elicited by NaCN. At this dose, NaCN responses are well above acute hypoxia responses obtained at a PaO_2 of 45 Torr. The mechanism of the CB response to histotoxic hypoxia (NaCN) may be different from that to hypoxemia (9) and thus may not be directly comparable.

The observation that CSN responses to acute hypoxia and asphyxia were significantly depressed in 1- and 2-wk postnatal hyperoxia exposure groups is consistent with phrenic response data (2). Both studies indicate that the first 2 wk of postnatal life constitutes a critical developmental window for the CB, CSN, and the ventilatory response to hypoxia. This timing is consistent with previous observations that the first 2 postnatal wk of life is a critical period for development of the ventilatory and CSN responses to hypoxia in the rat (1, 5, 12, 19). Our studies emphasize the importance of a normoxic environment to that development.

**CB volume.** CB growth is impaired by hyperoxia in the early postnatal period (7, 8). Our studies show that as little as 1 wk of hyperoxia causes persistent CB hypoplasia. Erickson et al. (6) studied neonatal rats treated with 30 or 60% O_2 for the first 7 or 28 postnatal days. In that study, the CBs were hypoplastic and the CSN exhibited a marked reduction in unmyelinated axons, suggesting a reduced number of chemoafferent neurons in the petrosal ganglion. The nearby nodose ganglion was unaffected. On the basis of earlier studies showing that chemoafferent neuron growth is dependent on target tissue (i.e., the CB), Erickson et al. suggested that the diminished chemoafferent development results from decreased type 1 cell numbers in the CB after hyperoxia. Chemoafferent neurons and their unmyelinated axons most likely remain deficient in adult animals if they are not present at 4 wk of age, but this has not been directly examined. Vidruk and colleagues were unable to record from single chemoafferent fibers in the CSN after developmental hyperoxia in rats (E. H. Vidruk, L. Ling, and G. S. Mitchell, personal communication). Some of the changes after 4 wk of hyperoxia are known to persist to old age in the rat (14–15 mo; e.g., the reduced volume of the CB with proportional reductions in cellular and vascular volume) (7). CB volume is only one aspect of structural change after postnatal hyperoxia. Our studies of CB volume are meant to show that significant structural changes are common to adult rats treated with 1, 2, or 4 wk of postnatal hyperoxia.

The permanent structural and functional deficits after postnatal hyperoxia do not prevent the CB from some responses known to occur in the normal CB. Fuller et al. (8) studied rats that had been treated with 4 wk of postnatal hyperoxia and then subjected to 1 wk of 12% O_2 as adults. They found a significant recovery of the minute phrenic response to hypoxia and an increased CB volume. The increased CB volume was primarily due to vasodilation. Both changes are typical of the normal CB response to chronic hypoxia (16). Another change typical of the normal CB response to sustained hypoxia, increased tyrosine hydroxylase expression within type 1 cells (18), was also present (Z.-Y. Wang and G. E. Bisgard, unpublished observations). It is not yet known whether any of these changes persist and contribute to a more permanent functional recovery.

Postnatal hyperoxia permanently impairs structural development in the CB and its afferent neural innervation. Such defects likely contribute to the depressed CSN responses to acute hypoxia. The central integration and motor output in response to CSN stimulation appear to be normal; thus impaired carotid chemoafferent function accounts for depressed hypoxic ventilatory or phrenic responses after developmental hyperoxia (14). It is not yet clear what specific changes may contribute to diminished carotid chemoafferent function. Erickson et al. (6) suggest that there could be disrupted synaptic contact between type 1 cells and their afferent neurons, or among type 1 cells themselves, or that type 1 cell transduction mechanisms may be affected. Recently, Prieto-Lloret et al. (17) found that dopamine synthesis and release by hypoxic stimulation are reduced in adult rat CBs after postnatal hyperoxia, whereas the K^+–induced dopamine release was the same in control and hypoxia-treated rats. They concluded that the O_2-sensing machinery of the adult CB was damaged by postnatal hyperoxia. Acute hypoxia-induced depolarization and intracellular calcium increase were diminished in type 1 cells from isolated rat type 1 cells from pups after 11–16 days of hyperoxia (13). This finding has not been studied in adult rats, but it clearly indicates a functional deficit that may persist later in life. Collectively, these recent reports strongly indicate that a deficit in chemosensory function accompanies CB, and possibly CSN, structural deficits after developmental hyperoxia. Thus both structural and functional deficits probably contribute to weak carotid chemoafferent responses in adult rats treated with postnatal hyperoxia.

**Significance.** Our results expand on previous studies concerning the consequences of postnatal hyperoxia from birth on carotid chemoafferent function (7, 14). Our data indicate that carotid chemoafferent function is attenuated after 1 wk of postnatal hyperoxia, that greater functional impairment occurs with 2 wk of postnatal hyperoxia, and that additional hyperoxia has no further effect on adult chemoafferent function. We
suggest that the first 2 wk of life constitutes a critical period of susceptibility to hyperoxia in rats.

These are only some of the research findings indicating that caution must be utilized when O₂ is used for resuscitation or for intensive care in infants. Indeed, depressed arterial chemoreceptor responses have been documented after prolonged use of O₂ therapy in infants (11). Suppressed chemoreflexes have the potential to contribute to poor defense reactions in response to hypoxic or asphyxic conditions and could contribute to ventilatory instability or even termination.

The authors thank Gordon Johnson for excellent technical assistance and Dr. Edward Vidruk for advice on carotid sinus nerve recording.

DISCLOSURES

This research was supported by National Heart, Lung, and Blood Institute Grants HL-68255 and HL-07654.

REFERENCES


