Chronic hypoxia abolished the postnatal increase in carotid body type I cell sensitivity to hypoxia

L. M. STERNI, O. S. BAMFORD, M. J. WASICKO, AND J. L. CARROLL
Department of Pediatrics, The Johns Hopkins Children's Center, Baltimore, Maryland 21287

Sterni, L. M., O. S. Bamford, M. J. Wasicko, and J. L. Carroll. Chronic hypoxia abolished the postnatal increase in carotid body type I cell sensitivity to hypoxia. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L645–L652, 1999.—The O2 sensitivity of carotid chemoreceptor type I cells is low just after birth and increases with postnatal age. Chronic hypoxia during postnatal maturation blunts ventilatory and carotid chemoreceptor neural responses to hypoxia, but the mechanism remains unknown. We tested the hypothesis that chronic hypoxia from birth impairs the postnatal increase in type I cell O2 sensitivity by comparing intracellular Ca2+ concentration ([Ca2+]i) responses to graded hypoxia in type I cell clusters from rats born and reared in room air or 12% O2. [Ca2+]i levels at 0, 1, 5, and 21% O2, as well as with 40 mM K+, were measured at 3, 11, and 18 days of age with use of fura 2 in freshly isolated cells. The [Ca2+]i response to elevated CO2/low pH was measured at 11 days. Chronic hypoxia from birth abolished the normal developmental increase in the type I cell [Ca2+]i response to hypoxia. Effects of chronic hypoxia on development of [Ca2+]i responses to elevated K+ were small, and [Ca2+]i responses to CO2 remained unaffected. Impairment of type I cell maturation was partially reversible on return to normoxic conditions. These results indicate that chronic hypoxia severely impairs the postnatal development of carotid chemoreceptor O2 sensitivity at the cellular level and leaves responses to other stimuli largely intact.

carotid chemoreceptors; maturation; calcium; birth

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THE CAROTID CHEMORECEPTORS, located bilaterally at the carotid bifurcation, are the major sensors of arterial O2 concentration and are largely responsible for driving the important ventilatory, cardiovascular, and behavioral responses to hypoxia. Functioning carotid chemoreceptors appear to be critically important for survival in the neonatal period. Although carotid denervation is well tolerated by adults (41), newborn mammals deprived of carotid chemoreceptor function just after birth exhibit hypventilation, abnormal breathing patterns, apnea, and high mortality rates (8, 10, 11, 21).

Despite its importance during postnatal development, carotid chemoreceptor sensitivity to O2 is not mature at birth. Neural activity from the fetal carotid chemoreceptors can be elicited only with extreme hypoxic stimuli, and the response to hypoxia is weak just after birth (4). Over the first few days of life, neonatal carotid chemoreceptor sensitivity to hypoxia increases, requiring weeks or even months in some species to reach full maturity (9, 24, 27). Although the mechanisms are poorly understood, the consensus view is that the fetal carotid chemoreceptors are adapted to the low levels of fetal arterial Po2 (PaO2) and, after birth, reset O2 sensitivity to the approximately fourfold higher PaO2 range seen during postnatal life (5).

The postnatal increase in carotid chemoreceptor O2 sensitivity is dependent on the rise in PaO2 after birth (5, 19), suggesting that postnatal chronic hypoxia could negate the fetal/newborn state of low chemoreceptor O2 sensitivity. Chronic hypoxia from birth has been shown to impair postnatal development of carotid chemoreceptor O2 sensitivity and ventilatory responses to acute hypoxia in all species studied, including humans (12, 16, 23, 25, 33). However, the mechanisms underlying the effects of chronic hypoxia on peripheral chemoreceptor sensitivity are unknown.

The O2-sensing element of the carotid chemoreceptors is believed to be the type I cell, which responds to hypoxia with a rise in intracellular Ca2+ concentration ([Ca2+]i), leading to neurotransmitter release and excitation of apposed carotid sinus nerve (CSN) endings (13). Our previous work on rabbit and rat carotid chemoreceptors showed that [Ca2+]i responses to hypoxia were weak in type I cells from newborns and increased during postnatal maturation (35, 40). Furthermore, maturation of type I cell O2 sensitivity followed approximately the same time course as maturation of carotid chemoreceptor neural responses to hypoxia (24), consistent with the hypothesis that maturation of carotid chemoreceptor function is due, at least in part, to maturation of type I cell O2 sensitivity.

We hypothesized that impaired resetting or maturation of type I cell O2 sensitivity is responsible for the blunting of the postnatal increase in chemoreceptor sensitivity by chronic hypoxia from birth. We tested this hypothesis by comparing [Ca2+]i responses to graded hypoxia in type I cells from rats reared in room air with responses from rats reared in 12% O2. The results indicate that exposure to peri- and postnatal chronic hypoxia abolishes the normal postnatal increase in type I cell O2 sensitivity. Furthermore, impairment of type I cell development appears to be largely specific for hypoxia sensing, and suppression of type I cell maturation is partially reversible on return to normoxic conditions.

METHODS

Type I cell isolation. Experiments were performed with carotid chemoreceptor type I cells isolated from Sprague-Dawley rats born and raised in normoxia or in a hypoxic chamber. The chamber was maintained at 12% O2 with CO2 removed by a soda lime canister. For chronic hypoxia, pregnant rats were transferred to the hypoxic chamber 1–2 days before delivery and maintained in the chamber for up to 3 wk after birth of the pups. Rats in the normoxic and hypoxic groups were killed and studied at 3, 11, and 18 days of age.
Glomus cells from seven litters of rats raised in normoxia and eight litters raised in hypoxia were studied. Several pups from two of the litters born into hypoxia were moved to normoxic conditions at 11 days of age and then killed for study at 18 days of age. Data for all other experimental and control groups were obtained from at least three litters (range 3–6 litters).

After rapid decapitation under surgical anesthesia (methoxyflurane), carotid bifurcations were dissected free and placed in ice-cold PBS. The cell isolation procedure was based on the method described by Buckler and Vaughan-Jones (6). The carotid bodies were dissected free from the bifurcation, cut in half, and placed in a solution containing 0.02% trypsin (Sigma) and 0.1% collagenase type II (Sigma) in PBS with 50 µM Ca2+. The carotid bodies were incubated for 20 min at 37°C, teased apart with forceps, then digested for an additional 5 min.

After dispersion by gentle rocking, the tissue was pelleted at 2,000 g for 2 min and then resuspended in a nutritive medium composed of Ham's F-12 (Mediatech) with 10% FCS, 33 mM glucose, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.08 U/ml insulin. The cells were then centrifuged as described above and resuspended in the nutritive medium. Cells were plated on poly-d-lysine-coated coverslips and maintained in a 5% CO2, 37°C incubator. Cells were studied ≥1.5 h and <6 h after dissociation. Type I cells were identified using well-described visual criteria, including a size of 10–15 µm, a rounded shape, and a tendency to occur in clusters. Only cells included in clusters of three or more cells were studied. Also, in cells chosen for study, viability was confirmed using the membrane-impermeant DNA stain propidium iodide (PI, 5 µg/ml; Molecular Probes). When membrane integrity is compromised, PI binds to nuclear DNA, resulting in fluorescence when the dye is excited at 487 ± 15 nm, with emission monitored at 645 ± 50 nm.

Measurement of [Ca2+]. Type I cells attached to coverslips were loaded with fura 2 by incubation for 8 min at 37°C with 4 mM fura 2-AM (Molecular Probes). Cells were studied on a Zeiss Axi'overt microscope with a ×40 Fluor (Zeiss) objective. Fura 2 fluorescence emission was measured at 505–540 nm in response to alternating excitation wavelengths of 340 and 380 nm. Pairs of images were collected every 8 s and stored on a computer using Metafluor (Universal Imaging). A charge-coupled device camera and image intensifier (Videosecope) at constant intensity and camera gain detected fluorescence. Background images were acquired at the end of each experiment from an area of the coverslip with no cells and subtracted, pixel by pixel, from the experimental images before the calculation of a fluorescence ratio of 340 to 380 nm. Data analysis. Baseline [Ca2+] was calculated as the average [Ca2+]i, over the 1 min before the stimulus and peak [Ca2+]i as the maximum value obtained during the 1- to 2-min challenge. Responses were calculated for each cell as the Δ[Ca2+]i, between the baseline and peak [Ca2+]i values. For baseline, peak, and Δ[Ca2+]i, data from cells in a single cluster were averaged and treated as one independent observation. Values are means ± SE; n refers to number of clusters. Statistical analysis for age-related effects was performed using ANOVA with Tukey's honestly significant difference post hoc testing. Comparisons in the 3- and 11-day age groups between room air and chronic hypoxia conditions were made using an unpaired t-test. In the 18-day age group, comparison between control, chronic hypoxia, and recovery groups was made using ANOVA with Dunnett's T3 post hoc testing (for unequal variance). P < 0.05 was considered significant.

RESULTS

Figure 1 shows typical [Ca2+]i responses of a type I cell cluster isolated from 11-day-old rats reared in normoxia (CON) and in chronic hypoxia (CH). In CON
clusters, acute exposure to 0 and 1% O_2 induced significant and consistent increases in [Ca^{2+}]_i responses to 0% O_2 was increased significantly (*) at 11 (n = 8) and 18 (n = 5) days compared with 3 days (n = 5). In contrast, in clusters from CH rats, there was no increase in [Ca^{2+}]_i response to 0% O_2 with age (n = 9, 12, and 6 at 3, 11, and 18 days, respectively).

Hypoxia. The mean Δ[Ca^{2+}]_i responses of CON and CH type I cell clusters to 0% O_2 at 3, 11, and 18 days are shown in Fig. 2. Consistent with our previous work (35, 40), Δ[Ca^{2+}]_i responses to 0% O_2 in the CON clusters were small at 3 days of age and increased by 11 days of age, with no further change noted at 18 days. In contrast, Δ[Ca^{2+}]_i responses of type I cell clusters were significantly smaller in CH than in CON rats, and there was no age-related increase in the response to the 0% O_2 challenge. Thus chronic hypoxia blunts the Δ[Ca^{2+}]_i response as early as 3 days of age and abolishes the normal developmental increase in the Δ[Ca^{2+}]_i response to hypoxia.

To determine the effect of chronic hypoxia on the development of O_2 sensitivity, [Ca^{2+}]_i at 0, 1, 5, and 21% O_2 was plotted (Fig. 3) to show the O_2 response profile ([Ca^{2+}]_i vs. O_2) at each age for clusters from CON vs. CH rats. Type I cell clusters isolated from CH rat pups clearly demonstrated significantly smaller [Ca^{2+}]_i responses to 0 and 1% O_2 challenge than clusters from the CON group at each age studied. In clusters from CON rats, the response profile became markedly steeper between 3 and 11 days. In sharp contrast, in cell clusters from CH rats, comparison of [Ca^{2+}]_i at the four O_2 levels by ANOVA revealed no significant hypoxic response above baseline (21% O_2) at any age. Compared with the CON group, the Δ[Ca^{2+}]_i response to 0% O_2 (response above 21% O_2 baseline) was reduced by 67% at 3 days and 90–95% by 11–18 days. Baseline [Ca^{2+}]_i measurements in type I cell clusters from CON rats were not significantly different from those from CH rats.

Elevated extracellular K^+. Chronic hypoxia abolished the postnatal development of the Δ[Ca^{2+}]_i response to acute hypoxia. To determine whether this effect was specific to hypoxia sensing or a nonspecific effect on Ca^{2+} influx during depolarization, the effect of chronic hypoxia on the Δ[Ca^{2+}]_i responses to other challenges was measured. Elevated extracellular K^+ causes depolarization and a rise in [Ca^{2+}]_i in type I cells. In all groups studied, the response to 40 mM K^+ was significantly larger than the Δ[Ca^{2+}]_i response to 0% O_2 (Fig. 1). At 3 days of age, the Δ[Ca^{2+}]_i responses to 40 mM K^+ were 848 ± 87 and 790 ± 70 nM in the CON and CH groups, respectively (not significant). At 11 days of age, the response was statistically significantly less in clusters from CH than in clusters from CON rats: 543 ± 60 and 758 ± 91 nM, respectively (P = 0.003; Fig. 4). In the 18-day-old group, the Δ[Ca^{2+}]_i response to elevated extracellular K^+ was reduced by approximately the same amount in the CH and CON groups (546 ± 94 and 767 ± 129 nM, respectively), but this difference was not statistically significant (P = 0.052; Fig. 4).

Hypercapnia. Hypercapnic acidosis raises [Ca^{2+}]_i in type I cells through membrane depolarization and extracellular Ca^{2+} entry through voltage-gated channels (7). The Δ[Ca^{2+}]_i response to 15% CO_2 measured at 11 days showed no difference between the CON and CH groups (Fig. 5). These data show that the type I cell
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Fig. 4. Δ(Ca^{2+})_i response to 40 mM K^+ at 3, 11, and 18 days of age in type I cell clusters from CH (solid bars) and CON (open bars) groups. At 3 days of age, Δ(Ca^{2+})_i response to 40 mM K^+ was not significantly different. Δ(Ca^{2+})_i response to high K^+ was significantly smaller (*) in CH than in CON cells at 11 days. A similar reduction in response to elevated extracellular K^+ was seen in CH group at 18 days, but this difference was not statistically significant (P = 0.052). At 3 days, n = 11 for CON and CH. At 11 days, n = 16 for CH and 10 for CON. At 18 days, n = 7 for CH and 5 for CON.

By ANOVA, the Δ(Ca^{2+})_i responses to 0 and 1% O_2 of the recovery group were intermediate, not statistically different from the CON or the CH group. Thus the effect of chronic hypoxia on the type I cell’s sensitivity to O_2 is at least partially reversible.

DISCUSSION

The major finding of this study is that the normal postnatal increase in type I cell O_2 sensitivity, as reflected by the Δ(Ca^{2+})_i response to graded hypoxia, is abolished by rearing rats in a hypoxic environment. We previously showed that Δ(Ca^{2+})_i responses of carotid chemoreceptor type I cells to hypoxia are weak just after birth and increase with postnatal age in rabbits (35) and rats (40) with the same developmental time course as CSN activity (24). This resetting of carotid chemosensitivity is modulated, at least in part, by the almost fourfold rise in PaO_2 after birth (5), suggesting that type I cell maturation may also be dependent on postnatal PO_2. Our results demonstrate that in type I cells of rats reared in a low O_2 environment from birth, sensitivity to hypoxia was significantly reduced, as evidenced by the remarkably smaller Δ(Ca^{2+})_i responses to hypoxic challenges than in type I cells from rats reared in room air. This novel finding raises the possibility that the level of PO_2 during infancy may regulate maturation of carotid chemoreceptor sensitivity by regulating the [Ca^{2+}]_i response to the type I cell. The findings that the type I cell response to elevated extracellular K^+ remained 75% intact and that the response to 15% CO_2 was not affected by exposure to chronic hypoxia suggest that chronic hypoxia affects a component specific to the O_2 transduction cascade.

Methodological issues. In studies of developing mammals, in which Δ(Ca^{2+})_i responses are low, it is important to ensure that differences between age groups are due to maturation rather than technical factors that affect cells from immature and mature rats differently. In our previous study on postnatal development of rat type I cell function, we used the same cell preparation methods to demonstrate that there were no age-related differences in Δ(Ca^{2+})_i responses to the Ca^{2+} ionophore ionomycin from full-term fetal to 21-day-old animals (40). This suggests that cells from immature and mature rats load and deesterify fura 2 in a similar manner. Similarly, the CON and CH groups exhibited large Δ(Ca^{2+})_i responses to 40 mM K^+ at all ages (Fig. 4). Finally, the viability of each cell was tested before study with PI. These results indicate that, under the conditions of study, observed differences were not due to age-related differences in the ability to measure or mobilize large [Ca^{2+}]_i, or to poor health of the cells.

Type I cells isolated from animals born and reared in chronic hypoxia are hypertrophied, as shown morphologically and by measurement of cell membrane capacitance (42). We also observed that the carotid bodies and individual type I cells isolated from CH pups appeared to be larger than those isolated from the CON group. Because Δ(Ca^{2+})_i responses in the CH group were severely blunted, it is important to demonstrate that...
the type I cells from this group were capable of mounting a Ca\(^{2+}\) response when stimulated. In the 11-day-old CH group, in which \(\Delta[Ca^{2+}]\) responses to 0% O\(_2\) were >90% blunted, type I cell \(\Delta[Ca^{2+}]\) responses to CO\(_2\)/acid were unaffected and essentially identical in the CON and CH groups. This indicates that type I cells in the CH group were able to load and deesterify fura 2 and mobilize Ca\(^{2+}\) in response to a natural stimulus yielding the same results as CON cells. In addition, although we did not quantify it, cells from the CH group appeared, after Ca\(^{2+}\) transients, to return [Ca\(^{2+}\)] to baseline as quickly as cells from CON rats. In summary, we found no evidence to indicate an impaired ability to measure [Ca\(^{2+}\)] in CH type I cells, nor did we find an impaired ability of type I cells from CH rats to mobilize large amounts of intracellular Ca\(^{2+}\).

Effects of chronic hypoxia on type I cell [Ca\(^{2+}\)] responses. There is controversy concerning the possible effects of chronic hypoxia on development of carotid chemosensitivity at the O2-sensing, type I cell level. Using whole cell patch-clamp recording, Hempleman (17, 18) found reduced K\(^{-}\)-induced Ca\(^{2+}\) currents in chronically hypoxic rats (18). In the present study, by 11 days of age, chronic hypoxia reduced the K\(^{-}\)-induced \(\Delta[Ca^{2+}]\) response by only ~25%. Therefore, although blunting of Ca\(^{2+}\) influx with depolarization may have contributed, it seems unlikely that it could account for the effect of chronic hypoxia, which nearly abolished the \(\Delta[Ca^{2+}]\) response to hypoxia.

There is strong evidence that hypercapnia raises [Ca\(^{2+}\)] through membrane depolarization and voltage-gated Ca\(^{2+}\) influx. We found that chronic hypoxia had no effect on the type I cell [Ca\(^{2+}\)] response to hypercapnia/acidosis. In our 11-day-old CH group, this suggests that hypercapnia/acidosis depolarized the type I cells, leading to Ca\(^{2+}\) influx, at a time when the type I cell [Ca\(^{2+}\)] response to hypoxia was abolished. Although the mechanisms by which hypoxia and hypercapnia/acidosis lead to membrane depolarization are unknown, our findings of a severely blunted [Ca\(^{2+}\)] response to hypoxia and intact [Ca\(^{2+}\)] response to CO\(_2\) in the same cells indicate that the component affected by chronic hypoxia is not shared by the O\(_2\) vs. CO\(_2\) transduction pathways or is not critical to the CO\(_2\) transduction cascade.

It is not surprising that the [Ca\(^{2+}\)] response to CO\(_2\) was unaffected by chronic hypoxia, whereas the response to 40 mM K\(^{+}\) was reduced by 25%. The likely explanation lies in the difference in magnitude of depolarization caused by the two stimuli. The type I cell \(\Delta[Ca^{2+}]\) response to 15% CO\(_2\) was ~100 nM vs. ~550–750 nM for 40 mM K\(^{+}\). Thus the \(\Delta[Ca^{2+}]\) response to CO\(_2\) was almost five- to sevenfold smaller than the response to 40 mM K\(^{+}\) and very likely associated with a much smaller cell membrane depolarization. Buckler and Vaughan-Jones (7) found that 20% CO\(_2\) depolarized type I cells from similar-aged rats, on average, to 37 mV. Our 15% CO\(_2\) stimulus would have caused even less depolarization. The beginning part of the Ca\(^{2+}\) current-voltage relationship (i.e., ~40 mV) tends to be minimally affected by small reductions in Ca\(^{2+}\) current, which mainly affect the voltage range of peak Ca\(^{2+}\) current (i.e., between about ~10 and +10 mV). Therefore, the lack of effect of CH on the [Ca\(^{2+}\)] response to CO\(_2\) was likely due to the small magnitude of depolarization underlying this response coupled with the characteristics of Ca\(^{2+}\) currents in the early part of the current-voltage curve.

Our previous study on the time course of type I cell [Ca\(^{2+}\)] response development, from full-term fetus to 3 wk of age, showed that [Ca\(^{2+}\)] responses to hypoxia are weak in the fetus and newborn and begin to increase by ~3 days, reaching a maximal level by 11–14 days of age.
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In sharp contrast, type I cells from rats born and reared in hypoxia show blunted [Ca^{2+}]i responses to hypoxia by 3 days and a complete lack of development of the [Ca^{2+}]i response to hypoxia over the following 2 wk. Responses to hypoxia at 3 days of age in the CH group resembled the [Ca^{2+}]i-O2 response curve of full-term fetal rat type I cells in our previous study (40). Thus it appears that chronic hypoxia from birth perpetuates the fetal state of low carotid chemoreceptor type I cell O2 sensitivity, and this is likely the mechanism leading to lack of resetting. To our knowledge, this is the first investigation specifically of the effects of chronic hypoxia on postnatal development of type I cell O2 sensitivity. These data provide new evidence that postnatal resetting of carotid chemoreceptor type I cell O2 sensitivity depends on the level of postnatal O2 exposure, extending the previous findings of Blanco et al. (5) in lambs, that resetting of carotid chemoreceptor type I cell O2 sensitivity was modulated by the perinatal rise in PaO2.

Studies of neonatal mammals exposed to chronic hypoxia demonstrate an attenuation of the ventilatory response to acute hypoxia, largely because of decreased sensitivity of the carotid chemoreceptors (15). Even short-duration exposure to chronic hypoxia, for a few days, results in blunting of O2 chemoreflex responses. These effects appear to be reversible in neonates on return to normoxia (15). In contrast, in mature mammals, carotid chemoreflex gain increases during the first few days of acclimatization to chronic hypoxia (3, 37), whereas more prolonged exposure to hypoxia leads to long-term blunting of the hypoxic ventilatory response (36), which is not readily reversible. These differences suggest that chronic hypoxia in the neonate may be affecting developmental aspects of O2 chemoreception that differ from the effects of chronic hypoxia on chemosensitivity in mature mammals.

It is generally accepted that the rise in PaO2 at birth is the major factor leading to carotid chemoreceptor resetting. Raising the PaO2 of the near-term sheep fetus initiates carotid chemoreceptor resetting in utero (5). Furthermore, animals hypoxic from birth have a weak arterial chemoreflex, which can then be increased by returning the animals to normoxia (19, 20). How Po2 modulates chemoreceptor resetting is unclear. One possibility is that O2 acts through regulation of dopamine, an abundant neuromodulator inhibitory to type I cells, via an autoreceptor mechanism (2). Development of the peripheral chemoreflex in newborn rats is accompanied by a decrease in carotid body dopamine turnover, suggesting a lifting of inhibition of the chemosensory mechanism (20). The postnatal fall in dopamine turnover and the development of the peripheral chemoreflex can be delayed in early life by rearing rats in hypoxia from birth (19). Carotid body and type I cell dopamine synthesis, turnover, and release have been shown to be significantly elevated by chronic exposure to hypoxia (22, 32), likely because of increased gene expression of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of dopamine (39). Acting via D2 receptors, dopamine inhibits type I cell Ca^{2+} currents, and, in other cell types, dopamine has been shown to acutely increase outward K+ currents, leading to cell hyperpolarization and decreased excitability (26). In rat lactotroph cells, long-term exposure to dopamine resulted in increased baseline activity of Ca^{2+}-dependent K+ channels and altered the signaling pathways between these K+ channels and modulators of hormone secretion. Thus considerable evidence suggests that dopamine may be an important component of carotid chemoreceptor resetting. However, its role is poorly understood.

It is difficult to speculate about mechanisms by which chronic hypoxia may perpetuate the fetal state of low O2 sensitivity, because the nature of the type I cell O2 sensor is unknown and the mechanisms by which hypoxia is transduced into CSN activity remain controversial. In the CH group, the low type I cell O2 sensitivity cannot be simple receptor-mediated inhibition, e.g., by dopamine, inasmuch as under the conditions of our study, type I cells were superfused with BSS at ~1 ml/min (chamber volume = 0.2 ml); therefore, secreted dopamine would have been rapidly washed away. Therefore, even when removed from the neuro-modulatory milieu of the intact carotid body, type I cells still exhibited remarkably blunted O2 sensitivity. If chronic high levels of dopamine play a role, it must be by inducing long-term changes in type I cell components that are critical for O2 transduction. Chronic hypoxia has been shown to induce a variety of long-term changes in second messenger systems, enzyme activity, and expression of ion channels in other O2-sensing cell types, such as pheochromocytoma (PC-12) cells (1) and pulmonary artery smooth muscle (38). Taken together, the absence of a Δ[Ca^{2+}]i response to hypoxia with preservation of Δ[Ca^{2+}]i responses to two other depolarizing stimuli strongly suggests that hypoxia fails to depolarize type I cells from CH rats, suggesting that chronic hypoxia affects the O2 sensor or its link with membrane potential.

Recovery from chronic hypoxia. The time course of recovery found in this study may provide further insight into the question of developmental plasticity of type I cell O2 sensitivity maturation. Type I cells from rats reared until 18 days of age in chronic hypoxia showed no sensitivity to acute hypoxia. However, when rats were reared until 11 days of age in chronic hypoxia and then returned to room air, the type I cell sensitivity to hypoxia at 18 days, as measured by the [Ca^{2+}]i response to graded hypoxia (Fig. 3C), had partially recovered. This time course, reaching ~60% of the mature response ~1 wk after return to room air, is similar to that previously reported for type I cell O2 sensitivity maturation after birth (40). These findings strongly suggest that the development of type I cell O2 sensitivity is modulated by Po2 and is, at least to some degree, independent of gestational or postnatal age. In other words, it appears that in the CH group at 11 days of age, once the chronic hypoxia was corrected by returning the litter to room air, the normal maturation increase in type I cell O2 sensitivity was triggered and/or disinhibited. However, Eden and Hanson (12)
suggested that rats may eventually reset their peripheral chemoreceptor sensitivity to $O_2$, despite chronic hypoxia (13–15%), on the basis of the finding that CSN recordings from chronically hypoxic 5- to 10-wk-old rats were not different from controls (12). These findings raise several questions with respect to the time course of full recovery, developmental time windows within which full recovery is possible, and the relationship between effects of chronic hypoxia and developmental stage, among others, which require further study.

Conclusions. Chronic hypoxia from birth in vivo up to 18 days of age abolishes the normal postnatal maturation of $O_2$ sensing by carotid chemoreceptor type I cells. The mechanism is unknown, but the effects of chronic hypoxia appear to be at least partially reversible. We speculate that the normal rise in $PaO_2$ at birth is required to promote expression of critically important components in the $O_2$ transduction cascade.

This work was supported by National Heart, Lung, and Blood Institute Grants K08-HL-03791 (L. M. Sterni) and R01-HL-54621 (J. L. Carroll). We also thank the Mount Washington Pediatric Hospital (IndaCordatory, MD) for their generous support. Address for reprint requests and other correspondence: L. M. Sterni, The Johns Hopkins Children’s Center, Park 316, 600 North Wolfe St., Baltimore, MD 21287-2533 (E-mail: lsterni@welchlink. welch.jhu.edu).

Received 2 October 1998; accepted in final form 13 April 1999.

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