Tyrosine hydroxylase expression and activity in the rat brain: differential regulation after long-term intermittent or sustained hypoxia

Evelyne Gozal,1,2 Zahoor A. Shah,1 Jean-Marc Pequignot,4 Jacqueline Pequignot,4 Leroy R. Sachleben,1 Maria F. Czyzyk-Krzeska,3 Richard C. Li,1 Shang-Z. Guo,1 and David Gozal1,2

1Department of Pediatrics, Kosair Children’s Hospital Research Institute, and 2Department of Pharmacology and Toxicology, University of Louisville, Louisville, Kentucky; 3Department of Genome Science, Genome Research Institute, University of Cincinnati, Cincinnati, Ohio; and 4Physiologie Integrative Cellulaire et Moleculaire, Unité Mixte de Recherche 5123, Centre National de la Recherche Scientifique, University of Lyon, Lyon, France

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Intermittent hypoxia, a characteristic feature of sleep apnea, leads to increased neuronal cell loss through activation of proapoptotic pathways (43, 46). A more frequent form of hypoxia, intermittent hypoxia (IH), which characteristically occurs in disease states such as sleep apnea, imposes substantial metabolic and cardiovascular consequences (10, 13, 34, 45). Mild IH during sleep, but not SH, is associated with substantial neurobehavioral deficits (12, 47, 48), mediated by increased neuronal cell loss through activation of proapoptotic pathways (11, 15). Furthermore, both IH and SH induce a wide range of genomic and proteomic alterations in neural tissue (2, 5, 14, 25, 35, 36). For example, when IH occurs during a critical period of brain development, it induces substantial cognitive and behavioral alterations (48) accompanied by regionally defined changes in dopaminergic pathways and resulting in increased sleep and locomotor activity as well as in impaired working memory (8). Despite the increasing body of evidence on the potential deleterious effects of hypoxia in the brain, the regulation of brain TH expression and catecholamine synthesis has not been systematically explored in vivo during long-term SH and IH.

TH activity is dependent on its cofactors, tetrahydrobipterin and molecular oxygen, and is regulated at the level of transcription, RNA stability. Furthermore, feedback inhibition, allosteric modifications, and posttranslational modifications such as phosphorylation at various sites play a role in the modulation of TH activity and thus in catecholamine turnover (4, 28, 29, 41, 44). Four phosphorylation sites have been identified in the mammalian TH amino terminal on Ser8, Ser19, Ser31, and Ser40, which may modulate enzyme activity (4, 18). Phosphorylation at these sites occurs after electrical stimulation (19) and after membrane depolarization (31), and it is involved in either directly activating TH or facilitating TH activation. With the exception of Ser8, all other phosphorylations sites appear to be physiologically relevant, and thus far, the main pathway of catecholamines biosynthesis begins with the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) catalyzed by tyrosine hydroxylase (TH), ultimately leading to the release of norepinephrine, epinephrine, and dopamine (9, 29, 31).

Sustained hypoxia (SH), as seen during high-altitude sojourns, is associated with substantial in vivo upregulation of the TH gene in the caudal brain stem, as well as with increased catecholamine turnover, and these changes have been associated with adaptive ventilatory mechanisms essential for respiratory homeostasis during hypoxia (43, 46). A more frequent form of hypoxia, intermittent hypoxia (IH), which characteristically occurs in disease states such as sleep apnea, imposes substantial metabolic and cardiovascular consequences (10, 13, 34, 45). Mild IH during sleep, but not SH, is associated with substantial neurobehavioral deficits (12, 47, 48), mediated by increased neuronal cell loss through activation of proapoptotic pathways (11, 15). Furthermore, both IH and SH induce a wide range of genomic and proteomic alterations in neural tissue (2, 5, 14, 25, 35, 36). For example, when IH occurs during a critical period of brain development, it induces substantial cognitive and behavioral alterations (48) accompanied by regionally defined changes in dopaminergic pathways and resulting in increased sleep and locomotor activity as well as in impaired working memory (8). Despite the increasing body of evidence on the potential deleterious effects of hypoxia in the brain, the regulation of brain TH expression and catecholamine synthesis has not been systematically explored in vivo during long-term SH and IH.

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at least eight different protein kinases phosphorylate TH in vitro (18, 29). Ser19 phosphorylation is calcium dependent and is catalyzed by a calcium/calmodulin-dependent protein kinase, but does not appear to influence directly TH activity in situ (21, 51). However, it has recently been demonstrated that Ser20 phosphorylation facilitates the phosphorylation of Ser40 in vitro (3). Ser31 is phosphorylated by ERKs, increasing the enzyme activity (17, 20). Whereas calcium/calmodulin-dependent protein kinase, c-AMP-dependent protein kinase (PKA), and protein kinase C all appear to play a role in Ser40 phosphorylation, PKA does so at a higher stoichiometry (28, 29). Furthermore, DA receptor D2 inhibition of Ser20 phosphorylation, without affecting Ser31 and Ser20 phosphorylation, results in the inhibition of 3,4-dihydroxyphenylalanine (DOPA) accumulation, suggesting that Ser19 phosphorylation is critical for TH activity and that DA regulates the rate-limiting enzyme for its own synthesis by inhibiting TH phosphorylation (31).

SH induces TH gene expression, thereby stimulating catecholamine synthesis in the dopaminergic pheochromocytoma 12 (PC-12) neuronal cell line and in corticot body type I cells (6, 7, 35, 36), through the c-Fos and junB binding to the activator protein-1 site (39) and through hypoxia-inducible transcription factor binding to the heat shock responsive element on the TH promoter (49). Both SH and IH stimulate TH phosphorylation on Ser31, Ser19, and Ser31 in cortic body, but IH is much less effective than SH (22). Short-term or very brief IH challenges in PC-12 cells increased TH activity by a mechanism involving serine phosphorylation without significant alterations in TH protein level (28). In addition, acute postnatal exposure to IH altered brain dopaminergic markers but did not induce changes in TH expression in a rat model (8). However, the effect of longer term IH and SH exposures on TH expression and regulation in the rat brain remains to be examined.

In the present study, we examined whether chronic IH and SH differentially regulate TH activity in different rat brain regions associated with cardiorespiratory or neurocognitive function. Selective increases in TH activity in the cortical region were identified with hypoxic exposure, particularly after SH. Furthermore, hypoxic regulation of cortical TH expression and of its Ser40 phosphorylation differed between long-term exposures to SH or to IH.

MATERIALS AND METHODS

Animal exposures. Animal experimental protocols were approved by the University of Louisville Institutional Animal Care and Use Committee and are in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Adult male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and were exposed in computer-controlled, commercially designed environment (30 in. × 20 in. × 20 in.; Oscycycler model A44XO, BioSpherix, Redfield, NY) to either normoxia [room air (RA); 21% O2], SH (10% O2), or IH (oscillating 10%–21% O2 every 90 s) for 6 h, 1 day, 3 days, 7 days, or 14 days. Rats were anesthetized and rapidly decapitated, and cortex, frontal cortex, brain stem, CA1, and CA3 regions were harvested and processed for TH activity assay. Cortical and brain stem tissue was also dissected and processed for either extraction of total cell lysate proteins, or for RNA extraction.

TH activity. In vivo TH activity was estimated using a classical and widely used assay of catecholamine biosynthesis, as previously described (33). Briefly, l-DOPA accumulation was measured after inhibition of aromatic amino acid decarboxylase (AADC), the enzyme catalyzing the conversion of l-DOPA to DA, using a specific inhibitor, m-hydroxymercaptohydrazide (MHBH; Sigma, St. Louis, MO). MHBH was injected intraperitoneally (100 mg/kg body wt) 20 min before the animals were killed. At this dose, MHBH is able to fully abolish DOPA decarboxylase activity, leading to DOPA accumulation, linear at least for 20 min, regardless of the brain area (30). Brains were dissected, and cortex, frontal cortex, brain stem, CA1, and CA3 tissues from rats exposed to 7 days of IH, SH, or normoxia (RA) were stored in 0.1 N perchloric acid with 0.1% wt/vol EDTA, and frozen at −80°C until analyzed. TH activity in each tissue was expressed as picomoles per milligram protein of l-DOPA formed during 20 min. Neurochemical determination of l-DOPA tissue content was performed using HPLC as previously described, and l-DOPA accumulation was expressed as picomoles per milligram of protein per 20 min (32). The recovery of DOPA eluted from the initial ion-exchange column and from the final acid-activated alumina columns was 68% as a mean (SE: 4%). The DOPA recovery was determined in each assay by adding to the sample an internal standard, 3,4-dihydroxybenzylamine, which was measured on HPLC chromatogram. The data were then corrected for recovery.

Semiquantitative RT-PCR. Total RNA was prepared from cortical tissue samples using the TRizol reagent (Gibco BRL) and was quantitated spectrophotometrically. RNA (1 µg) from each sample was reverse transcribed using oligo(dT)18 primers for TH and GAPDH (Biosource, Camarillo, CA) and Superscript II-Reverse Transcriptase. cDNA equivalent to 20 ng of total RNA was subjected to subsequent PCR analysis following standard protocol. Controls included RNA subjected to the RT-PCR procedure without addition of reverse transcriptase and PCR performed in the absence of cDNA that always yielded negative results. PCR products were separated on 2% agarose gels containing 10 µg/ml ethidium bromide and photographed using the GelPrint 2000i (Genomic Solution), and densitometric analysis was performed with Densitometer SI (Molecular Dynamics).

Total cell lysate preparation and Western blot analysis. Tissue was homogenized with a tissue tearer in 20 mM Tris-HCl buffer, pH 7.5, containing 2 mM EDTA, 0.5 mM EGTA, 10 µg/ml aprotinin, 20 µg/ml leupeptin, and 1 mM PMSF. The lysate was centrifuged for 10 min at 14,000 rpm at 4°C to remove cellular debris. Protein content was measured using a modified Lowry method (DC-Bio-Rad, Hercules, CA).

TH expression and phosphorylation were assayed by Western blot analysis: 50 µg protein/sample were separated by SDS-PAGE (10% Tris-glycine acrylamide gels, Invitrogen, Camarillo, CA) and Superscript II-Reverse Transcriptionase. cDNA equivalent to 20 ng of total RNA was subjected to the RT-PCR procedure with addition of reverse transcriptase and PCR performed in the absence of cDNA that always yielded negative results. PCR products were separated on 2% agarose gels containing 10 µg/ml ethidium bromide and photographed using the GelPrint 2000i (Genomic Solution), and densitometric analysis was performed with Densitometer SI (Molecular Dynamics).

Two-dimensional gel analysis of TH phosphorylation: Tissue was homogenized in ~5 volumes of RIPA buffer (PBS, 1% NP-40, 0.5% deoxycholate, 1% SDS, 1 mM sodium orthovanadate, 0.5 mM PMSF, 10 µg/ml aprotinin, and 20 µg/ml leupeptin). Proteins (150 µg) were separated by two-dimensional gel electrophoresis (Invitrogen, Carlsbad, CA). Proteins were first separated by isoelectric focusing using a pH range of 4–7 running precast immobilized pH gradient strips as first dimension (Zoom, Invitrogen), according to manufacturer’s instructions. The strip was then loaded on NU-PAGE second-dimension mini-gel (Invitrogen) to separate proteins by molecular weight. After
electrophoresis, proteins were transferred and membranes immunoblotted with a TH antibody (Protos, Burlingame, CA).

Data analysis. Data in the text and Figs. 1 and 4 are expressed as means ± SE. Means were compared with the mean values in normoxic animals (RA) using t-test analysis. Differences were considered statistically significant for \( P < 0.05 \).

RESULTS

**Hypoxia regulates TH activity in selected areas of the brain.** To determine whether the intermittent character of the hypoxic stimulus affected TH activity, we selected a 7-day exposure, to allow for recovery from acute effects of hypoxia, and compared the effects of IH or SH on TH activity in the various brain regions. Because of the alternating profile characteristic of IH, the overall duration of hypoxia is significantly lower in IH than in SH. However, IH is physiologically more detrimental than SH (15, 22). On the basis of these observations, we hypothesized that the intermittent character of IH may affect brain TH response to hypoxia, resulting in enhanced neuronal vulnerability. Therefore, we compared TH activity in different areas of the brain from animals exposed to 7 days of SH, IH, or normoxia. Exposure to 7 days of SH significantly increased TH activity in the cortex, as measured by DOPA accumulation during 20 min after inhibition of aromatic amino acid decarboxylase [17 ± 2.6 (SH) vs. 10 ± 1.3 (RA) pmol DOPA/mg protein; \( P < 0.05; n = 8 \); Fig. 1]. Exposure to 7 days of IH induced a slight, albeit not statistically significant, increase in cortical TH activity (\( P = \) not significant; \( n = 8 \); Fig. 1). In the brain stem, 7 days of SH decreased TH activity [2.8 ± 0.29 (SH) vs. 7 ± 0.55 (RA) pmol DOPA/mg protein; \( P < 0.0001; n = 8 \); Fig. 1], whereas 7 days of IH did not significantly affect TH enzymatic activity (Fig. 1). No significant changes were apparent in the frontal cortex, CA1, or CA3 hippocampal regions after 7 days of IH or SH (\( n = 8 \); Fig. 1). Thus long-term SH modulates TH activity in selected areas of the brain, whereas IH does not significantly affect TH activity. These findings suggest that hypoxic modulation of TH brain activity exhibits regional and stimulus specificity.

**Time course of hypoxia-induced changes in cortical TH expression and Ser\(^{40}\) phosphorylation.** To examine whether cortical changes in activity were a consequence of hypoxia-induced changes in TH expression, alterations in TH mRNA expression relative to RA were assessed in the cortex after 1, 3, 7, or 14 days of SH or IH, by semiquantitative RT-PCR. Both IH and SH increased TH mRNA expression, relative to basal expression in RA, starting at 1 day for IH and at 3 days for SH, and this persisted until 14 days for both IH and SH (Fig. 2). To determine whether these changes in mRNA expression correlated with parallel changes in protein expression, cortical proteins were resolved by SDS-PAGE, and TH protein expression and its Ser\(^{40}\) phosphorylation were assessed by Western
moderately increased cortical TH Ser40 phosphorylation (Fig. 5). Changes in TH activity could correlate with changes in Ser40, suggesting multiple phosphorylation sites. To confirm whether catecholamine neurotransmitter system 

To corroborate these findings, we further examined TH phosphorylation in the cortex of rats exposed to 7-day SH or 7-day IH, by separating proteins by two-dimensional gel electrophoresis to allow for adequate electrophoretic separation of the different TH phosphorylated species. The first-dimension gel, or isoelectric focusing, will concentrate the different TH species according to their isoelectric point. Increased phosphorylation will result in a shift toward the acidic part of the pH gradient. The isoelectric point of unphosphorylated TH is 5.74 and decreases with each additional phosphorylation, reaching 5.28 with eight phosphorylated sites, slightly shifting the mobility of the protein toward the acidic part of the gradient. The different phosphorylated TH species can then be detected by SDS-PAGE second-dimension gel and immunoblotting with a TH antibody. Fig. 5 shows the area of the second-dimension gel containing the TH immunoreactivity spots. Differences in the relative abundance of TH immunoreactivity spots migrating at different isoelectric points were detected. In agreement with the Western blot data, TH was more extensively phosphorylated in SH-exposed than in IH-exposed rats, as evidenced by the increased amount of immunoreactivity shifted toward the more acidic area of the gel [Fig. 5, middle (IH) and (bottom) (SH)], whereas in RA the highest level of TH immunoreactivity was found in the more basic area of the gel [Fig. 5, top (RA)]. These findings indicate that IH and SH exposure induce a different pattern of TH phosphorylation, and differentially regulate TH activity.

**DISCUSSION**

Catecholamines are important neurotransmitters, ubiquitously distributed, and released in the circulation, thereby contributing to stress-induced responses and extending their physiological roles beyond those of neurotransmitters. Regulation of catecholamine synthesis plays a role in brain adaptation to hypoxic stress and may differ according to the type and extent of hypoxia. In the present study, long-term SH induced increased TH activity in the cortex, a region of substantial hypoxic susceptibility, whereas decreased TH activity occurred in the more hypoxia-resistant dorsal brain stem area. These findings suggest that intrinsic properties of neuronal susceptibility to hypoxia may trigger a defense response in the cortex that is, at least in part, mediated by induction of the catecholaminergic system. In contrast, in hypoxia-tolerant brain regions such as the brain stem, neurons may not require rapid induction of the stress-activated catecholamine response to hypoxia and may downregulate TH activity to prevent DA-induced cellular injury. Furthermore, modulation of TH activity in brain stem nuclei may also reflect the prominent role

**Fig. 2.** Expression of TH (top) and GAPDH (bottom) mRNA in the cortex of rats exposed to normoxia or 1 day (1d), 3 days (3d), 7 days (7d), and 14 days (14d) of IH or SH, as determined by RT-PCR. Representative gel of PCR products separated on a 2% agarose gel and visualized by ethidium bromide fluorescence (n = 3 rats) is shown.

**Fig. 3.** Protein expression (TH) and Ser40 phosphorylation (Phospho-TH), analyzed by SDS-PAGE and immunoblotting, using antibodies to TH and Ser40-phospho-TH in cortical (left) and brain stem (right) of proteins from rats exposed to normoxia or 6 h (6h), 1 day, 3 days, and 7 days of SH or IH. Membranes were reprobed for β-actin to show equal sample loading. Representative data are shown (n = 4 rats).
of this brain region in respiratory and cardiovascular control. Indeed, several studies have identified increases in TH expression in specific substructures of the brain stem after SH exposure, more specifically in those regions involved in respiratory and baroreflex activity (1, 43, 46). However, our data show no change in TH expression or phosphorylation in brain stem, whereas in the cortex, posttranslational modifications and changes in TH activation occurred in SH but not in IH.

Although studies addressing the temporal dependencies of TH regulation during hypoxia are scarce, TH expression was recently examined in fetal sheep brain. Acute hypoxic exposures increased TH expression in the brain stem; however, sustained SH exposure, lasting throughout pregnancy, failed to modify TH expression (1). In contrast, decreased TH immunoreactivity was found in the brain stem of sudden infant death syndrome deceased infants, a condition in which a putative pathophysiological role has been related to hypoxia and more specifically to IH (23, 32, 40). The emerging differences in TH expression relative to the type of hypoxic exposure were attributed to the nature of the hypoxic stimulus per se (i.e., sustained or repeated) as well as to other potentially contributing factors such as immaturity of neurons underlying cardiovascular regulation (40). Of note, the studies of both Pepin et al. (43) and Ozawa and Takashima (40) failed to measure TH enzymatic activity. Furthermore, the discrepancies in the detection of hypoxia-induced changes in TH expression in the different studies may result from differences in the hypoxic profiles and duration, as well as from the different methods used for the determination of TH expression (mRNA vs. protein vs. activity). Thus decreased TH activity in the brain stem, as identified in our present study, may as well reflect alterations induced by SH on brain stem regions mediating respiratory and cardiovascular function. In contrast, IH failed to induce a similar catecholamine response or alternatively did not modify catecholamine turnover. The absence of such changes could in turn contribute to the enhanced IH-induced neuronal vulnerability in the cortical brain regions. However, neither SH nor IH affected TH activity in the CA1 and CA3 hippocampal regions, even though these regions markedly differ in their sensitivity to hypoxia (14, 26, 27). Thus inappropriate catecholamine response may contribute to increase the neuronal susceptibility to hypoxia in certain areas of the brain, whereas in other brain regions, the catecholamine response may not be critical, or may be compensated by other defense and/or adaptive mechanisms.

Although TH mRNA was moderately increased in both SH- and IH-exposed animals, there was no significant difference in
SH- or IH-induced TH protein expression. This discrepancy
between TH mRNA content and its immunoreactivity has been
previously described in transgenic mice overexpressing human
TH, with ~50-fold induction in brain mRNA but only a 2- to
3-fold increase in immunoreactivity, suggesting that posttran-
scriptional regulation of TH is critical to its function (24).

TH is the rate-limiting enzyme in the catecholamine biosyn-
thesis pathway, and catalytic activity of other enzymes in this
pathway, such as AAADC and dopamine-β-hydroxylase
(DBH), is considered to be high relative to TH (9). However,
catecholamine turnover may be affected by the regulation of
other enzymes, such as AAADC, DBH, or phenylethano-
lamine-N-methyl transferase. Furthermore, changes in the rel-
ative rates of catecholamine degradation, or differences in the
fluctuations of the activity and stability of such enzymes, may
also markedly alter catecholamine levels in various brain
tissues and may regulate TH activity by substrate inhibition,
allosteric enzyme regulation, or activation of inhibitory or
stimulatory signaling pathways (9, 44). Therefore, interactions
with additional hypothia-modulated signaling pathways may
underlie the regional differences in catecholamine response
observed in our study. Our findings suggest the catecholamin-
ergic regulation may also play a role in the regulation of these
pathways, and additional studies will be required to elucidate
these complex interactions.

TH or DBH deletion in knockout mice results in perinatal
lethality, indicating that both these enzymes are critical to
normal development. Accumulation of DA and l-DOPA can
lead to neuronal cell death by both oxidative and nonoxidative
mechanisms (15, 42, 50). In addition to being a precursor of
NE and epinephrine, DA serves as an important and rather
ubiquitous neurotransmitter. However, at high concentrations,
DA may induce apoptosis in many neuronal and nonneuronal
cells, including chick sympathetic neurons, PC-12, and pri-
mary neurons, and such phenomenon has also been docu-
mented in vivo (50). The enhanced DA production resulting
from increased cortical TH activity in SH that is not associated
with increased apoptosis may reflect the concomitant induction
of anti-apoptotic pathways such as growth factor-induced sig-
aling pathways. Miyazaki et al. (37) recently reported that
glial-derived growth factor modulates TH expression in the rat
hippocampus after transient forebrain ischemia to allow dopa-
mineergic neuronal activity while controlling excess TH expres-
ion that may lead to DA-induced neurotoxicity. Our labora-
tory has recently reported that sustained hypoxia elicits an
autocrine-paracrine growth factor response, secreting growth
factor and initiating a survival response in RN-46A neuronal
cells exposed to severe hypoxia (52). Thus growth factor
release may contribute to neuroprotection by moderating
TH upregulation, allowing dopaminergic neurons to mount an
appropriate response to hypoxic stress but preventing excessive
DA release. The equilibrium between these different pathways
determine the outcome of the injury in a specific brain
area.

In addition to transcriptional and translational regulation,
posttranslational modifications of TH have recently been de-
scribed as an essential step in TH activity regulation. TH
phosphorylation by a variety of kinases is the main posttrans-
lational mechanism regulating TH enzymatic activity. How-
ever, it is still unclear whether phosphorylation of the different
sites is independently regulated and whether the different
phosphorylations have antagonistic or synergistic effects on
TH enzymatic activity. In this study, we show that, in specific
areas of the rat brain, a very moderate TH phosphorylation
induced by IH results in unchanged or decreased TH activa-
tion, whereas more extensive phosphorylation, as occurring in
the cortex after SH exposure, results in increased TH activity.
Identification of these phosphorylation sites and the order of
phosphorylation events required for TH activation remain to be
eclucidated. Phosphorylation on different sites of the TH mol-
ecule requires activation of several kinases. Both IH and SH
result in genomic and proteomic alterations in neural tissue and
cells (14, 25, 35, 36, 41). Hypoxic durations in IH are charac-
teristically reduced compared with SH; however, IH induces
greater neurotoxicity that may be due to its recurrent hypoxia-
reoxygenation characteristics. Our laboratory has previously
shown that, in PC-12 cells, IH induces different cell death
pathways than SH (15), and others have shown that TH
phosphorylation is impaired in IH in these cells (28). Further-
more, our laboratory has shown differential TH regulation by
IH and SH in vivo, using a rat model (22). Thus, whereas SH
allows upregulation of specific genes and proteins to induce
hypoxic adaptation, and recruit multiple protein kinases acting
in concert to phosphorylate and activate TH. IH exposure,
because of its intrinsic intermittent characteristic, may not be
able to induce a similarly orchestrated adaptive response. Thus
IH could result in disorganized and improper kinase activation,
whereas SH could induce a coordinated response, activating
the different pathways required for TH phosphorylation and
activation. Carefully balanced rates of catecholamine biosyn-
thesis and degradation, exquisitely regulated by TH activity,
appear to be critical to the integrity of the nervous system
during long-term hypoxic stress. Furthermore, regionally spe-
cific requirements and fluctuations in TH activity may be
related to their respiratory and cardiovascular response to
hypoxia. In contrast to cortical areas, catecholamine response
does not appear to be required in the more hypoxia-tolerant
brain stem. The lack of hypoxic induction of TH phosphory-
lation in the brain stem coincides with a decrease in TH
activity, which does not result in increased susceptibility to
hypoxia in this notoriously more resistant area. Although TH
activation appears to contribute to the adaptive response to
hypoxia in the cortex, this mechanism is not recruited in the
brain stem. Thus TH and catecholamine response to hypoxia
appear to be elicited in response to hypoxia in more vulnerable
areas of the brain rather than playing a role promoting this
increased regional vulnerability. The ability of cortical cells to
upregulate hypoxia-induced defense mechanisms and regulate
TH activity may underlie the differences between their adap-
tation to IH and SH, whereas other mechanisms may be
recruited in different brain regions. Defining these pathways
can be critical for the understanding of our present findings
and to avoid oversimplified interpretations of the role of TH
regulation in the hypoxic response. Thus various brain regions
may have developed a variety of mechanisms that regulate
hypoxic adaptation and tolerance and that interact to preserve
brain integrity and function in pathophysiological conditions
involving long-term SH or IH.

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