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

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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 (11). Nonetheless, 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 (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 (11, 15).

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at least eight different protein kinases phosphorylate TH in vitro (18, 29). Ser\(^{19}\) 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 Ser\(^{19}\) phosphorylation facilitates the phosphorylation of Ser\(^{40}\) in vitro (3). Ser\(^{31}\) 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 Ser\(^{40}\) phosphorylation, PKA does so at a higher stoichiometry (28, 29).

Furthermore, DA receptor D\(_{2}\) inhibition of Ser\(^{19}\) phosphorylation, without affecting Ser\(^{11}\) and Ser\(^{31}\) phosphorylation, results in the inhibition of 3,4-dihydroxyphenylalanine (DOPA) accumulation, suggesting that Ser\(^{40}\) 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 Ser\(^{31}\), Ser\(^{19}\), and Ser\(^{31}\) 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 Ser\(^{40}\) phosphorylation differed between long-term exposures to SH or to IH.

**MATERIALS AND METHODS**

*Animal exposure.* 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, com-

**TH activity.** In vivo TH activity was estimated using a classical and widely used assay of catecholamine biosynthesis, as previously described (33). Briefly, 1-DOPA accumulation was measured after inhibition of aromatic amino acid decarboxylase (AAADC), the enzyme catalyzing the conversion of L-DOPA to DA, using a specific inhibitor, m-hydroxyphenylhydrazine (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 1-DOPA formed during 20 min. Neurochemical determination of 1-DOPA tissue content was performed using HPLC as previously described, and 1-DOPA accumulation was expressed as picomoles per milligrams 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.

**Semi-quantitative 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)\(_{20}\) primers for TH and GAPDH (Biosource, Camarillo, CA) and Superscript II-Reverse Transcribease. 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 aproitin, 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, Carlsbad, CA) and transferred on a 0.2-μm nitrocellulose membrane. Membranes were blocked for 1 h in a 5% nonfat dry milk solution in Tris-buffered saline-Tween and incubated overnight at 4°C with specific antibodies against TH (Protos, Burlingame, CA; or Zymed, San Francisco, CA) and phospho-TH (Ser\(^{40}\)) (Cell Signaling Technology, Beverly, MA; or Zymed). Membranes were washed, incubated 1 h with horseradish peroxidase-labeled goat anti-rabbit or anti-mouse antibody as appropriate, and washed extensively. Proteins were visualized by enhanced chemiluminescence (Amersham/Pharmacia, Piscataway, NJ) and analyzed by scanning densitometry. To verify equal transfer of proteins, Ponceau-S staining of the nitrocellulose membranes was performed. Equal loading of proteins was verified by reprobing the membranes with a β-actin antibody (Sigma).

**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 aproitin, 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 over 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 immuno-
blotted 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 nor-
moxic 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 com-
pared 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 detrimen-
tal 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 decar-
boxylase [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 Ser40 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 corre-
lated with parallel changes in protein expression, cortical
proteins were resolved by SDS-PAGE, and TH protein expres-
sion and its Ser40 phosphorylation were assessed by Western

Fig. 1. Tyrosine hydroxylase (TH) activity estimated by measure of L-3,4-dihydroxyphenylalanine (DOPA) accumulation during 20 min after aromatic amino
acid decarboxylase enzymatic inhibition in cortex, frontal cortex, brain stem, CA1, and CA3 from rats exposed to 7 days of normoxia (room air (RA)), intermittent
hypoxia (IH), or sustained hypoxia (SH). Values are means ± SE; n = 8 rats. Data are expressed as picomoles DOPA per milligram protein per 20 min. *P <
0.05.
moderately increased cortical TH Ser40 phosphorylation (Fig. 3, left). IH moderately increased cortical TH Ser40 phosphorylation (Fig. 3, left, and Fig. 4, top right). In contrast, SH significantly induced TH phosphorylation at 7 days (Fig. 3, left, and Fig. 4, top left). Multiple bands with close molecular weights, indicating several species of phosphorylated TH, were detected, suggesting multiple phosphorylation sites. To confirm whether changes in TH activity could correlate with changes in Ser40 TH expression, we also examined the time course of TH expression and phosphorylation in the brain stem. Total TH expression remained unchanged during a time course of IH or SH (Fig. 3, right). In contrast to our findings in cortex, SH did not induce significant changes in Ser40 phospho-TH expression in brain stem, although a decrease trend was detected in some of the experiments (Fig. 3, right, Fig. 4, bottom). Thus the decrease in TH activity in the brain stem of SH-exposed animals did not correlate with a significant decrease in TH phosphorylation. However, increased TH activity in the cortex of SH animals may result from increased TH phosphorylation in this brain region; in the absence of TH phosphorylation increase in the brain stem, SH did not induce an increase in brain stem TH activity. Therefore, SH may differentially affect TH phosphorylation compared with IH in the cortex but not in the brain stem, suggesting that differential catecholaminergic response to hypoxia may be required in these brain regions.

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.

![Image](https://example.com/image1.png)

**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.

![Image](https://example.com/image2.png)

**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).

![Image](https://example.com/image3.png)

**Fig. 4.** Protein expression (TH) and Ser40 phosphorylation (Phospho-TH), analyzed by SDS-PAGE and immunoblotting, using antibodies to TH and Ser40-phospho-TH in cortical (top left and top right) and GAPDH (bottom left and bottom right) mRNA in the cortex of 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 = 3 rats).

![Image](https://example.com/image4.png)

**Fig. 5.** Western blot analysis of TH protein expression and phosphorylation in the brain stem of 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 = 3 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 posttranscriptional regulation of TH is critical to its function (24).

TH is the rate-limiting enzyme in the catecholamine biosynthesis 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 phenylethanolamine-N-methyl transferase. Furthermore, changes in the relative 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 hypoxia-modulated signaling pathways may underlie the regional differences in catecholamine response observed in our study. Our findings suggest that the catecholaminergic 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 primary neurons, and such phenomenon has also been documented 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 signaling 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 dopaminergic neuronal activity while controlling excess TH expression that may lead to DA-induced neurotoxicity. Our laboratory 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 may 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 described as an essential step in TH activity regulation. TH phosphorylation by a variety of kinases is the main posttranslational mechanism regulating TH enzymatic activity. However, 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 activation, 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 elucidated. Phosphorylation on different sites of the TH molecule 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 characteristically 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). Furthermore, 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 kinetic activation, whereas SH could induce a coordinated response, activating the different pathways required for TH phosphorylation and activation. Carefully balanced rates of catecholamine biosynthesis 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 specific 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 phosphorylation 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 adaptation to IH and SH, whereas other mechanisms may be recruited in different brain regions. Defining these pathways may 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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