Hypoxia. 3. Hypoxia and neurotransmitter synthesis

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Submitted 20 January 2011; accepted in final form 20 January 2011

Kumar GK. Hypoxia. 3. Hypoxia and neurotransmitter synthesis. Am J Physiol Cell Physiol 300: C743–C751, 2011. First published January 26, 2011; doi:10.1152/ajpcell.00019.2011.—Central and peripheral neurons as well as neuroendocrine cells express a variety of neurotransmitters/modulators that play critical roles in regulation of physiological systems. The synthesis of several neurotransmitters/modulators is regulated by O2-requiring rate-limiting enzymes. Consequently, hypoxia resulting from perturbations in O2 homeostasis can affect neuronal functions by altering neurotransmitter synthesis. Two broad categories of hypoxia are frequently encountered: continuous hypoxia (CH) and intermittent hypoxia (IH). CH is often seen during high altitude sojourns, whereas IH is experienced in sleep-disordered breathing with recurrent apneas (i.e., brief, repetitive cessations of breathing). This article presents what is currently known on the effects of both forms of hypoxia on neurotransmitter levels and neurotransmitter synthesizing enzymes in the central and peripheral nervous systems.

THE MAMMALIAN BRAIN represents 2% of the total body weight and consumes 20% of the total body oxygen for its normal function (108). This high level of oxygen consumption makes the brain an extremely sensitive tissue to changes in arterial blood oxygen levels. Many physiological systems are regulated by the nervous system, and neurotransmitters are critical for neuronal function. The rate-limiting enzymes associated with the synthesis of several neurotransmitters require molecular oxygen for their activity. Consequently, hypoxia (i.e., reduced oxygen availability) resulting from perturbations in O2 homeostasis can profoundly impact neurotransmitter synthesis resulting in altered neuronal functions and consequently impact physiological systems.

Hypoxia is experienced in a variety of physiological and pathological conditions. Two broad categories of hypoxia are most frequently encountered: continuous hypoxia (CH) and intermittent hypoxia (IH). For instance, CH is experienced by healthy humans during high altitude sojourns, whereas IH is encountered during sleep-disordered breathing with recurrent apneas; i.e., brief repetitive cessations of breathing wherein each episode lasts for tens of seconds. The purpose of this article is to provide a brief review of the effects of both forms of hypoxia on neurotransmitter levels, rate-limiting enzymes associated with neurotransmitter synthesis and the underlying mechanisms. Several of these molecules, which function as transmitters/modulators in the nervous system, are also expressed in nonneuronal tissues. However, because of space constraints, this review will focus on the effects of hypoxia on neurotransmitters in the central and peripheral nervous systems.

Based on the chemical properties, neurotransmitters are divided into various groups as shown in Table 1. This review focuses on the impact of CH and IH on the synthesis of biogenic amines, acetylcholine, excitatory and inhibitory amino acids, bioactive peptides, and gasotransmitters (for examples see Table 1).

BIOCHEMICALLY Active Small Molecules

Examples include cotransmitters/modulators that play critical roles in regulation of physiological systems. The synthesis of several neurotransmitters/modulators is regulated by O2-requiring rate-limiting enzymes. Consequently, hypoxia resulting from perturbations in O2 homeostasis can affect neuronal functions by altering neurotransmitter synthesis. Two broad categories of hypoxia are frequently encountered: continuous hypoxia (CH) and intermittent hypoxia (IH). CH is often seen during high altitude sojourns, whereas IH is experienced in sleep-disordered breathing with recurrent apneas (i.e., brief, repetitive cessations of breathing). This article presents what is currently known on the effects of both forms of hypoxia on neurotransmitter levels and neurotransmitter synthesizing enzymes in the central and peripheral nervous systems.

Impact of Hypoxia on Catecholamines

Catecholamines comprising dopamine (DA), norepinephrine (NE), and epinephrine (Epi) are expressed in many regions of the brain, adrenal medulla, and the carotid body, which is a peripheral neuronal sensory organ specialized for detecting changes in arterial blood O2. In addition to their roles in development and energy metabolism, catecholamines play important roles in regulation of cardio-respiratory functions both centrally and peripherally (16, 109). Catecholamines are synthesized enzymatically from L-tyrosine through a sequence of reactions as outlined in Table 2. Tyrosine hydroxylase (TH) expressed in the cytosol catalyzes hydroxylation of L-tyrosine to produce L-dihydroxyphenylalanine (L-Dopa). L-Dopa is then enzymatically converted to DA, NE, and Epi by the sequential actions of cytosolic L-aromatic amino acid decarboxylase (AADC), vesicular dopamine-β-hydroxylase (DBH), and cytosolic phenylethanolamine-N-methyltransferase (PNMT), respectively.

The effects of CH, lasting minutes to hours, on catecholamine synthesis were investigated in various brain regions, adrenal medulla, and carotid bodies. In rats, short-term exposure (30 min) to either moderate (10 or 12% O2) or severe hypoxia (6% O2) during different stages of development (postnatal days P0 to P28) produced an initial decrease in brain catecholamine synthesis, which returned to control levels despite continuing the hypoxic challenge (11, 12, 14, 41, 42). A similar biphasic response of catecholamine synthesis to CH (7.5% O2 for 4–12 h) was also reported in the adrenal medulla of guinea pigs and rats (110). On the other hand, CH (10% O2 for 3 h) facilitates DA and NE synthesis in rabbit and rat carotid bodies, an effect seen only with tyrosine but not with DOPA as substrate, suggesting a role for altered TH activity in CH-induced facilitation of catecholamine synthesis (22, 23). These studies suggest that CH exerts differential effects on catecholamine synthesis in the central (brain) versus peripheral nervous system (i.e., carotid body).

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The impact of more prolonged form of CH, lasting days to weeks, on catecholamine levels was investigated in the rat carotid body. Exposure to either normobaric (10% O2 for up to 28 days) or hypobaric (0.45 atm; high altitude simulation for 7 days) hypoxia elevated DA and NE levels (37, 84, 88, 113). After 28 days of normobaric CH, there was a 27- and 51-fold increase in DA and NE levels, respectively (88).

The effects of IH, consisting of alternating cycles of 5% O2 for 15 s followed by 21% O2 for 5 min for 8 h/day (86) on catecholamine levels in rat brainstem and adrenal medulla were studied. Exposure to 10 days of IH significantly increased DA levels in rat brainstem (98) and NE levels in adrenal medulla (57) compared with normoxic controls. Similarly, IH (1% O2 for 15 s and 21% O2 for 4 min; 60 cycles) also increased DA levels in pheochromocytoma 12 (PC12) cell cultures (56). These studies demonstrate that both CH and IH elevate tissue and cellular levels of DA and NE.

Effects of Hypoxia on Catecholamine Synthesizing Enzymes

TH is the rate-limiting enzymes of catecholamine synthesis and DBH is the NE-synthesizing enzyme (Table 2). Both enzymes require molecular oxygen for their catalytic activity (24). Consequently, several studies examined the effects of hypoxia on TH and DBH.

Tyrosine hydroxylase. CH (10% O2 for 1–14 days) increased TH activity in the rat carotid body (36, 45) and brain cortex (34). The CH-induced increase in TH activity in the carotid body was associated with an upregulation of TH protein expression (45, 47, 117, 118). CH also elevated TH mRNA levels in the rat carotid body, which can be seen as early as 6 h of CH treatment (9). Studies on PC12 cell cultures showed that CH increases the TH gene transcription as well as the stability of TH mRNA. The increased TH transcription by CH requires activation of hypoxia-inducible transcription factors (HIFs) that interact with a specific hypoxia-responsive element (HRE) in the TH promoter region (10, 103).

In addition to its effect on transcription, CH (10% O2 for 30 days) affects TH activity via posttranslational mechanisms. Phosphorylation of serine-19, serine-31, and serine-40 at the NH2-terminal regulatory domain of TH alters the $K_m$ and maximal velocity ($V_{max}$) leading to more active form of TH with increased activity (19, 25). CH increased phosphorylation of serine-19, serine-31 and serine-40 residues in TH in rat carotid bodies but not in adrenal medullae and superior cervical ganglion (45). A similar increase in TH phosphorylation was also reported in the cortex and brainstem of rats exposed to CH (10% O2 for 14 days) (34). The mechanisms underlying increased phosphorylation of TH by CH, however, are not known.

Studies on PC12 cell cultures revealed that IH (1% O2 for 15 s and 21% O2 for 4 min; 60 cycles) progressively increased TH activity (56). Although IH increased TH mRNA levels via HIF-1 activation (123), TH protein levels were unaffected in IH-treated PC12 cells (56, 123). Despite unaltered TH protein, IH markedly increased TH activity. The IH-induced increase in TH activity is primarily due to its effects on posttranslational modification involving phosphorylation of TH at serine-40 mediated by calcium/calmodulin-dependent protein kinase (CaMK) and protein kinase A (PKA) (56). Studies in rats showed that IH (5% O2 for 15 s followed by 21% O2 for 5 min for 8 h/day; termed as “15 s IH”) also increases TH activity in the dorsal and ventral medullary but not in the pontine regions of the brainstem (98). IH increased TH activity in brainstem regions via phosphorylation at serine-31 and serine-40 residues without altering TH protein expression (98). However, the effects of IH are critically dependent on the severity of hypoxia and duration of hypoxia-reoxygenation of the IH paradigm. Thus exposing rats to 10% O2 and 21% O2 for 90 s each for 12 h/day (termed as “90 s IH”; 34) had virtually no effect either on TH activity or on TH phosphorylation in the brainstem regions as opposed to robust activation observed with 15 s IH (98).

Table 2. Enzymatic reactions associated with the synthesis of biogenic amines

<table>
<thead>
<tr>
<th>Transmitters</th>
<th>Synthesizing Enzyme(s)</th>
<th>Enzymatic Reactions</th>
<th>Requirement for Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>Tyrosine hydroxylase</td>
<td>Hydroxylation: l-Tyrosine $\rightarrow$ 3,4-dihydroxyphenylalanine $+$ H2O</td>
<td>Tetrathiodofluorobetain (BH4), Fe2+, and O2</td>
</tr>
<tr>
<td></td>
<td>l-Aromatic amino acid</td>
<td>Decarboxylation: 3,4-dihydroxyphenylalanine $\rightarrow$ Dopamine $+$ H2O</td>
<td>Pyridoxal 1-phosphate (PLP)</td>
</tr>
<tr>
<td></td>
<td>decarboxylase (AADC)</td>
<td></td>
<td>Cu2+, O2, and ascorbic acid</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Dopamine-β-hydroxylase</td>
<td></td>
<td>S-Adenosylmethionine</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Phenylenalanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Hydroxy tryptamine (5-HT, serotonin)</td>
<td>Tryptophan hydroxylase</td>
<td>Hydroxylation: l-Tryptophan $\rightarrow$ 5-hydroxytryptophan</td>
<td>BH4 $+$ O2</td>
</tr>
<tr>
<td></td>
<td>(rate-limiting) AADC</td>
<td>Decarboxylation: 5-hydroxytryptophan $\rightarrow$ 5-HT</td>
<td>PLP</td>
</tr>
</tbody>
</table>

AJP-Cell Physiol • VOL 300 • APRIL 2011 • www.ajpcell.org
Raghuraman et al. (98) examined the mechanisms underlying IH-induced TH phosphorylation. These authors found that 15 s IH increases reactive oxygen species (ROS) generation and the ensuing ROS signaling facilitates PKA, CaMK, and ERK activation with a simultaneous inhibition of protein phosphatase 2A activity in the brainstem. The resulting imbalance between kinase and phosphatase activities sustains phosphorylation of TH at multiple serine residues. The effects of IH on TH phosphorylation, like its effect on TH activity, depend on IH paradigm. Thus 90 s IH paradigm leads to decreased TH phosphorylation in the brainstem with a modest augmentation in the cerebral cortex (34). A comparison of ROS levels revealed that ROS generation is significantly lower in 90 s IH than with 15 s IH paradigm in rat brainstem (98), suggesting that 90 s IH is less potent than 15 s IH paradigm in stimulating ROS generation in the brainstem. However, 90 s IH increased TH phosphorylation at serine-19, serine-31, and serine-40 in the rat carotid body, without affecting TH phosphorylation levels in other catecholamine expressing peripheral tissues including superior cervical ganglion and adrenal medulla (45). It is likely that the stimulatory effects of 90 s IH on the carotid body can be attributed to greater hypoxic sensitivity of the carotid body relative to other tissues. Nonetheless, the above results suggest that IH, depending on the duration and severity of hypoxia and period of reoxygenation, induces oxidative stress, which via increased serine phosphorylation activates TH and augments catecholamine synthesis. Thus the effects of IH on TH activity primarily involve posttranslational modifications rather than transcriptional mechanisms. Since IH-mediated activation of TH is coupled to elevated DA levels in the medullary brainstem regions, it is conceivable that IH-evoked changes in DA may, in part, contribute to cardiorespiratory abnormalities associated with recurrent apneas.

Dopamine-β-hydroxylase. Exposure of ewes to high altitude (3, 820 m) from 30 to 138 days gestation reduced DBH protein and mRNA expression in the adrenal medulla (17). In contrast, exposure to 15 s IH paradigm (96) but not 90 s IH paradigm (45) increased DBH protein in the rat adrenal medulla with a concomitant increase in NE levels (57). Future studies are, however, needed to determine whether IH-induced increases in NE levels in the adrenal medulla is due to upregulation of DBH activity and changes in DBH mRNA.

Effects of Hypoxia on 5-HT Synthesis

5-HT, another well-established monoamine neurotransmitter, is synthesized from L-tryptophan via two enzymatic reactions involving tryptophan hydroxylase (TPH) and AADC (Table 2). The TPH-mediated reaction is the rate-limiting step in 5-HT biosynthesis and requires molecular oxygen (48). Two isoforms of TPH, i.e., TPH1 and TPH2, are known. While TPH1 is expressed in several tissues, TPH2 is expressed only in the brain (115).

As short as 30 min of CH decreased TPH activity in many brain regions in neonatal and adult rats (13, 40). On the other hand, prolonged exposures to CH affected TPH activity in the brain in a region-dependent manner. Thus CH (10% O2 for 14 days) decreased TPH activity in the dorsal and median raphe, striatum, dorsomedian medulla oblongata, the locus coeruleus, and the anterior hypothalamic nucleus, whereas increased TPH activity in the ventrolateral medulla oblongata and the preoptic area (91). The effects of CH on TPH 1 and TPH 2 isoforms, however, remain to be studied.

ACETYLCHOLINE

Acetylcholine (ACh) functions as an important neurotransmitter in the autonomic nervous system, central nervous system (CNS), and at the neuromuscular junction. ACh is synthesized in the cytosol from acetyl-coenzyme A (formed via mitochondrial oxidation of pyruvate and transported via an acyl carrier into the cytosol) and choline (produced via lipid metabolism) by the catalytic action of choline acetyltransferase. ACh synthesis was assessed by monitoring the incorporation of [3H]choline and [14C]glucose into ACh.

CH (10% O2), as short as 15 min, decreases ACh synthesis in adult and developing rat brains (5, 27, 28, 30 55). The mechanisms and functional consequences of CH-evoked decreases in ACh synthesis have not been elucidated. Since the acetyl group of ACh is derived from pyruvate oxidation and pharmacological inhibition of pyruvate oxidation with either bromopyruvate or 2-keto acids reduced ACh synthesis (29), it is conceivable that reduction in precursor availability might have contributed to the decreased ACh synthesis by CH. Also, little is known on the consequences of prolonged CH (for days) on ACh synthesis. Such studies, especially in the brain will be of value in understanding the effects of CH associated with stroke on cholinergic transmission.

On the other hand, IH (consisting of alternating cycles of 1% O2 for 15 s and 21% O2 for 4 min for 60 cycles) had no significant effect on ACh levels in PC12 cell cultures, an oxygen-sensitive cell line derived from rat adrenal medullary tumors (50). Whether IH affects ACh synthesis in intact animals remains to be studied.

EXCITATORY AND INHIBITORY AMINO ACIDS

Amino acids such as glutamate and γ-amino butyric acid (GABA) are synthesized in the central and peripheral nervous systems, and they function as fast-acting neurotransmitters. Glutamate provides the major excitatory drive to neurotransmitter systems, and they function as fast-acting neurotransmitters. Glutamate provides the major excitatory drive to neurotransmitter systems, and they function as fast-acting neurotransmitters.

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Effects of Hypoxia on GABA Synthesis

GABA is synthesized via enzymatic decarboxylation of L-glutamate involving glutamic acid decarboxylase (GAD) and pyridoxal-5-phosphate (PLP) as cofactor. Two forms of GAD, i.e., GAD65 (vesicle associated) and GAD67 (localized in the cytosol) with molecular masses of 65 and 67 kDa, respectively, are known (49). GAD67, which has a greater affinity for PLP than GAD65, exists in vivo in a PLP-bound active form (74). The activities of GAD67 and GAD65 are regulated in vivo via reversible phosphorylation and dephosphorylation reactions (119).

CH (10% O2 for days) increased GABA levels in neurons but not in glial cells (35, 121). Although the effects of CH on GAD activity were not assessed in intact animals, studies on PC12 cells showed that CH (10% O2 for 24 h) increased GAD
activity, which was in part due to increases in GAD65 and GAD67 mRNA as well as protein (53). In striking contrast, GAD activity markedly decreased in IH-treated PC12 cells (97). This decrease in GAD activity by IH was due to cAMP-protein kinase A (PKA)-dependent phosphorylation of GAD67 involving activation of dopamine 1 receptor but not as a result of changes in either GAD67 mRNA or protein expression (97). Thus studies on PC12 cell cultures revealed striking differences in the regulation of GAD activity by CH and IH, wherein the former form of hypoxia stimulates, whereas the later inhibits the enzyme activity. Furthermore, studies by Raghuraman et al. (97) suggest a cross-talk between dopaminergic and GABA-ergic systems contributing to IH-induced GAD inhibition and GABA synthesis.

GABA is involved in the regulation of blood pressure and sympathetic activity (104) and endocrine functions (32, 76, 78, 89). Since GABA is involved in regulation of cardiorespiratory functions, further studies on intact animals might unravel the role of GABAergic system in CH-evoked adaptation versus IH-induced maladaptation of cardiorespiratory systems.

Effects of Hypoxia on Glutamate Synthesis

Glutamate is primarily produced from glutamine by the action of phosphate-activated glutaminase (PAG), a mitochondrial enzyme. Alternatively, glutamate can also be generated in the cytosol via alanine aminotransferase-catalyzed reaction involving α-ketoglutarate and alanine. Glutamate generated in the cytoplasm is transported into vesicles by vesicular glutamate transporters.

Studies in PC12 cell cultures showed that CH (10% O2 for 24 h) decreased PAG activity in a time-dependent manner with a concomitant reduction in extracellular glutamate levels (53). The hypoxia-evoked reduction in PAG activity was in part due to decreased expressions of PAG mRNA and protein. In striking contrast, IH increased PAG activity and glutamate levels in PC12 cell cultures as well as in dorsal and ventral brainstem regions in rats (Kumar and Raghuraman; unpublished observations). This increase in PAG activity was associated with elevated PAG protein levels.

BIOACTIVE PEPTIDES

A variety of bioactive peptides are expressed in the central and peripheral nervous systems. The peptides expressed in neuronal tissues require α-amidation of the carboxy-terminus for their biological activity (21, 77). Examples of α-amidated peptides include neuropeptide Y (NPY) and substance P (SP). These peptides are often referred to as neuropeptides and exert powerful modulatory effects on neuronal transmission.

α-Amidated peptides are synthesized initially as prepro-peptide precursor molecules, which then undergo posttranslational endoproteolytic processing inside the secretory vesicles involving prohormone convertase (PC), cathepsin L (CtsL), carboxypeptidase E (CPE), and peptidyl glycine α-amidating monoxygenase (PAM) as shown in Fig. 1 (left). In the final step of α-amidated peptides synthesis, PAM catalyzes the conversion of the COOH-terminal glycine of glycine-extended peptides to a carboxy terminal amide group.

Effects of Hypoxia on α-Amidated Peptide Synthesis

Neuropeptide Y. NPY is expressed in specific cell bodies and processes of the central and peripheral nervous systems (102, 111). In addition to being a potent vasoconstrictor, NPY also plays important roles in the regulation of food intake (via paraventricular nucleus in the hypothalamus), energy balance (120), and sympathetic neurotransmission (69).

The effects of CH (10% O2 for 14 days) on NPY expression were investigated in the rat brain and carotid bodies (58, 59, 92). CH selectively increased NPY-like immunoreactivity in the ventrolateral medulla oblongata, striatum, and anterior pituitary without affecting NPY expression in other brain regions (92). A similar increase in NPY-like immunoreactivity was also reported in rat carotid bodies exposed to CH (10% O2 for 3 mo) (58, 59, 92). It has been suggested that CH-evoked changes in NPY-like immunoreactivity in the carotid body and specific brain regions may contribute to adaptive mechanisms involving morphological changes in carotid bodies and alterations in sympathetic control and neuroendocrine function, respectively (92).

IH (15 s paradigm) augmented NPY-like immunoreactivity in the rat brainstem (105) and markedly upregulated NPY expression in DBH-expressing adrenal chromaffin cells (96). IH-induced increase in NPY in the adrenal medulla was associated with ROS-dependent upregulation of prepro-NPY mRNA and protein as well as increased prepro-NPY processing. Furthermore, IH-induced increase in blood pressure (57, 87) was reversed following in vivo inhibition of NPY amidation suggesting an important role for amidated peptides including NPY in blood pressure regulation during IH.
**Substance P.** SP, a member of the tachykinin neuropeptide family, is involved in the regulation of pain and nociception (125), mood disorders, anxiety, stress (20), and respiratory rhythm (4).

CH (10–12% O₂ for 1 h) increased SP content in the cat carotid body in vivo compared with hyperoxic and normoxic controls (94). On the other hand, severe CH (5% O₂ for 1 h) reduced SP content in the rabbit carotid body (38). Since peptide secretion requires stronger stimulus than that required for biogenic amines (26), the reduced SP content by severe CH reported by Hanson et al. (38) is likely due to enhanced release of SP. Mechanisms underlying the elevated SP content in the cat carotid body by moderate CH, however, have not been examined.

Longer exposures to CH exert diverse effects on SP levels in the central and peripheral nervous systems. For instance, CH (10% O₂) exposure for 3 mo leads to increased density of SP-immunoreactive nerve fibers in the nasal mucosa, especially in the intra- and subepithelial and lamina propria regions (75). Given that SP is one of the predominant signal peptides of primary sensory neurons, the increased density of SP expressing fibers may represent enhanced sensory mechanisms in the hypoxic nasal mucosa. On the other hand, long-term CH (10% O₂ for 2–12 wk) decreased SP-like immunoreactivity in the cat (118) and rat (58, 59) carotid bodies. Based on these findings, Kusakabe et al. (58, 59) suggested that SP might play a role in carotid body adaptations to chronic hypoxia. In the central nervous system, 2 wk of CH (10% O₂) increased SP expression in the fetal rabbit brainstem (33) but not in the adult rat brainstem (92). A recent study showed that IH increases SP expression in various regions of the rat brainstem via ROS-mediated endoproteolytic activation of PAM, the rate-limiting enzyme in the synthesis of α-amidated peptides (105).

**Adrenomedullin.** Adrenomedullin (ADM) is an important regulator in the renal and cardiovascular systems, where it exerts a dose-dependent increase in vasodilation (51). Although ADM is expressed in several brain regions including the brainstem and hypothalamus (46), the effects of hypoxia on ADM expression in the central nervous system have not been studied. However, studies on non-neuronal cells showed that CH (1% O₂ for 3–12 h) stimulated ADM expression in cardiac myocytes (8) and human coronary artery endothelial cells (82). The CH-induced ADM expression was associated with increases in ADM mRNA levels (81) and activation of HIF-1 transcription factor (8). The effects of IH on ADM expression have not been determined.

**Effects of Hypoxia on Non-α-Amidated Peptides**

Although bioactive peptides such as endothelin 1 (ET-1) and angiotensin II (ANG II) undergo prepro-peptide processing as illustrated in Fig. 1 (right and middle, respectively), the mature peptide formation in the final step is not catalyzed by PAM but involves a converting enzyme that is specific for a given type of bioactive peptide. For example, ET-converting enzyme (ECE) is the rate-limiting enzyme in ET-1 synthesis, whereas ANG-converting enzyme (ACE) catalyzes the conversion of ANG I to ANG II (Fig. 1, right and middle, respectively). The following section summarizes hypoxia-evoked changes on ET-1 and ANG II.

**Endothelin-1.** ET-1, a 21-amino acid peptide with potent vasoconstrictor property, is synthesized and secreted primarily by vascular endothelial cells and several cell types in the lung. ET-1 is implicated in the regulation of vascular and airway tone and has mitogenic properties (107). In addition, ET-1 is also expressed in the central nervous system and has been shown to play important roles in the central neural control of circulation and respiration (60). Available evidence suggests that ET-1 synthesis in vascular endothelium and central nervous system is regulated by hypoxia.

Prolonged exposure to hypobaric CH (380 Torr for 14 days) increased ET-1 levels in type I cells of rat carotid bodies, which was in part due to marked upregulation of prepro-ET-1 mRNA (39). A similar increase in prepro-ET-1 mRNA was also reported in lungs of rats exposed to 4 wk of CH (10% O₂; 65). CH decreases ECE expression, the rate-limiting enzyme in ET-1 synthesis, in the rat brain cortex and striatum (83).

IH significantly increased ET-1-like immunoreactivity in the carotid body of adult cats (100) and rats (15). Although ET-1 levels were not altered in IH-treated neonatal rat carotid body, its release was augmented by acute hypoxia and the ensuing ET-1 signaling mediates the sensitization of the carotid body response to hypoxia (85), a finding that is similar to that previously reported in adult cats (100). Studies in the rat heart and renal medulla suggested that IH-induced increase in ET-1 levels in adult rats may in part be due to increase in ET-1 mRNA (2). Although the molecular mechanism by which CH elevates ET-1 levels has not been delineated, the human ET-1 promoter region contains a consensus HIF-1 binding site that may contribute to the upregulation of prepro-ET-1 mRNA expression in endothelial cells (43). In addition to the HIF-1 binding site, a flanking sequence containing binding sites for the factors activator protein-1 (AP-1), GATA-2, and CAAT-binding factor (NF-1) are also identified in prepro-ET-1 promoter, which may also contribute to enhanced ET-1 mRNA expression in response to hypoxia (122).

**Angiotensin II.** Angiotensins, a family of peptide hormones with potent vasoregulatory properties, contribute to the pathogenesis of hypertension. They are formed via sequential proteolytic processing of angiotensinogen, a protein precursor, as shown in Fig. 1, middle. Among angiotensins, ANG II, the principal effector of the renin-angiotensin cascade, is primarily synthesized by a pathway requiring ACE.

Acute lowering of oxygen levels attenuated ACE activity in dog cerebral vasculature (90). Prolonged CH (10% O₂ for 4 wk) increased ACE activity in rat carotid bodies, which was associated with significant increase in ACE mRNA (62, 63).

**GASOTRANSMITTERS**

Gasotransmitters such as nitric oxide (NO) and carbon monoxide (CO) are membrane-permeable, low-molecular-weight signaling molecules (31, 112, 116). They differ from classical neurotransmitters in that they are not stored in secretory vesicles but are enzymatically generated on demand as outlined in Table 3. The synthesis of NO and CO requires molecular oxygen and, therefore, their cellular production can be altered by hypoxia.
Effects of Hypoxia on NO Synthase

NO, a freely diffusible gas, is involved in regulation of blood vessel homeostasis and neuronal cell function. NO is synthesized from L-arginine via NO synthase (NOS) involving molecular O2, NADPH (cosubstrate), and several cofactors including calmodulin, tetrahydrobiopterin (BH4), flavin adenine dinucleotide, flavin adenine mononucleotide, and heme (1). Three isoforms of NOS are known. Two isoforms are constitutively expressed in neuronal tissues (neuronal NOS; nNOS) and in endothelial cells (endothelial NOS; eNOS). They produce in a Ca2+-dependent manner low levels of NO, which play important roles in neurotransmission, vasodilatation, and inhibition of platelet and leukocyte adhesion (6, 7). The third Ca2+-independent isofrom (inducible NOS; iNOS) is induced by inflammatory cytokines and LPS in many cell types but mostly in monocytes/macrophages (54). All three isofroms catalyze the conversion of arginine and O2 into NO and citrulline. eNOS is also capable of releasing NO from nitrite (79). The effects of NO on target cells are mediated via activation of the soluble guanylate cyclase (sGC) isofrom, generating cGMP.

The apparent K_m values for O2 for nNOS, eNOS, and iNOS were 23.2 ± 2.8, 7.7 ± 1.6, and 6.3 ± 0.9 µM, respectively (99). The regulation and mechanisms of action of NOS have been well characterized (52, 67, 68). NOS activity and the ensuing NO production by NOS in addition to substrate and cofactor availability also depends on dimeric state of NOS, which facilitates the formation of high-affinity binding sites for BH4 and L-arginine.

Neuronal NOS. Short-term exposure (40 mmHg for 1 h) to CH decreased nNOS activity in the cat carotid body in vitro (93). Since NO inhibits carotid body activity, it was proposed that the hypoxia-induced decrease in NO results in sensory excitation via “disinhibition” of the carotid body. A similar decrease in nNOS activity was also reported in immature rat cerebellum in vivo (114) and in bovine cerebellum extracts in vitro (99). The inhibitory effect of short-term CH on nNOS activity was due to reduced oxygen availability (99) but not as a result of either lack of BH4, arginine, and NADPH availability or reduction in the amount of NO dimers (101, 114).

On the other hand, Prabhakar et al. (95) demonstrated that prolonged CH increased nNOS activity and mRNA in rat cerebellum and nodose ganglion, a finding that is confirmed later in the rat brain (61). These observations suggest that hypoxia induces nNOS, which is constitutively expressed, similar to inducible NOS isofrom; i.e., iNOS.

IH (90 s paradigm) had no significant effect on nNOS expression in the cortex of the adult rat brain (66). However, another pattern of IH comprising 5 s 7% O2 and 115 s 21% O2 for 35 days reduced nNOS mRNA and protein expression in rat paraventricular and periventricular hypothalamic nuclei and in the subformical organ (44). These results suggest that IH-induced alterations in nNOS expression may depend on IH paradigm and brain regions under investigation.

Inducible NOS. The effects of IH on iNOS activity and expression have been studied in mice and rats. IH increased iNOS activity in wake-active brain regions in mice and was associated with increased sleep times and shortened sleep latencies (124). The IH-induced alteration in sleep behavior was absent in mice treated with iNOS inhibitor or with genetic absence of iNOS activity, suggesting a critical role for iNOS activity in IH-evoked disturbances in sleep behavior (124). Also, IH transiently elevated iNOS expression and activity in the cortex of adult rat brains (66). These authors further showed that IH-induced neurobehavioral deficits were absent in iNOS knockout mice (66), suggesting that NO generated by iNOS is an important mediator of neurobehavioral deficits evoked by IH.

Effects of Hypoxia on Heme Oxygenase

Heme oxygenase (HO) catalyzes the cleavage of the heme ring via oxidation at the α-meso-carbon with the formation of biliverdin, gaseous carbon monoxide, and free iron (80). Three isoforms of HO have been identified: the inducible HO-1, also known as Hsp32, and the constitutive HO-2 and HO-3, which are products of individual genes (71, 73, 106). HO-2 and HO-3 share a very high level of homology, whereas they differ from HO-1 in their amino acid sequence (71). HO-1 gene expression is induced by transcription factors, such as the NF-E2-related factor-2 (Nrf2), which binds anti-oxidant response elements (ARE) in the gene promoter region (70). The ARE is critical to enzyme induction by several stimuli including LPS, cytokines, heat shock, hyperoxia, oxidants, and hypoxia ischemia. Furthermore, HO-1 is also induced by hypoxia via hypoxia-inducible factor-1 (HIF-1; 64).

Very little is known on the effects of hypoxia on HO expression in the nervous system. However, studies on rat aortic smooth muscle cells (SMC) and rat pulmonary artery SMCs showed that CH (P02 of 18–20 Torr for up to 12 h) augmented CO production and HO activity, which was inhibited by SnPP-9, an inhibitor of HO in a dose-dependent manner (80). The CH-induced upregulation of HO activity was associated with increased HO-1 mRNA expression. This study further showed that hypoxia increased cGMP levels, an important regulator of vascular tone in SMC, and SnPP-9 inhibited hypoxia-evoked cGMP. Based on these findings, it has been suggested that hypoxia-induced CO production regulates SMC function and vascular tone (80). No information on the effects of CH or IH on other HO isoforms (i.e., HO-2 and HO-3) is available.

SUMMARY AND CONCLUSIONS

In summary, the above-described studies demonstrate that both CH and IH, depending on the duration and severity,
profoundly impact neurotransmitter levels and neurotransmitter-synthesizing enzymes. The effects of CH and IH often show regional variations in the central and peripheral nervous system and often differ between neonates and adults. Studies on TH and GAD indicate that CH primarily utilizes transcriptional mechanisms as opposed to posttranslational regulation by IH. It would be interesting to explore whether similar differential mechanisms also operate with other transmitters under the conditions CH and IH. Although much attention has been focused in delineating the effects of CH and IH on transmitter levels, very little is known on the functional significance of altered neurotransmitters on physiological responses to both forms of hypoxia.

ACKNOWLEDGMENTS
The author thanks Drs. Gayatri Raghuraman, Vandana Rai, and Suresh Sharma for their scientific contributions to studies related to glutamic acid decarboxylase, tyrosine hydroxylase, and peptidyl glycine amidating mono-oxygenase, respectively reported in this review. The author also expresses gratitude to Dr. Nanduri R. Prabahkar for several decades of constant support and exciting scientific collaborations.

GRANTS
This work was supported by the National Heart, Lung, and Blood Institute Grants PO1HL-90554 and RO1HL-89616.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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