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New insights into nNOS regulation of vascular homeostasis

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Commentary

An important physiological response to changes in local or systemic oxygenation is the modulation of vascular tone, which is mediated in part by changes in the activities of the 3 NO synthase (NOS) isoforms. In arterial smooth muscle cells, acute hypoxia induces increased vascular tone, which is attenuated if hypoxia persists. In this issue of the *JCI*, Ward et al. demonstrate that changes in O_2 concentration have effects on neuronal NOS enzymatic activity and gene expression that contribute to vascular homeostasis under conditions of acute and chronic hypoxia.

for any of these conditions at any time of life anywhere in the world. Until this happens, there will still be patients who present with recurrent infections who have undiagnosed, genetically determined immunodeficiency, the basis of which is unidentified. Failure to make these diagnoses early in life results in high rates of mortality and morbidity that could be prevented.

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New insights into nNOS regulation of vascular homeostasis

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An important physiological response to changes in local or systemic oxygenation is the modulation of vascular tone, which is mediated in part by changes in the activities of the 3 NO synthase (NOS) isoforms. In arterial smooth muscle cells, acute hypoxia induces increased vascular tone, which is attenuated if hypoxia persists. In this issue of the *JCI*, Ward et al. demonstrate that changes in O_2 concentration have effects on neuronal NOS enzymatic activity and gene expression that contribute to vascular homeostasis under conditions of acute and chronic hypoxia (see the related article beginning on page 3128).

Every cell in the human body is dependent upon the delivery of adequate concentrations of O_2 to maintain normal cellular functions, which are principally powered by ATP derived from mitochondrial oxidative phosphorylation. The anatomical matching of O_2 delivery to demand is determined by the production of secreted factors that stimulate blood vessel growth, most notably VEGF (1). Every step of its biogenesis, from transcription of *VEGF* gene sequences in the nucleus and protection of the resulting mRNA against degradation to the ribosomal translation and folding of VEGF protein in the endoplasmic reticulum and transport via the Golgi system to the plasma membrane for secretion, is O_2 regulated, as is the expression of its cognate receptor on vascular endothelial cells (1–3).

Anatomical responses to changes in O_2 demand occur on a scale of days, whereas other physiological responses resulting in alterations in O_2 delivery occur on a scale of seconds. Systemic responses are mediated by chemoreceptor cells in the carotid body that depolarize in response to reduced arterial O_2 tension, leading to reflex changes in ventilation, heart rate, and vascular tone (4). The vasculature within tissues also responds to acute regional hypoxia by dilation of arterioles that control the flow of blood into each capillary bed, as in the case of increased O_2 consumption in skeletal muscle during exercise (5). In contrast, when systemic hypoxia occurs as a result of vascular hypotension (shock), the adrenergic nervous system directs redistribution of blood flow to maintain the perfusion of

Nonstandard abbreviations used: HIF-1, hypoxia-inducible factor–1; nNOS, neuronal NOS; NOS, NO synthase.

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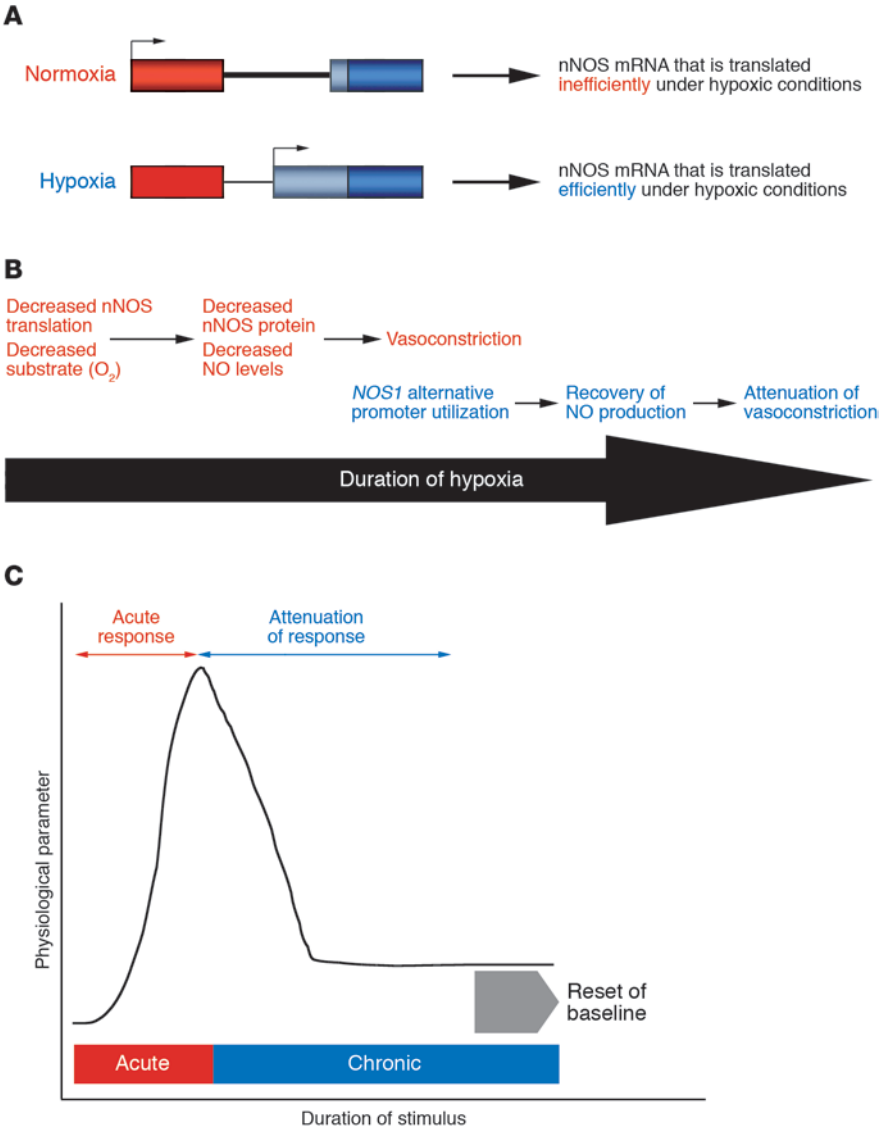

Figure 1
Oxygen-dependent regulation of nNOS activity and vasomotor tone. (**A**) Hypoxia-induced transcription of an alternative nNOS mRNA species. The 2 panels depict nNOS mRNA transcription under normoxic (upper) and hypoxic (lower) conditions. In each panel, the mRNA transcription start site is indicated by a bent arrow; 5′-untranslated sequences are indicated by a light blue box; translated sequences are indicated by a dark blue box; intervening sequences are indicated by a thick line; and 5′-flanking sequences are indicated by a thin line. Top panel: Under normoxic conditions, transcription is initiated at exon 1 (red box), which consists of 5′-untranslated sequences. Exon 2 contains additional 5′-untranslated sequences (light blue box) and the beginning of the protein coding sequence (dark blue box). Lower panel: In this issue of the *JCI*, Ward et al. (9) demonstrate that under hypoxic conditions, transcription is initiated downstream of exon 1, resulting in the synthesis of nNOS mRNA containing an alternative 5′-untranslated region (light blue box). Under hypoxic conditions, the translation of mRNA containing exon 1 is inhibited, whereas the alternative mRNA is efficiently translated into protein. Thus, a qualitative change in the structure of nNOS mRNA based upon alternative transcription initiation results in a quantitative change in the levels of nNOS protein synthesized under hypoxic conditions. (**B**) Time-dependent responses to hypoxia in vascular smooth muscle cells. (**C**) Attenuation of physiological responses with chronic stimulation. A physiological stimulus (e.g., hypoxia) induces an acute physiological response (e.g., increased vascular tone). However, if the stimulus persists, the response is attenuated, resulting in the establishment of a new (reset) steady state.

the heart and brain at the expense of other organs such as the gastrointestinal tract. However, under conditions of chronic hypoxia, the contractile response of arteries to hypoxia is blunted in an attempt to maintain tissue viability.

NO business like flow business

The endothelium plays a critical role in the production of vasoactive molecules that regulate blood flow. NO produced in the endothelium by eNOS (encoded by the *NOS3* gene in humans) has been shown to play a major role by binding to soluble guanylate cyclase in vascular smooth muscle cells, resulting in cGMP production and the activation of signal transduction pathways leading to vasodilation (6). Activation of eNOS is also required for endothelial cell responses to angiogenic factors. Under hypoxic conditions, iNOS mRNA (encoded by the human *NOS2* gene) is also expressed in endothelial cells, macrophages, and other cell types (7, 8).

In this issue of the *JCI*, Ward et al. report that chronic hypoxia also induces increased activity of neuronal NO synthase (nNOS; encoded by the human *NOS1* gene) in arterial smooth muscle cells (9). Increased nNOS activity is a consequence of increased nNOS protein synthesis, which occurs against the background of a global reduction in protein synthesis that serves to conserve ATP by reducing the synthesis of all nonessential proteins in hypoxic cells. A major mechanism mediating inhibition of protein synthesis is the phosphorylation of eukaryotic translation initiation factor–2α by pancreatic endoplasmic reticulum kinase (PERK), which inhibits cap-dependent mRNA translation (10). The mRNAs encoding VEGF and several other proteins that are expressed in response to hypoxia contain an internal ribosomal entry site, which provides a mechanism for bypassing the inhibition of cap-dependent translation (11).

Table 1
Apparent K_m of NOSs for O_2

Gene	Protein	K_m (μM)
NOS1	nNOS	350
NOS2	iNOS	130
NOS3	eNOS	4

Data represented in this table are from ref. 14.

Making the best of a bad situation

Ward et al. (9) report that under hypoxic conditions, an alternative promoter located in the first intron of the *NOS1* gene directs the transcription of mRNA species lacking 5′-untranslated sequences encoded by exon 1, which when present inhibit the translation of nNOS mRNA into protein, especially under hypoxic conditions (Figure 1A). Thus, the synthesis of an mRNA species that escapes translational repression allows efficient synthesis of nNOS protein in hypoxic cells. A similar strategy of alternative promoter utilization has been reported for the *VEGF* gene (12). Ward et al. also generated transgenic mice in which *lacZ* coding sequences were under the control of a 2.5-kb *Nos1* genomic region upstream of the translation initiation site. When these mice were exposed to chronic ambient hypoxia (8% O_2 for 48 hours), arterial expression of β-galactosidase was induced (9).

With the delineation of the *cis*-acting sequences controlling hypoxia-induced expression of nNOS, attention will turn to the identification of *trans*-acting factors. Hypoxia-inducible factor–1 (HIF-1) regulates the expression of *VEGF* as well as hundreds of other genes in response to hypoxia (13), including *NOS2* (7, 8). Ward et al. (9) report the presence of potential HIF-1 binding sites within the *NOS1* genomic sequences that were sufficient for hypoxia-inducible *lacZ* expression. Chromatin immunoprecipitation assays can be performed to investigate whether HIF-1 binds to specific sites in the *NOS1* gene in response to hypoxia.

NO synthase performance: O_2 does matter

Even more intriguing is the significance of nNOS protein synthesis under hypoxic conditions. NO synthase (NOS) catalyzes the reaction of arginine plus O_2 to yield citrulline plus NO. The apparent K_ms of eNOS, iNOS, and nNOS allow for NO production over a 2-log range of O_2 concentrations (ref. 14; Table 1), which provides important insight into the physiological functions of the NOS isoforms. The eNOS isoform has a low K_m for O_2, which insures that the enzyme will remain active in endothelial cells that are invading hypoxic tissue during angiogenesis. In contrast, the high K_m of nNOS suggests that even modest reductions in O_2 concentration will result in a significant loss of enzyme activity and there is a linear relationship between O_2 concentration and nNOS activity over the entire physiological range (15). These results suggest that the generation of NO by nNOS may represent a signal transduction mechanism in which signal intensity is directly related to O_2 concentration. For example, O_2-dependent NO production inhibits sensory discharge from the carotid body under normoxic conditions (16). The fruit fly *Drosophila melanogaster* also utilizes NO signaling for a variety of adaptive responses to hypoxia (17).

Homeostasis: respond and reset

These properties of nNOS enzyme activity and gene regulation lead to a paradoxical situation in which hypoxia induces increased synthesis of nNOS protein, which has reduced activity due to substrate deprivation. Indeed, Ward et al. show that nNOS protein levels increased approximately 10-fold (see Figure 4 in ref. 9), whereas NOS activity increased less than 1.5-fold (see Figure 3 in ref. 9) in the aortae of rats subjected to hypoxia. The data suggest a homeostatic mechanism (Figure 1B) by which acute hypoxia induces vasoconstriction, which is attenuated under conditions of chronic exposure to hypoxia (9, 18). During acute hypoxia, vascular nNOS activity and NO levels decline due to the combined effect of decreased translation of exon 1–initiated nNOS mRNA into protein and decreased enzyme activity due to substrate (O_2) deprivation, leading to an acute increase in vasomotor tone. This acute response is followed by the induction of an alternative nNOS mRNA isoform that is not subject to hypoxia-induced translational inhibition, leading to increased nNOS protein levels, increased nNOS activity, and attenuated vasoconstriction. Attenuation of the response under conditions of chronic stimulation is an important characteristic of many homeostatic systems (Figure 1C). The delineation of this feedback circuit provides both an elegant illustration of the complex mechanisms that mediate adaptive responses to hypoxia and a foundation for further analysis of how these responses are dysregulated in the setting of cardiovascular disease.

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