Control of Adenosine Transport by Hypoxia

Agnes Görlach

The extracellular accumulation of the nucleoside adenosine is one of the first steps in a protective auto/paracrine signaling cascade aimed at limiting cellular damage in response to adverse conditions including hypoxia or ischemia. This adenosine acts as a signal molecule that is able to mediate numerous physiological and metabolic effects that could be beneficial to hypoxic cells including vasodilation, stimulation of glycolysis breakdown to provide glucose for ATP production via anaerobic glycolysis and reduction of neuronal excitability as well as neurotransmitter release to reduce neuronal energy requirements.

These intracellular effects of adenosine are mediated by 4 subtypes of G-protein–coupled adenosine receptors (AR) (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>) which differ in their expression profiles in defined cell types, the type of G-proteins to which they are coupled and their sensitivity to control by receptor phosphorylation. Endothelial cells predominantly express the A<sub>2A</sub> and A<sub>2B</sub> AR subtypes. The expression of both subtypes is regulated by hypoxia, such that low pO<sub>2</sub> induces A<sub>2A</sub>AR expression, which promotes the expression of angiogenic factors and reduction of A<sub>2B</sub>AR expression. The expression of the AR is further stimulated by increased extracellular adenosine during hypoxia. Adenosine released from the endothelium during systemic hypoxia acts back on AR on the endothelium to increase the synthesis of nitric oxide (NO) which then causes vasodilatation. Therefore, the regulation of extracellular adenosine levels is critical for the interaction of adenosine with its receptors and subsequent responses that modify cell function in response to hypoxia.

Adenosine can be generated and metabolized both intra- and extracellularly (Figure). Cytosolic S<sup>-</sup>- and membrane-bound ecto-S<sup>-</sup>-AMP nucleotidases (CD73) produce adenosine from AMP. Alternatively, adenosine can be generated from hydrolysis of S-adenylylhomocysteine by S-adenosylhomocysteine hydrolase. On the other hand, adenosine deaminase which is widely distributed in many cells and tissues converts adenosine to inosine, whereas adenosine kinase catalyzes the formation of AMP from adenosine.

Hypoxia leads to the breakdown of ATP resulting in nucleotide catabolism predominantly via dephosphorylation of AMP by S<sup>-</sup>-nucleotidases. Hypoxia can upregulate an adenosine nucleotide-metabolizing ecto-enzyme cascade comprising ecto-ATP apyrase (CD39) and CD73, whereby de novo synthesis of functional active CD73 is dependent on the hypoxia-inducible transcription factor HIF-1α. Gene targeting studies in mice demonstrated that functional CD39 and CD73 are necessary to maintain endothelial barrier function and to prevent vascular leakage after hypoxia. In addition, extracellular adenosine concentrations may be further potentiated by preventing reutilization through hypoxic inhibition of adenosine kinase and adenosine deaminase. Thus, hypoxia appears to induce a program which shifts the cellular phenotype toward an increase in intracellular adenosine.

Importantly, adenosine flux across the membrane depends on the concentration gradient between extra- and intracellular nucleoside levels. In human umbilical vein endothelial cells (HUVEC), adenosine is generated continuously but is immediately recycled via adenosine kinase thus leaving the cytosolic adenosine concentrations low and allowing adenosine uptake. The capacity to take up adenosine from the extracellular space plays a prominent role in adenosine homeostasis. Because adenosine is not lipophilic, release and uptake of adenosine requires nucleoside membrane transport which is conducted by 2 families of unrelated nucleoside transporter proteins. Active sodium-dependent nucleoside transport is found primarily in specialized epithelial tissues and is mediated by members of the concentrative nucleoside transporter family (SLC28). Passive nucleoside transport processes are ubiquitous and are mediated by members of the equilibrative nucleoside transporter (ENT) family (SLC29). ENTs are bidirectional, allowing adenosine release and uptake by facilitated diffusion along its concentration gradient. The human and rodent genomes encode 4 ENT isoforms, designated ENT1–4. The best characterized of these isoforms, ENT1 and ENT2, have broad selectivity and have been classified on the basis of their sensitivity to inhibition by nitrobenzylmercaptopurine riboside (NBMPR). The importance of ENTs in controlling adenosine levels is apparent from coronary vasodilatory and cardioprotectant pharmacological agents such as dipyridamole which inhibit ENTs thereby preventing reuptake of adenosine and potentiating AR activation.

In this issue of Circulation Research, Casanello et al investigated the role of hypoxia in the regulation of ENT expression and activity in HUVEC. In contrast to other studies routinely exposing HUVEC to 20% O<sub>2</sub>, normoxia was here set to 5% O<sub>2</sub> to model the physiological oxygen concentration in the umbilical vein and to 1% to 2% O<sub>2</sub> for hypoxic incubations. Local changes in pO<sub>2</sub> are important determinants controlling umbilical vein tone thereby regulating blood flow from the placenta to the fetus. Whereas hypoxia induces vasodilation, pO<sub>2</sub> levels exceeding 5% O<sub>2</sub>...
result in vasoconstriction. Because the human umbilical vein is thought to lack autonomic innervation, local mechanisms regulating vascular tone appear to be of particular relevance in this response.  

Casanello et al showed increased extracellular adenosine in HUVEC exposed to hypoxia. This effect was attributed to reduced human equilibrative nucleoside transporter 1 (hENT1)-mediated adenosine uptake attributable to decreased hENT1 expression and transport activity associated with a diminished number of transporters under hypoxia. This response was at least partially mediated by the MAP kinase ERK1/2. Hypoxic downregulation of ENT1 via a PKC-dependent mechanism leading to decreased adenosine uptake has been shown in mouse cardiomyocytes and in rat PC12 cells. Unlike the situation in cardiomyocytes but similar to PC12 cells, hENT2 was not affected by hypoxia in HUVEC. Whereas NBMPR decreased extracellular adenosine levels in hypoxic cardiomyocytes suggesting an outward flux of adenosine by mENT1, this substance did not affect extracellular adenosine levels in HUVEC. Although this is the first study describing the role of hypoxia on hENT1 expression and activity in a physiologically relevant model of hypoxia in HUVEC, the underlying mechanisms of this observation are still to be determined. In addition to decreased adenosine uptake, an increase in extracellular adenosine may also result from breakdown of nucleotides released independently of hENT1. Hypoxia-induced ATP release as well as induction of CD39 and CD73 have been found in endothelial cells. Further studies may address whether downregulation of hENT1 by hypoxia is accompanied by a change in the expression and activity of adenosine-forming and metabolizing enzymes to fully appreciate the molecular mechanisms underlying the increased accumulation of extracellular adenosine by hypoxia in this model system.

Because this group and others showed induction of NO synthesis by adenosine, one would expect that increased extracellular adenosine under hypoxia would activate NO synthesis. Conversely, NO production was decreased by hypoxia although eNOS protein was upregulated. Decreased NO production accompanied by reduced eNOS levels has been reported in hypoxic HUVEC and in umbilical veins in vivo. In contrast, a recent study showed increased levels of NO in hypoxic HUVEC. Furthermore, inhibition of NO synthesis by N(ω)-nitro-L-arginine methyl ester (l-NAME) counteracted hypoxic vasodilatation of umbilical veins in vivo. Thus, the differences observed between these studies cannot be accounted for by “physiological” versus “nonphysiological” culture conditions of HUVEC or varying origins of endothelial cells. Similar to the situation with NO, elevated or decreased levels of reactive oxygen species (ROS) have been found under hypoxic conditions indicating that the redox balance of the cell determined by NO/ROS is delicately regulated under hypoxic conditions and that modulation of ROS and NO levels has profound effects on hypoxia-dependent gene expression. In light of these findings, Casanello et al investigated the role of NO in the regulation of hENT1. Although a NO-donor downregulated and l-NAME upregulated hENT1 expression under normoxia, these substances had no effect under hypoxia indicating that NO does not contribute to hypoxic adenosine accumulation mediated by hENT1 downregulation in HUVEC.

Because adenosine is important for adaptation to inadequate oxygen supply, the findings that hENT1 is controlled by oxygen availability provides an additional element in our understanding of the oxygen sensitive pathways. Further studies are required to demonstrate the in vivo relevance of this observation with regard to the proposed role as a key process to maintain blood flow from the placenta to the fetus in situations such as intrauterine growth restriction or gestational diabetes, as well as in other situations of hypoxia and ischemia.

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