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HIF-1α in the heart: Remodeling nucleotide metabolism

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ABSTRACT

These studies have examined the effect of hypoxia inducible factor 1α (HIF-1α) on nucleotide metabolism in the ischemic heart using a genetic mouse model with heart-specific and regulated expression of a stable form of HIF-1α. We find that AMP deaminase (AMPD), the entry point of the purine nucleotide cycle (PNC), is induced by HIF-1α at the level of mRNA, protein, and activity. AMP that accumulates during ischemia can be metabolized to adenosine by 5′-nucleotidase or to IMP by AMPD. Consistent with the finding of AMPD induction, adenosine accumulation during ischemia was much attenuated in HIF-1α-expressing hearts. Further investigation of nucleotide salvage enzymes found that hypoxanthine phosphoribosyl transferase (HPRT) is also upregulated in HIF-1α-expressing hearts. Treatment of hearts with an inhibitor of the PNC, hadacidin, hastens the fall of the adenylate energy charge during ischemia and the accumulation of AMP. The results provide new insight into the role of the PNC in the heart, especially as it relates to ischemia, and indicate that HIF-1α regulates nucleotide metabolism as a compensatory response to hypoxia.

1. Introduction

These studies have used a mouse model as described by Bekeredjian et al. [1] containing a cardiac-specific, oxygen-stabilized, doxycycline (Dox)-off regulated HIF-1α transgene (HIF-1α-PPN) to probe the role of HIF-1α in purine metabolism. Hypoxia inducible factor 1α (HIF-1α) is a master regulatory transcription factor that directs the transcription of a multitude of genes that provide adaptive responses when O_2 levels decrease. The cardio-specific role of HIF-1α is less understood, however recent evidence indicates that HIF-1α plays a central role in the protection of myocardium against hypoxic stress. Several studies indicate that HIF-1α is necessary for preconditioning protection [2,3] and our previous work showed that the overexpression of HIF-1α in cardiomyocytes confers robust protection in adult hearts subjected to ex vivo ischemia [4]. We attributed the protection by HIF-1α to the cardiomyocyte's ability to maintain mitochondrial polarization during anoxia or when cytochrome c oxidase was inhibited with cyanide [4]. We also demonstrated preservation of electron transport chain (ETC) activity by utilization of fumarate as a terminal electron acceptor in the absence of O_2 [5]. Fumarate is reduced to succinate at complex 2 and allows for the continued pumping of H^+ by complex 1 in the absence of cytochrome c oxidase activity. This reduction of fumarate with the concomitant production of succinate has been shown to operate in kidney tubule and heart tissue previously; albeit at low levels [6,7]. Thus, the capacity to employ fumarate reduction is inducible through HIF-1α stabilization [5,8].

Further studies identified the source of fumarate used for anaerobic ETC activity as the purine nucleotide cycle (PNC) and incidentally suggested that PNC activity might be higher in cardiomyocytes treated with prolyl hydroxylase inhibitors to induce HIF-1α levels [5]. The previous suggestion that the PNC metabolic pathway is upregulated by HIF-1α largely motivates the present work; a systematic examination of the enzymes of the PNC and the catabolism of adenonucleotides during ischemia in HIF-1α-over-expressing hearts. The entry reaction of the PNC is catalyzed by AMP deaminase and converts AMP into IMP (Fig. 1). In a series of two reactions, catalyzed by adenylosuccinate synthetase and adenylosuccinate lyase, aspartate and GTP are consumed and fumarate and AMP are produced to complete the cycle. The PNC has been mostly studied in skeletal muscle where it has been suggested to help maintain the energy charge (EC), where EC = ([ATP] + 0.5[ADP]) / ([ATP] + [ADP] + [AMP]) by preventing the rise of AMP and ADP during strenuous exercise [9]. The energy charge ranges from 0 to 1 as proposed by Atkinson and is an index of the cellular energy state [10]. A high energy charge is indicative of favorable conditions for ATP utilization to carry out cellular work. The PNC may also act as an anaplerotic pathway that generates fumarate for the citric acid cycle [11]. Direct confirmation of the function of the PNC in skeletal muscle is lacking, whereas a significant metabolic role for the PNC in the heart has been discounted entirely by some investigators [12].

HIF-1α is stabilized through inhibition of the prolyl hydroxylase domain-containing (PHD) enzymes at [O_2] far above those that limit respiration, and can thus be regarded as anticipatory in nature. In this scheme HIF-1α responds to sublethal levels of hypoxia, directing

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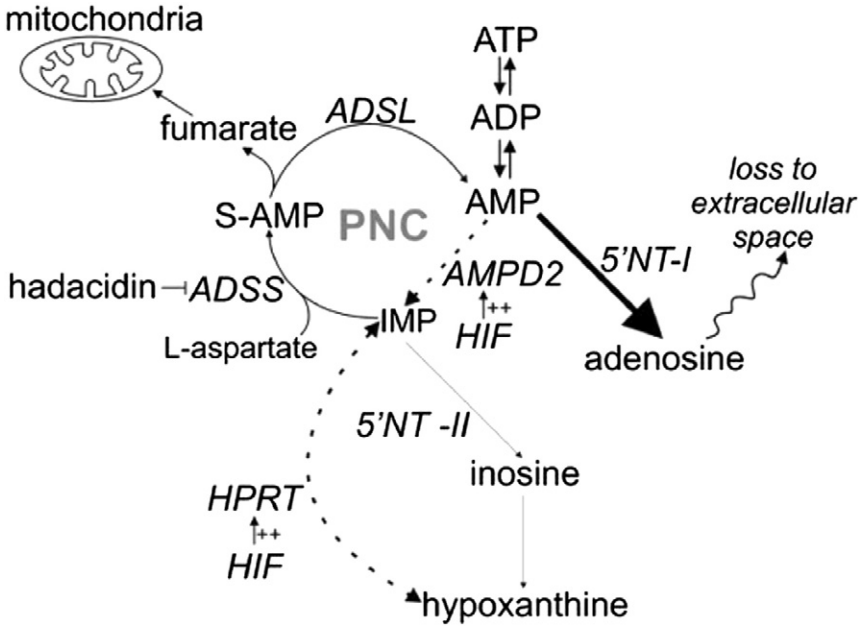

Fig. 1. Nucleotide degradation pathways. During ischemia, the rate of ATP consumption exceeds its synthesis from glycolysis. As a result, there is a net degradation of ATP and the heart's nucleotide pool while nucleosides and nucleobases accumulate. PNC = purine nucleotide cycle. AMPD2 = AMP deaminase isoform 2. ADSS = adenylosuccinate synthetase. ADSL = adenylosuccinate lyase. 5′NT-I = 5′-nucleotidase isoform 1 (AMP specific). 5′NT-II = 5′-nucleotidase isoform 2 (IMP specific). HPRT = hypoxanthine phosphoribosyl transferase. Major pathways are indicated by bold arrows. HIF-1α induced pathways are indicated by dashed arrows.

compensatory changes that equip the cardiomyocyte with the ability to survive ischemic stress. For example, HIF-1α upregulates glycolytic enzymes, thereby, increasing the capacity to generate ATP anaerobically [13]. Thus, the upregulation of the PNC by HIF-1α might suggest a protective role for the PNC against ischemic stress. This is certainly in line with our finding that fumarate derived from the PNC allows for the anaerobic operation of respiratory complex 1. These considerations motivate the present studies where we have 1) evaluated the effects of HIF-1α overexpression on the enzymes that constitute the PNC, as well as other nucleotide salvage enzymes; and 2) followed the adenylate nucleotide breakdown products during a bout of ischemia in hearts with, and without, enhanced HIF-1α expression. In the heart, ischemia limits ATP production via oxidative phosphorylation and glycolysis is used to generate ATP anaerobically. However, the rate of glycolytic ATP synthesis is insufficient to meet the ATP demands of the working heart. As in skeletal muscle during intense exercise, ATP and the nucleotide pool becomes rapidly depleted while nucleosides and nucleobases accumulate (Fig. 1) [14,15]. Taken together, the above considerations may suggest that the PNC plays a role in the heart during ischemia similar to skeletal muscle during intense work; albeit after the pathway is induced by HIF-1α.

2. Materials and methods

2.1. Animal model

In order to examine the effect of HIF-1α on nucleotide metabolism in the ischemic heart, we utilized B6C3F1 mice that contain a doxycycline inducible HIF-1α transgene (HIF-1α-PPN) that has been previously described [1]. All mice used were males between 2 and 4 months of age. For experiments in which the HIF-1α transgene was not induced (Non-I), mice were maintained on a 625 mg/kg doxycycline-replete diet throughout (Harlan Research Laboratories, Madison, WI). In experiments requiring 2 days of HIF-1α expression (2D), mice were switched from doxycycline food to doxycycline-replete water containing 73 mM sucrose (Mallinckrodt Baker, Phillipsburg, NJ) and 0.416 mM doxycycline hydrochloride (Research Product International, Mount Prospect, IL) for 2 days followed by maintenance of mice on regular food and water for two additional days. In experiments requiring 6 days of HIF-1α expression (6D), mice were maintained on doxycycline-free food and water for 5–7 days before experimentation. Results from HIF-1α-PPN mice were compared to those from B6C3F1 wildtypes obtained from Harlan Research Laboratories. Animals were handled in accordance to a protocol reviewed and approved by the East Tennessee State University Committee on Animal Care.

2.2. Realtime PCR mRNA quantification

RNA was extracted from hearts using TRIzol reagent (Life Technologies, Carlsbad, CA) and concentration as well as integrity were determined using the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). cDNA was synthesized using the Superscript III cDNA synthesis kit (Life Technologies). For each cDNA synthesis reaction, 2 μg of RNA was added to 1.25 μM oligo$(dT)_{20}$, 50 ng random hexamers, 0.5 mM dNTP mix, 80 U RNaseOUT, 5 mM DTT, 1.25 mM $MgCl_2$, and 400 U reverse transcriptase in a final volume of 40 μl. The cDNA synthesis reaction was allowed to proceed for 1 h at 50 °C and then terminated by heating at 70 °C for 15 min. Quantitative PCR was performed in triplicate for all samples in a 96 well plate format on a CFX96 real-time PCR detection system (BioRad Laboratories, Richmond, CA). 40 cycles were carried out. Each reaction consisted of 50 ng cDNA and 1.5 μl of Quantitect AMPD2, HPRT, or transferrin primer from Qiagen in 1 × SsoFast Evagreen supermix (Bio-Rad Laboratories). AMPD2 and HPRT mRNA expression was normalized to that of reference gene transferrin and results were reported as a percent change in gene expression relative to WT.

2.3. Western blot — protein expression

Hearts obtained from WT, Non-I, 2D, and 6D mice were ground into a fine powder over liquid nitrogen. The frozen heart powder was then homogenized in RIPA buffer composed of 50 mM Tris · HCl, pH 7.4 (Calbiochem, Darmstadt, Germany), 1% Triton X-100 (Fisher, Pittsburgh, PA), 1% w/v sodium deoxycholate (Fisher), 0.1% w/v SDS (EMD, Billerica, MA), and 1 mM EDTA (Fisher) with 1:40 protease inhibitor cocktail mix (Sigma, St. Louis, MO). The homogenates were centrifuged at 12,000 *g* at 4 °C for 10 min and the supernatant was collected. Protein