Adenosine Deaminase Inhibition Prevents Free Radical-mediated Injury in the Postischemic Heart*

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In the presence of its substrates hypoxanthine and xanthine, xanthine oxidase generates oxygen free radicals that cause postischemic injury. Recently, it has been demonstrated that the burst of xanthine oxidase-mediated free radical generation in the reperfused heart is triggered by a large increase in substrate formation, which occurs secondary to the degradation of adenine nucleotides during ischemia. It is not known, however, whether blocking this substrate formation is sufficient to prevent radical generation and functional injury. Therefore, studies were performed in isolated rat hearts in which xanthine oxidase substrate formation was blocked with the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), and measurements of contractile function and free radical generation were performed. Chromatographic measurements of the intracellular adenine nucleotide pool showed that preischemic administration of EHNA blocked postischemic hypoxanthine, xanthine, and inosine formation. Electron paramagnetic resonance spin trapping measurements of free radical generation showed that inhibition of adenosine deaminase with EHNA blocked free radical generation and that it also increased the recovery of contractile function by more than 2-fold. Exogenous infusion of hypoxanthine and xanthine totally reversed the protective effects of EHNA. These results demonstrate that blockade of xanthine oxidase substrate formation by adenosine deaminase inhibition can prevent free radical generation and contractile dysfunction in the postischemic heart.

Oxygen free radical generation has been shown to be an important mechanism of cellular injury in ischemic and reperfused tissues (1, 2). Studies in a variety of tissues including heart, lung, kidney, and brain have demonstrated that intracellular administration of antioxidant enzymes or free radical-scavenging drugs can prevent reperfusion damage and improve postischemic function (3, 4). These studies have provided indirect evidence of free radical generation in postischemic tissues. More recently, free radical generation has been measured in ischemic tissues with EPR spectroscopy. Both direct and spin trapping EPR techniques have confirmed that there is a burst of oxygen free radical generation in the heart after postischemic reperfusion (5–8). Although several mechanisms have been proposed to be involved in the generation of oxygen free radicals, xanthine oxidase has been shown to be a central mechanism in a variety of postischemic cells and tissues (9–14). In reperfused tissues, xanthine oxidase in the presence of its substrate hypoxanthine or xanthine reduces molecular oxygen to \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \), which can further react to form the more reactive \( \cdot\text{OH} \) (12, 13). The \( \cdot\text{OH} \) and \( \text{O}_2 \) radicals produced by the enzyme can then in turn oxidize cellular proteins and membranes resulting in cellular injury.

Over the last decade the therapeutic use of inhibitors of xanthine oxidase has been proposed as an approach to prevent reperfusion injury (15). This was based on evidence that xanthine oxidase is a major source of reperfusion-associated free radical generation; therefore, blocking the enzyme would be expected to inhibit radical generation. The importance of this approach was further supported by the hypothesis that in ischemic tissues xanthine dehydrogenase, which reduces NAD to NADH, may be converted via proteolytic cleavage to xanthine oxidase with this increase in xanthine oxidase activity triggering postischemic free radical generation (9, 14, 16). Indeed, xanthine oxidase blockers have been reported to decrease radical generation and prevent postischemic heart injury (12, 17–20). But there was also evidence of possible adverse effects of these drugs, and a number of reports failed to show in vivo efficacy in preventing cell death. It has been reported that both the xanthine oxidase blockers allopurinol and oxypurinol failed to decrease infarct size in vivo regional models of myocardial necrosis (21–23). There is also evidence that these drugs may either exert direct dose-dependent toxicity or toxicities due to increased xanthine or hypoxanthine concentrations (12). Therefore, there has been a need to look for other alternative ways to prevent free radical-mediated injury.

Recently, the kinetics of free radical generation and the relationship of substrate and enzyme control in the mechanism of free radical generation from xanthine oxidase during ischemia and reperfusion have been elucidated in isolated rat hearts (24). It was demonstrated that the burst of xanthine oxidase-mediated free radical generation in the reperfused heart is triggered and its time course controlled by a large increase in substrate formation, which occurs secondary to the degradation of ATP during ischemia.

Since the availability of xanthine oxidase substrates was shown to be the primary factor that triggers and controls the burst of oxygen free radical generation in the postischemic heart, blocking the formation of these enzyme substrates should inhibit radical generation and prevent radical-mediated myocardial dysfunction. Therefore, studies were performed to determine the effect of adenosine deaminase inhibition on the formation of xanthine oxidase substrates as well as upon free radical generation and free radical-mediated contractile dys-
function. These studies demonstrate that inhibition of xanthine oxidase substrate formation by blockade of adenosine deaminase can greatly decrease free radical generation and contractile dysfunction in the posts ischemic heart.

MATERIALS AND METHODS

Isolated Heart Perfusion—Female Sprague-Dawley rats (250–350 g) were heparinized and anesthetized with intraperitoneal pentobarbital. The hearts were excised, the aorta was cannulated, and retrograde perfusion was initiated. Hearts were perfused at a constant pressure of 80 mm Hg using Krebs bicarbonate buffer consisting of 120 mM NaCl, 17 mM glucose, 25 mM NaHCO3, 5.9 mM KCl, 1.2 mM MgCl2, 1.25 mM CaCl2, 0.5 mM EDTA and bubbled with 95% O2 and 5% CO2 gas at 37 °C as described previously (24). A sidearm in the perfusion line allowed infusion of different agents according to the experimental purpose. To monitor cardiac contractile function, a latex balloon was inserted through an opening in the left atrium across the mitral valve into the left ventricular cavity and connected to a pressure transducer as described previously (12). The balloon was initially inflated with a volume of distilled water sufficient to produce an end diastolic pressure in the range of 8–14 mm Hg. Subsequent measurements of developed pressures were calculated as the difference between the peak systolic and end diastolic pressure. Left ventricular pressure was recorded with a Gould RS3400 four-channel recorder. Coronary flow and heart rates were measured periodically every 5 min prior to ischemia and after 30 min of global ischemia for 45 min of reperfusion.

High Performance Liquid Chromatography (HPLC) Measurement—After 15 min of perfusion with equilibration of contractile function, hearts were frozen in liquid nitrogen as a preischemic control. Per ischemic and reperfusion measurements, hearts were subjected to further 30-min periods of global ischemia or 30 min of ischemia followed by variable duration of reflow. The hearts were immediately frozen in liquid nitrogen at the desired time point and then ground under liquid nitrogen. Then the frozen tissue was transferred to a glass homogenizer with a Teflon pestle and allowed to thaw with homogenization in 10 ml of 0.5 M perchloric acid at 4 °C. A small amount of homogenate was removed for assay of total protein content by Lowry assay (25). Acetic acid extraction continued on ice for 15 min, at which time cellular debris was pelleted by centrifugation at 14,000 × g for 1 min. The acid extract was neutralized by mixing with 20 ml of 0.5 M Tris(hydroxymethyl)aminomethane (4:1) for 30 s. The mixture was centrifuged at 14,000 × g for 1 min, and the upper aqueous layer was recovered and passed through a 0.45-μM Millipore filter and then stored at −80 °C for analysis.

Reversed-phase HPLC was performed using a procedure similar to that of Hull-Ryde et al. (26) using a Waters µBondapak C18 column and a Waters HPLC system (Waters Associates, Milford, MA) with a model 484 UV detector, model 510 reciprocating pumps, and Maxima software, as described previously (27).

EPR Spectroscopy and Spin Tracking—Studies were performed using the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). The DMPO (>97% pure) was purchased from Aldrich and further purified by distillation. For the in vitro studies assessing the effects of EHNA as a radical scavenger, 50 mM DMPO was used. Superoxide was generated from 0.05 units/ml of xanthine oxidase and 0.5 mM xanthine in 50 mM phosphate buffer containing 1 mM deferoxamine at pH 7.4, as described previously (13). To generate the hydroxyl radical, deferoxamine was excluded and 50 μM Fe(III)-nitrilotriacetate (1:3) was added to facilitate rapid formation of the hydroxyl radical via the iron-mediated Fenton reaction (6, 13). For studies measuring free radical generation in the isolated heart, DMPO was infused through a sidearm located just proximal to the heart perfusion cannula with a final concentration of 50 mM. Spin trap containing effluent was collected in 20-s aliquots prior to ischemia and at different times of reflow (12, 14).

EPR spectra were recorded in flat cells at room temperature with a Bruker-IBM ER 300 spectrometer operating at X-band with a TM 110 cavity using a modulation frequency of 100 KHz, modulation amplitude of 0.5 G, microwave power of 20 milliwatts, microwave frequency of 9.77 GHz, and acquisition of 10-1 min scans. Quantitation of the free radical signals was performed by comparing the double integral of the observed signal with that of a known concentration of the 2,2,6,6-tetramethylpiperidinyloxy free radical in aqueous solution as described previously (6, 11).

The abbreviations used are: HPLC, high performance liquid chromatography; EHNA, erythro-9-(2-hydroxy-3-nonyl) adenine; DMPO, 5,5-dimethyl-1-pyrroline-N-oxide; LVDP, left ventricular developed pressure; RPP, rate-pressure production.

FIG. 1. Schematic diagram illustrating the reaction pathways by which adenine nucleotides are degraded to form substrates for the oxygen free radical-generating enzyme xanthine oxidase. The deamination of adenosine is the rate-determining factor for the subsequent generation of xanthine oxidase substrates. Adenosine deaminase inhibition with EHNA blocks the formation of these substrates and thus may decrease free radical generation.

Statistical Analysis—Data are expressed as mean ± S.E. Differences in the recovery of various physiologic parameters among the groups were tested using a repeated measures two-way analysis of variance. Individual comparisons were analyzed by Student’s t test for independent means. The values of p < 0.05 were accepted as statistically significant.

RESULTS

Effect of EHNA on the Adenine Nucleotide Pool in the Posts ischemic Heart—It is well established that ATP depletion during myocardial ischemia leads to accumulation of the diffusible adenine nucleotides, adenosine and inosine, as well as the nucleopurines, hypoxanthine and xanthine (Fig. 1). In these freeze conditions, a limiting step is conversion of adenosine to inosine catalyzed by adenosine deaminase (28, 29).

EHNA has been demonstrated to be an adenosine deaminase blocker with K_i = 1 nm (30, 31). Studies were performed to confirm that EHNA administration prior to ischemia effectively blocked adenosine deaminase with decreased formation of hypoxanthine and xanthine. Either untreated or EHNA-pretreated (250 μM infusion for 10 min prior to ischemia) hearts were studied, with 4 hearts in each group. The hearts were subjected to 30-min global ischemia, and reversed-phase HPLC of heart extracts was performed to measure the intracellular adenosine nucleotide pool. As shown in Fig. 2a and reported previously (27), each of the cellular nucleotides and their metabolites have unique retention times, allowing measurement and quantitation. Myocardial adenine nucleotide levels in EHNA-treated and vehicle control groups before and after ischemia are shown in Figs. 2 and 3. The mean preischemic levels of adenine nucleotides in EHNA and control groups were similar, with no significant differences observed. At the end of 30-min ischemia, ATP and ADP levels in control hearts markedly dropped to 7.2 ± 0.4 and 4.9 ± 1.1 nmol/mg protein from the preischemic values of 33.4 ± 0.5 and 16.3 ± 0.2 nmol/mg protein (p < 0.01, versus preischemia). While pre-
chemic administration of EHNA resulted in higher mean ATP and ADP levels after ischemia with values of 12.6 ± 2.7 and 9.6 ± 2.4 nmol/mg protein, respectively, due to interexperiment variation these differences did not reach statistical significance (p = 0.17 and p = 0.09, respectively, for EHNA versus untreated hearts). Myocardial AMP and NAD levels were also not significantly affected by the inhibitor (Fig. 3A).

Only trace amounts of inosine and adenosine could be detected in preischemic heart. At the end of 30 min of ischemia, the concentration of inosine and adenosine increased to 9.9 ± 0.8 and 8.6 ± 2.6 nmol/mg protein, respectively. In the presence of EHNA, a marked further increase in myocardial adenosine level was observed to a concentration of 17.1 ± 0.8 nmol/mg protein (p < 0.01 versus control hearts). However, the large rise of inosine that occurred in untreated ischemic hearts was dramatically decreased to 2.6 ± 0.2 nmol/mg protein (p < 0.001, versus control) (Figs. 2 and 3B). These data confirmed that adenosine deaminase had been inhibited by the preischemic infusion of EHNA.

Inosine can be further degraded by inosine phosphorylase to form the xanthine oxidase substrates hypoxanthine and xanthine. While no hypoxanthine or xanthine was detected in preischemic hearts, after 30 min ischemia significant hypoxanthine and xanthine formation and accumulation occurred with average concentrations of 2.2 ± 0.3 and 2.4 ± 0.2 nmol/mg protein. In EHNA-treated hearts, hypoxanthine and xanthine concentrations were much lower than in untreated hearts with mean concentrations of 0.6 ± 0.3 and 0.5 ± 0.4 nmol/mg protein (p < 0.01). Thus, EHNA infusion was effective at blocking adenosine deaminase within the heart, and it prevented the formation and accumulation of xanthine oxidase substrates, hypoxanthine and xanthine, in postischemic myocardium.

Fig. 3. Effect of EHNA preischemic administration on the adenine nucleotide pool in hearts subjected to 30-min ischemia. As shown, preischemic infusion with 250 μM EHNA significantly blocked the formation of hypoxanthine and xanthine during ischemia. Each bar represents the mean ± S.E. values obtained from measurements of four hearts.

Effect of EHNA on Free Radical Generation in the Postischemic Heart—Preischemic administration of EHNA significantly reduces the formation and accumulation of radical-generating substrates hypoxanthine and xanthine. In order to determine whether this results in inhibition of free radical generation, experiments were performed comparing radical generation in untreated and EHNA-treated hearts, with three hearts in each group. The coronary effluent containing the spin trap DMPO was collected in 20-s aliquots during both preischemic and postischemic infusion, and EPR measurements were performed. No significant signal was observed prior to ischemia.
and 23.3 indexes were continuously recorded. 250 ischemia followed by 45 min of reperfusion, and hemodynamic fusion, a series of 14 hearts were subjected to 30 min of global and enhanced the recovery of contractile function after reperfusion, with 50 mM DMPO. In untreated hearts (circles) a burst of radical generation during the first 2 min of reperfusion was observed and gradually declined over the next 5 min. In EHNA-treated hearts (squares) radical concentrations were significantly decreased.

in either untreated control or EHNA-treated hearts. In control hearts, a prominent spectrum was seen immediately after the onset of reflow. As reported previously, this spectrum consisted of a large 1:2:2:1 quartet signal, \(a_H = a_N = 14.9 \text{ G} \), indicative of DMPO-OH, though small trace amounts of the six peaked DMPO-R adducts were also present. As shown, the radical signals were markedly attenuated by the preischemic infusion of 250 \(\mu\text{M} \) EHNA.

To evaluate if EHNA in the concentration used could have any direct effects in scavenging free radicals, studies were performed evaluating the effect of 250 \(\mu\text{M} \) EHNA on both superoxide and hydroxyl free radicals generated in vitro from xanthine oxidase using the spin trap DMPO, 50 mM. In the presence of xanthine, xanthine oxidase generates superoxide radical with a prominent DMPO-OOH signal, and EHNA had no effect on the magnitude of this radical generation (Fig. 4, A and B). Similarly in the presence of the iron chelate Fe(III)-nitrilotriacetate, xanthine oxidase and xanthine generate the hydroxyl radical with a prominent DMPO-R signal, and this radical generation was not altered by EHNA (Fig. 6, C and D). Thus, EHNA had no measurable direct effect as a free radical scavenger, which confirms that the observed decrease in radical generation is due to its effect on decreasing the formation of xanthine oxidase substrates.

Effect of EHNA on Postischemic Injury—To determine if adenosine deaminase inhibition prevented postischemic injury and enhanced the recovery of contractile function after reperfusion, a series of 14 hearts were subjected to 30 min of global ischemia followed by 45 min of reperfusion, and hemodynamic indexes were continuously recorded. 250 \(\mu\text{M} \) EHNA or vehicle were infused into the heart prior to ischemia. Preischemic administration of EHNA had no effect on contractile function or coronary flow. However, EHNA-treated hearts had significantly improved contractile performance with higher recovery of left ventricular developed pressure (LVDP) and rate-pressure product (RPP). EHNA-treated hearts exhibited more than 2-fold increased recovery of LVDP and RPP over the entire course of reflow (Figs. 7 and 8). Average final recovery of LVDP and RPP after 45 min of reperfusion was 48.2 \(\pm\) 8.4% and 44.6 \(\pm\) 8.9% in the EHNA group, compared with only 25.0 \(\pm\) 2.5% and 23.3 \(\pm\) 3.7% in the untreated controls (p < 0.01). EHNA pretreatment did not significantly alter the recovery of coronary flow upon reperfusion (Fig. 9).

While EHNA pretreatment resulted in significant beneficial effects on the recovery of contractile function, with inhibition of the formation of the xanthine oxidase substrates hypoxanthine and xanthine, and decreased free radical generation, it also increased the concentrations of adenosine, and this also might exert protective effects. To further confirm the importance of the decreased formation of radical-generating substrates in the protective action of EHNA, additional experiments were performed to determine whether supplementation of hypoxanthine and xanthine within the heart could reverse this protection. As reported previously, the concentrations of hypoxanthine and xanthine within the heart were approximately 500 \(\mu\text{M} \) after 30 min of global ischemia (24). In a series of seven hearts, hypoxanthine
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It has been demonstrated that reactive oxygen species including 
\( \text{O}_2^\bullet \), \( \text{H}_2\text{O}_2 \), and \( \text{OH} \) are produced at the time of postischemic reperfusion (5–7). These oxidants can be cytotoxic to cells by initiating lipid peroxidation of cell membranes and reacting with proteins causing amino acid oxidation and polypeptide chain scission. While several mechanisms have been described for the production of oxygen free radicals in postischemic tissues, xanthine oxidase has been widely accepted as a major primary source of this radical generation (9–14). This theory was initially supported by many studies that demonstrated that administration of xanthine oxidase inhibitors reduced the incidence of reperfusion arrhythmias and improved functional recovery (17). More recently, further direct evidence for the presence of xanthine oxidase-mediated free radical generation in postischemic cells and tissues has been provided by EPR studies that demonstrate that xanthine oxidase inhibition markedly decreases radical generation in both reoxygenated endothelial cells and in the isolated rat heart (12, 13). While it had been questioned whether this mechanism of radical generation occurs in humans tissues (32, 33), recently it has been demonstrated that xanthine oxidase is present in both human arterial and venous endothelial cells and that under conditions of anoxia and reoxygenation the enzyme gives rise to free radical generation, which can result in cellular injury and death (27, 34).

In order to develop a rational and effective approach to prevent free radical generation and radical-mediated injury in the postischemic heart, it is necessary to understand the biochemical process that triggers and controls this radical generation. It has been demonstrated that xanthine oxidase is present and is a major source of radical generation in the isolated rat heart (12). While this was known for some time, questions remained regarding the magnitude and relative importance of substrate formation versus substrate formation in triggering the burst of radical generation seen upon reperfusion. It was originally proposed that in ischemic tissues xanthine dehydrogenase undergoes proteolytic cleavage to form the oxidase and that a large proportion of enzyme conversion would trigger the oxidant burst. It was also recognized that substrate formation would concomitantly occur, further supporting this process of radical generation (9). Recently, studies performed in the isolated rat heart demonstrated that xanthine oxidase-mediated radical generation is triggered and controlled primarily by the formation of large concentrations of the substrates xanthine and hypoxanthine due to the breakdown of ATP during myocardial ischemia (24). While no substrate was detectable prior to ischemia, xanthine oxidase was present, but in the absence of its substrates no radical generation was observed. After ischemia only modest 30% increases were seen. Thus, it was demonstrated that the burst of radical generation in the ischemic heart was triggered and controlled primarily by the formation of xanthine and hypoxanthine during ischemia.

In principle, either blocking xanthine oxidase or inhibiting
the formation of enzyme substrates could have identical effects on radical generation, but the latter strategy would be expected to quench the initiator that triggered the free radical burst. A large number of experiments have been performed to evaluate the effect of xanthine oxidase blockers on reperfusion damage, and variable efficacy has been reported (17–23). In contrast, relatively little research has been reported to assess the potential of inhibiting enzyme substrate formation in attenuating oxidative reperfusion injury. Abd-El-fattah and colleagues (35, 36) have noted with in vivo surgical canine models of global ischemia and reflow that pharmacological interventions aimed at decreasing substrate concentrations can decrease the severity of injury. However, the exact mechanism by which this protection occurred was not elucidated. In the present study, we systematically evaluated how blocking substrate formation affected the burst of free radical generation and radical-mediated heart dysfunction.

While from prior studies it was clear that radical generation was triggered by xanthine oxidase substrate formation, it was not known if blocking this substrate formation could be effective at preventing free radical generation and free radical-mediated reperfusion injury. To confirm the functional importance of this substrate formation in the pathogenesis of postischemic injury and in an effort to elucidate effective pharmacological approaches to prevent this tissue injury, the present studies were performed evaluating the effects of adenosine deaminase inhibition on xanthine oxidase substrate formation, free radical generation, and functional injury in the isolated rat heart. It was observed that the adenosine deaminase inhibitor EHNA was effective at blocking the formation of the xanthine oxidase substrates xanthine and hypoxanthine. More than 70% block of this substrate formation was seen in the presence of 250 μM EHNA, with no adverse functional effects. EPR spin trapping measurements demonstrated that this inhibition of xanthine oxidase substrate formation was sufficient to decrease radical generation by more than 80%. While radical generation was inhibited in the heart, EHNA had no intrinsic efficacy as a scavenger of superoxide or hydroxyl radicals and no measurable effect on xanthine oxidase-mediated radical generation in the presence of added xanthine. Studies of heart contractile function demonstrated that this blockade of xanthine oxidase substrate formation and radical generation resulted in more than a 2-fold increase in the recovery of contractile function upon reperfusion. Therefore, these findings suggest that decreasing the formation of xanthine oxidase substrates by blocking adenosine deaminase can inhibit oxygen free radical generation and prevent myocardial reperfusion injury.

Inhibition of adenosine deaminase not only decreased the concentration of hypoxanthine and xanthine, but it also increased the levels of adenosine within the ischemic heart. This elevation of adenosine could have also potentially exerted a protective effect on the heart beyond that which occurs due to prevention of xanthine oxidase-mediated radical generation. It is well known that adenosine is a potent coronary vasodilator, and adenosine-mediated increases in coronary flow could potentially result in enhanced recovery of cardiac function (37). Indeed, it has been suggested that adenosine may have an important role in the process of myocardial preconditioning, by which short periods of ischemia protect the heart from subsequent longer periods of ischemia (38). In untreated hearts, it was observed that the adenosine concentration after 30 min of ischemia was increased by about 20-fold, while in the presence of EHNA a 40-fold increase was seen. Even though the greater increase in adenosine concentrations in the EHNA-treated hearts could have potentially resulted in increased coronary flow and subsequent protection, no such increase occurred, and the recovery of coronary flow was identical in EHNA and untreated hearts. This suggested that the further increases in adenosine seen with EHNA did not exert significant functional effects on myocardial circulation and function. It is probable that the large increase of adenosine in untreated hearts is more than sufficient to saturate adenosine receptors within the heart. In the EHNA-treated hearts a higher recovery of ATP was also observed, with values of 22% of preischemic values in the untreated hearts (corresponding to an intracellular concentration of 2.0 mM), while values of 36% were measured in the EHNA-treated hearts (intracellular concentration of 3.2 mM). Since it has previously been shown that ATP concentrations of greater than 1 mM are more than sufficient to saturate the myocardial ATPases (39, 40), this change in ATP concentration in itself would not be expected to explain the enhanced recovery of contractile function that was observed with EHNA.

In order to further demonstrate that the beneficial effects of adenosine deaminase inhibition were due to the decreased formation of xanthine oxidase substrates, experiments were performed to determine if the EHNA-induced protection could be reversed by hypoxanthine and xanthine. We observed that an exogenous supply of hypoxanthine and xanthine at concentrations equal to those that occur in the absence of EHNA completely reversed the EHNA-induced protection of the postischemic heart. These results confirm that the EHNA-induced protective effects on postischemic heart function were mainly due to decreased formation of xanthine oxidase substrates.

Adenosine deaminase blockade is only one of several ways to inhibit the formation and accumulation of hypoxanthine and xanthine in ischemic tissues. As shown in the ATP degradation diagram, interrupting any link in the chain of reactions prior to hypoxanthine formation would limit the production of hypoxanthine and xanthine (Fig. 1). In fact, these enzymatic steps provide multiple possible sites of pharmacological intervention. For instance, it has been reported that blocking adenine nucleotide transport protein with p-nitrobenzylthioinosine significantly reduced the formation of hypoxanthine as well as xanthine and improved postischemic heart function (35, 36). Several studies have demonstrated that inhibition of 5’-nucleotidase exerted protective effects on the postischemic heart and suggested that this protection was related to enhanced ATP resynthesis during reperfusion (41, 42). Our observations could provide an alternative explanation for these beneficial effects on postischemic function, since inhibition of 5’-nucleotidase would also be expected to decrease the formation of hypoxanthine and xanthine, which would decrease free radical generation.

In conclusion, we have demonstrated that the formation and accumulation of xanthine oxidase substrates in ischemic myocardium could be largely inhibited by blocking the degradation of adenine nucleotides using the adenosine deaminase blocker EHNA. Limiting substrate formation greatly decreased free radical generation, which in turn resulted in decreased reperfusion injury with increased recovery of contractile function. Thus, inhibition of adenine nucleotide breakdown was found to be highly effective at preventing xanthine oxidase-mediated free radical generation and subsequent contractile dysfunction in the postischemic heart.

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