NITRITE CONFERS PROTECTION AGAINST MYOCARDIAL INFARCTION: ROLE OF XANTHINE OXIDOREDUCTASE, NADPH OXIDASE AND K_{ATP} CHANNELS

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Abstract

Reduction of nitrite to nitric oxide during ischemia protects the heart against injury from ischemia/ reperfusion. However the optimal dose of nitrite and the mechanisms underlying nitrite-induced cardioprotection are not known. We determined the ability of nitrite and nitrate to confer protection against myocardial infarction in two rat models of ischemia/reperfusion injury and the role of xanthine oxidoreductase, NADPH oxidase, nitric oxide synthase and K_{ATP} channels in mediating nitrite-induced cardioprotection. *In vivo* and *in vitro* rat models of myocardial ischemia/reperfusion injury were used to cause infarction. Hearts (n=6/group) were treated with nitrite or nitrate for 15 min prior to 30 min regional ischemia and 180 min reperfusion. Xanthine oxidoreductase activity was measured after 15 min aerobic perfusion and 30 min ischemia. Nitrite reduced myocardial necrosis and decline in ventricular function following ischemia/reperfusion in the intact and isolated rat heart in a dose or concentration-dependent manner with an optimal dose of 4 mg/kg *in vivo* and concentration of 10 μM *in vitro*. Nitrate had no effect on protection. Reduction in infarction by nitrite was abolished by inhibition of flavoprotein reductases and the molybdenum site of xanthine oxidoreductase, and was associated with an increase in activity of xanthine dehydrogenase and xanthine oxidase during ischemia. Inhibition of nitric oxide synthase had no effect on nitrite-induced cardioprotection. Inhibition of NADPH oxidase and K_{ATP} channels abolished nitrite-induced cardioprotection. Nitrite but not nitrate protects against infarction by a mechanism involving xanthine oxidoreductase, NADPH oxidase and K_{ATP} channels.

Keywords

nitrite; xanthine oxidoreductase; NADPH oxidase; K_{ATP} channels; infarction

INTRODUCTION

Until recently the nitrite anion (NO_2^-) has been considered to be an oxidation product of nitric oxide, useful as an index of the catalytic activity of nitric oxide synthase [1]. Approximately 80–90% of plasma nitrite is derived from endothelial nitric oxidase synthase derived nitric oxide [2]. Recently it has been shown that plasma cerulloplasmin has a nitric oxide oxidase activity, and may play a role in the conversion of nitric oxide to nitrite *in vivo* [3]. The nitrite anion, which is present between 0.2 and 10 μM in the blood and tissues [4,5], may represent a storage form of nitric oxide that is made available under conditions of oxygen deprivation to maintain cell survival. Cardioprotective effects of nitrite have recently been demonstrated. Reduction of nitrite to nitric oxide during ischemia protects the heart against injury from ischemia-reperfusion [6,7]. The underlying mechanisms by which nitrite is reduced to nitric oxide have not yet been fully defined. Of the candidates for nitrite reduction *in vivo*, deoxyhemoglobin, deoxymyoglobin and xanthine oxidoreductase have been shown to have an oxygen-sensitive nitrite-reductase activity [8–10]. Data suggest that deoxyhemoglobin plays a role in nitrite-dependent control of vascular tone during hypoxia [11], while xanthine oxidoreductase has been implicated in the cardioprotective effects of nitrite under conditions of oxygen deprivation [7]. In mammals, xanthine oxidoreductase exists in two interconvertible forms, xanthine dehydrogenase (EC 1.1.1.204), which predominates *in vivo*, and xanthine oxidase (EC 1.1.3.22). The extent to which nitrite alters the activity of xanthine dehydrogenase and xanthine oxidase during myocardial ischemia is not known.

In this study we have selected *in vitro* and *in vivo* models of regional myocardial ischemia and reperfusion to determine the ability of nitrite to confer acute cardioprotection in both the presence and absence of hemoglobin, The objectives of our study were to determine 1) the ability of nitrite and nitrate to confer protection against injury from regional ischemia-reperfusion *in vivo* and *in vitro* and 2) to assess the contribution of xanthine oxidoreductase, nitric oxide synthase, NADPH oxidase and the sarcolemmal and mitochondrial ATP-sensitive potassium (K_{ATP}) channels to nitrite-induced cardioprotection.

MATERIALS AND METHODS

Male Sprague Dawley rats at 8 weeks of age used in this study received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" formulated by the National Research Council, 1996.

Nitrite, nitrate and cardioprotection studies *in vivo*

An *in vivo* anesthetized rat model was used for these experiments with the general surgical protocol and determination of infarct size described previously [12]. For infarct size studies, rats underwent 30 minutes of regional ischemia followed by 2 hours of reperfusion. Rats were treated with K_{ATP} channel blockers as needed.

Nitrite, nitrate and cardioprotection studies *in vitro*

Isolated rat hearts were perfused in a retrograde manner with bicarbonate buffer at constant perfusion pressure (98 mmHg) with a balloon inflated in the left ventricle. End-diastolic pressure was set to 5 mmHg and developed pressure was recorded during steady-state conditions [13]. Hearts were allowed to beat spontaneously. Hearts were kept in temperature-controlled chambers to maintain myocardial temperature at 37°C during periods of perfusion and ischemia. Hearts were subjected to 30 minutes of regional no-flow ischemia and 3 hours of reperfusion. Inhibitors of xanthine oxidoreductase, nitric oxide synthase, NADPH oxidase and scavengers of nitric oxide were added to the coronary perfusate as needed.

Xanthine oxidoreductase studies/analysis

The frozen free wall of the left ventricle from perfused rat hearts was powdered in a prechilled mortar and pestle with liquid nitrogen. Ice-cold homogenization buffer (0.25 M sucrose, 10 mM DTT, 0.2 mM PMSE, 0.1 mM EDTA, and 50 mM K^+-phosphate, pH 7.4) was added to the powdered tissue (200mg/ml) and homogenized in a Wheaton 903475 homogenizer. The homogenate was centrifuged at a of speed $40,000 \times g$ at 4 °C for 30 min and xanthine dehydrogenase and xanthine oxidase activities were measured in the supernatant according to the method of Beckman et al [14]. Data (means ± SE) are shown as mIUs of xanthine oxidase/ xanthine dehydrogenase per gram protein.

Statistical analysis

Data reported are mean ± SD. Statistical analysis was performed by use of repeated measures ANOVA with the Greenhouse-Geisser adjustment used to correct for the inflated risk of a Type I error [15]. If significant, the Mann-Whitney test was used as a second step to identify which groups were significantly different [15]. Significance was set at $P < 0.05$.

RESULTS

Nitrite, nitrate and cardioprotection *in vivo*

We determined the dose-response relationship for nitrite to confer protection against injury from myocardial infarction using an *in vivo* model of regional myocardial ischemia and reperfusion. Rats were treated with an I.V. bolus of nitrite (0.04, 0.4, 1.0, 4.0, 7.0 or 10.0 mg/ kg) 15 minutes prior to 30 minutes occlusion of the left anterior descending coronary artery and 2 hours of reperfusion. The effects of increasing nitrite dosage on hemodynamic parameters in the *in vivo* rat model of ischemia reperfusion injury are shown in Table 1. Heart rate, mean arterial pressure, and rate-pressure product showed significant differences in baseline heart rate for the vehicle-treated group and the experimental groups when nitrite was administered 15 minutes prior to ischemia. Rate pressure product was better maintained in nitrite treated animals both 15 minutes and 3 hours after ischemia as compared to vehicle-treated controls.

Figure 1A shows the effect of nitrite dose on infarct size. The area of the left ventricle at risk for myocardial infarction was equivalent in all experimental groups (data not shown). Increased resistance to myocardial ischemia was determined by a reduction in infarct size. The dose-dependent effect of nitrite on infarct size was biphasic, with protection being afforded up to a dose of 4 mg/kg which was lost at higher doses. The dose of nitrite that afforded maximum reduction in infarct (4.0 mg/kg) resulted in an infarct size of 42±3% of the area at risk which corresponds to a 32% reduction in infarct size over controls. Equivalent doses of nitrate (0.04, 0.4, 1.0, 4.0, 7.0 or 10.0 mg/kg I.V.) had no effect on infarct size (Figure 1A).

Timing of nitrite administration

Clinically, patients generally receive medical treatment for acute myocardial infarction after the onset of symptoms. Thus, we determined whether nitrite is able to protect the heart against injury after the onset of ischemia or when administered at the time of reperfusion. Rats were treated with a bolus of nitrite (4 mg/kg I.V.) 15 minutes prior to ischemia, 15 minutes after the onset of ischemia or 10 seconds after the onset of reperfusion. Nitrite was able to reduce infarct size when administered before and during ischemia but not immediately at the onset of reperfusion (Figure 1B).

Nitrite, nitrate and cardioprotection *in vitro*

Recent studies have suggested hemoglobin can act as a nitrite reductase under conditions of oxygen deprivation such as that which occurs during ischemia [11]. To examine if cardiac

tissue contains an intrinsic ability to realize the protective effects of nitrite, we used a buffer perfused isolated heart model of regional ischemia and reperfusion where hemoglobin is absent. Rat hearts were isolated and perfused with nitrite (1.0, 10.0, 30.0, 100.0 or 300 μM) for 15 minutes prior to 30 minutes of regional ischemia and 3 hours of reperfusion. Nitrite (10 μM) had no effect on coronary flow rate prior to ischemia, but increased left ventricular developed pressure (LVDP) from 138±6 mmHg to 149±7 mmHg and decreased heart rate from 238±23 beats/minute to 208±14 beats/minute (Table 2). Increased cardioprotection was measured by a reduction in infarct size and an increase in recovery of developed pressure. In a similar way to the *in vivo* response, nitrite exhibited a biphasic concentration-dependent effect on post-ischemic infarct size (Figure 2A). The protection afforded by lower concentrations of nitrite was lost at the highest concentration. The presence of 10 μM nitrite resulted in an infarct size of 23±5% which corresponds to a 47% reduction in infarct size over control hearts. Nitrite also increased the recovery of LVDP following ischemia and reperfusion, reaching significance between 10 and 100 μM nitrite; an effect that was again lost at the highest nitrite concentration (Figure 2B). Recovery of heart rate and coronary flow was unaffected in hearts treated with 10 μM nitrite compared with untreated hearts. Nitrate (10 μM) had no effect on infarct size or on post-ischemic recovery of LVDP (Figure 2A and B).

Role of xanthine oxidoreductase in nitrite-induced cardioprotection

It has been previously demonstrated that xanthine oxidoreductase can reduce nitrite at the molybdenum site [16]. To determine the role of xanthine oxidoreductase in mediating nitrite-induced cardioprotection hearts were perfused with oxypurinol, an inhibitor of the molybdenum site of xanthine oxidoreductase, or DPI, a non-specific flavoprotein inhibitor, prior to ischemia. Oxypurinol (20 μM) abolished the cardioprotective effect of nitrite (10 μM) but had no effect when used alone (Figure 3A and B). DPI also inhibited nitrite-mediated cardioprotection suggesting a role for both the molybdenum site of xanthine oxidoreductase and a flavoprotein (Figure 3A and B). These data implicate a major role for xanthine oxidoreductase in mediating nitrite reduction.

Nitrite can also act as a suicide substrate for XOR, inactivating the enzyme to the desulfo form [17]. To examine if nitrite affected xanthine oxidoreductase activity, the activity of both the dehydrogenase and oxidase activities of xanthine oxidoreductase were determined before and after ischemia. Aerobic perfusion of hearts with nitrite (10 μM) had no effect on xanthine oxidase or xanthine dehydrogenase activity. Interestingly, ischemia alone (without nitrite) increased the activity of xanthine dehydrogenase but not xanthine oxidase, whereas ischemia plus nitrite increased the activity of xanthine oxidase and almost doubled xanthine dehydrogenase activity. DPI (2 μM) inhibited both xanthine oxidase and xanthine dehydrogenase activity in hearts subjected to ischemia with or without nitrite (Figure 3C).

Role of nitric oxide synthase in nitrite-induced cardioprotection

To determine the role of nitric oxide synthase in mediating nitrite-induced cardioprotection hearts were treated with L-NMA (100 μM), a general nitric oxide synthase inhibitor, prior to ischemia. L-NMA did not prevent nitrite-induced cardioprotection (Figure 4). L-NMA alone had no effect on cardioprotection.

Role of nitric oxide in mediating nitrite-induced cardioprotection

To determine whether the heart generates nitric oxide from nitrite to confer cardioprotection hearts were treated with the nitric oxide scavenger, C-PTIO. C-PTIO (100 μM) abolished nitrite-induced cardioprotection. C-PTIO alone had no effect on cardioprotection (Figure 4).

Role of NADPH oxidase in nitrite-induced cardioprotection *in vitro*

To determine a possible role for NADPH oxidase in mediating nitrite-induced cardioprotection hearts were treated with apocynin (30 μM), an inhibitor of NADPH oxidase. Apocynin abolished nitrite-induced cardioprotection. Apocynin alone had no effect on cardioprotection (Figure 5).

Role of K_{ATP} channels in nitrite-induced cardioprotection *in vitro*

K_{ATP} channels, highly expressed in myocardial sarcolemma and thought to be expressed in myocardial mitochondria, have been found to serve as triggers and mediators of cardioprotection [18–20]. To investigate a role for K_{ATP} channels in mediating nitrite-induced cardioprotection, rats were treated with and without nitrite (4 mg/kg) 15 minutes prior to 30 minutes regional myocardial ischemia and 120 minutes reperfusion. The mitochondrial K_{ATP} channel blocker 5-HD (10 mg/kg) or sarcolemmal K_{ATP} channel blocker HMR 1098 (3 mg/kg) were administered either 20 minutes prior to ischemia or 5 minutes prior to reperfusion. Prior administration of 5-HD or HMR 1098 before ischemia abolished infarct size reduction with nitrite. 5-HD and HMR 1098 given alone before ischemia had no effect on infarct size (Figure 6A). Administration of 5-HD but not HMR 1098 prior to reperfusion abolished infarct size reduction produced by nitrite (Figure 6B). Administration of 5-HD and HMR 1098 alone prior to reperfusion had no effect on infarct size.

DISCUSSION

The present data indicate that nitrite protects the heart against injury from infarction and decreased post-ischemic ventricular function in both *in vivo* and *in vitro* models of ischemia/ reperfusion. This effect of nitrite is dose- and concentration-dependent with optimal protection occurring at 4 mg/kg I.V. *in vivo* and 10 μM *in vitro.* Nitrite conferred cardioprotection over the range of 10–100 μM *in vitro.* One hundred micromolar nitrite also vasodilates aortic ring preparations [21], with subsequent studies showing the effect is mediated by soluble guanylyl cyclase [22,23]. These observations suggested nitrite is being reduced to nitric oxide which elicits protection. Our studies on cardioprotection by nitrite are in strong agreement with the studies of Webb et al [7] and Duranski et al [6] and in addition describe novel findings on the mechanisms underlying nitrite-induced cardioprotection.

We calculated the nitrite concentration in the plasma at the time the heart becomes ischemic 15 minutes after nitrite administration. Previous pharmacokinetic studies of nitrite in the rat [24] predict a plasma concentration of 30 μM 15 minutes after intravenous administration at the optimal dose of 4 mg/kg. Thirty micromolar nitrite conferred cardioprotection in our *in vitro* studies. Thus the optimal dose of 4 mg/kg for the *in vivo* studies is within the range of concentrations (10–100 μM) for nitrite that confer protection in the *in vitro* studies.

Nitric oxide is an important defense against injury from myocardial ischemia/reperfusion. Generation of nitric oxide from L-arginine by nitric oxide synthase requires molecular oxygen as an essential substrate. Under conditions of oxygen deprivation, as encountered during ischemia, oxygen levels are rapidly depleted so that nitric oxide synthase activity will be inhibited. Generation of nitric oxide from the reduction of nitrite under ischemic conditions may represent an important mechanism to maintain nitric oxide production for cell survival and function until aerobic conditions are restored, following reperfusion, so that nitric oxide synthase can resume production of nitric oxide. In control studies, nitrate administered in an identical protocol did not confer cardioprotection in both *in vivo* and *in vitro* models of myocardial ischemia/reperfusion indicating that the protective effect is specific to nitrite.

Xanthine oxidoreductase is a molybdoflavin enzyme present in the highest concentrations in gut, liver and breast milk as well as in plasma from patients with inflammation [25]. Xanthine dehydrogenase is responsible for the conversion of xanthine to uric acid. Xanthine dehydrogenase activity can be converted to xanthine oxidase, under reducing conditions present during ischemia, and it is the oxidase form of the enzyme that is responsible for superoxide production. The activity of xanthine dehydrogenase in the rat hearts we studied accounts for approximately 80% of total enzymatic activity with the balance contributed by xanthine oxidase. This distribution in catalytic activity between the two forms of the enzyme is in agreement with previous studies on xanthine oxidoreductase activity [26,27]. The protective effects of nitrite against infarction and decreased post-ischemic ventricular dysfunction were abolished by oxypurinol, an inhibitor of the molybdo-pterin sites of xanthine oxidoreductase. This site is responsible for the binding and oxidation of purine substrates and has also been shown, in *in vitro* studies to be the site of nitrite reduction [28]. Surprisingly, the effect was also inhibited by DPI, an inhibitor of flavoprotein reductases, which will block electron flow from the molybdenum site to electron acceptors (oxygen or NAD^+). It has been shown previously that DPI is unable to inhibit nitrite reduction by xanthine oxidoreductase using xanthine as a substrate, but could inhibit NADH-driven nitrite reduction [28]. However, DPI is a relatively non-specific inhibitor and it is possible that other flavoprotein reductase enzymes are contributing to nitric oxide formation. We show that apocynin, an inhibitor of the NADPH oxidase, a flavoprotein reductase [29], abolishes nitrite-induced cardioprotection. This novel finding may explain why DPI abolishes the protective effect of nitrite and identifies a component of another mechanism by which nitrite may confer its salubrious actions. Although the reduction of nitrite by NADPH oxidase has not previously been observed, the reduction of nitrite by ferrous heme proteins, such as hemoglobin, is well established and may be mechanistically similar [30].

Our study suggests that nitrite can act as a substrate for both xanthine dehydrogenase and xanthine oxidase during ischemia to produce nitric oxide. Harrison has also shown that nitric oxide formation results from reduction of nitrites, both inorganic [16,31] and organic [32], catalyzed by xanthine oxidoreductase. Activity of xanthine oxidase and xanthine dehydrogenase is increased by nitrite during ischemic conditions, where oxygen and pH are reduced, but not during aerobic conditions. Increased xanthine oxidoreductase activity as a consequence of ischemia has been reported previously [33–36]. Alternately, ischemia may repair the inactive desulfo form of the enzyme to the fully active form as previously suggested [25].

The spin trap carboxy-PTIO abolished nitrite-induced cardioprotection indicating that nitric oxide is responsible for protection against post-ischemic ventricular dysfunction and necrosis. Inhibition of the enzyme nitric oxide synthase with L-NMA did not abolish cardioprotection by nitrite, suggesting nitrite-induced cardioprotection occurs by a nitric oxide synthase-independent mechanism. In addition it has been shown that NOS itself may function as a nitrite reductase in the absence of oxygen [37,38], but the lack of effect of L-NMA rules out this possibility in the isolated cardiac preparation used in these studies.

The signaling pathway by which nitrite protects against injury from ischemia/reperfusion has not been established. We show here that nitrite-mediated protection is mediated in part by the activation of K_{ATP} channels. Currently there are two K_{ATP} channels thought to be present in the cardiac myocyte, one in the sarcolemma (sarc K_{ATP} channel) and one in the inner mitochondrial membrane (mito K_{ATP} channel). Our data show that the sarcolemmal K_{ATP} channel appears to act as trigger and the mitochondrial K_{ATP} channel as an effector of nitrite-induced cardioprotection. There is evidence to suggest that opening either channel may be important in producing cardioprotection, although the bulk of evidence suggests that it is the mito K_{ATP} channel that is responsible for mediating the protective effect of ischemic

preconditioning [39,40]. The role of additional components in the signaling pathway by which the heart is protected by nitrite against injury remains unknown.

Furthermore, we found that a single treatment with nitrite before ischemia reduces cardiac necrosis and increases the recovery of ventricular function following reperfusion. The cardioprotective effect of nitrite is observed immediately after treatment suggesting that the production of new protein is not necessary to manifest its cardioprotective effect. Nitrite also confers protection against injury when given after the onset of ischemia, i.e. after the onset of symptoms. In patients experiencing symptoms of a myocardial infarction, or those who are about to undergo cardiac surgery, nitrite could be administered acutely to decrease injury to the heart from ischemia/reperfusion. Thus, nitrite may represent an important and potent agent for immediately conferring cardioprotection. Nitrite is well tolerated and does not require an elaborate drug delivery system as is needed for several gene-based therapies to confer cardioprotection.

Sodium nitrite, a naturally occurring chemical and common meat preservative, is only used medically to treat cyanide poisoning. Our study suggests this chemical can be used to protect and preserve organs such as the heart in the setting of myocardial infarction and preservation of the heart for transplantation. In support of this notion, nitrite is undergoing clinical trials to increase nitric oxide production in patients with coronary artery disease [41] and in preconditioning mediated tolerance to ischemia [42]. These clinical trials will determine whether dietary nitrite and nitrates can produce nitric oxide to dilate peripheral blood vessels and whether protection by nitrite is equivalent to preconditioning in the setting of ischemia/ reperfusion in the human forearm.

We conclude that nitrite, a simple inorganic anion, is reduced by xanthine oxidoreductase to nitric oxide to increase resistance of the heart to injury from ischemia/reperfusion. The cardioprotective effects of nitrite are also mediated by NADPH oxidase and K_{ATP} channels suggesting nitrite acts by triggering multiple pathways. These observations contribute to a re-evaluation of how we view the mechanisms and biological effects of nitrite.

Acknowledgments

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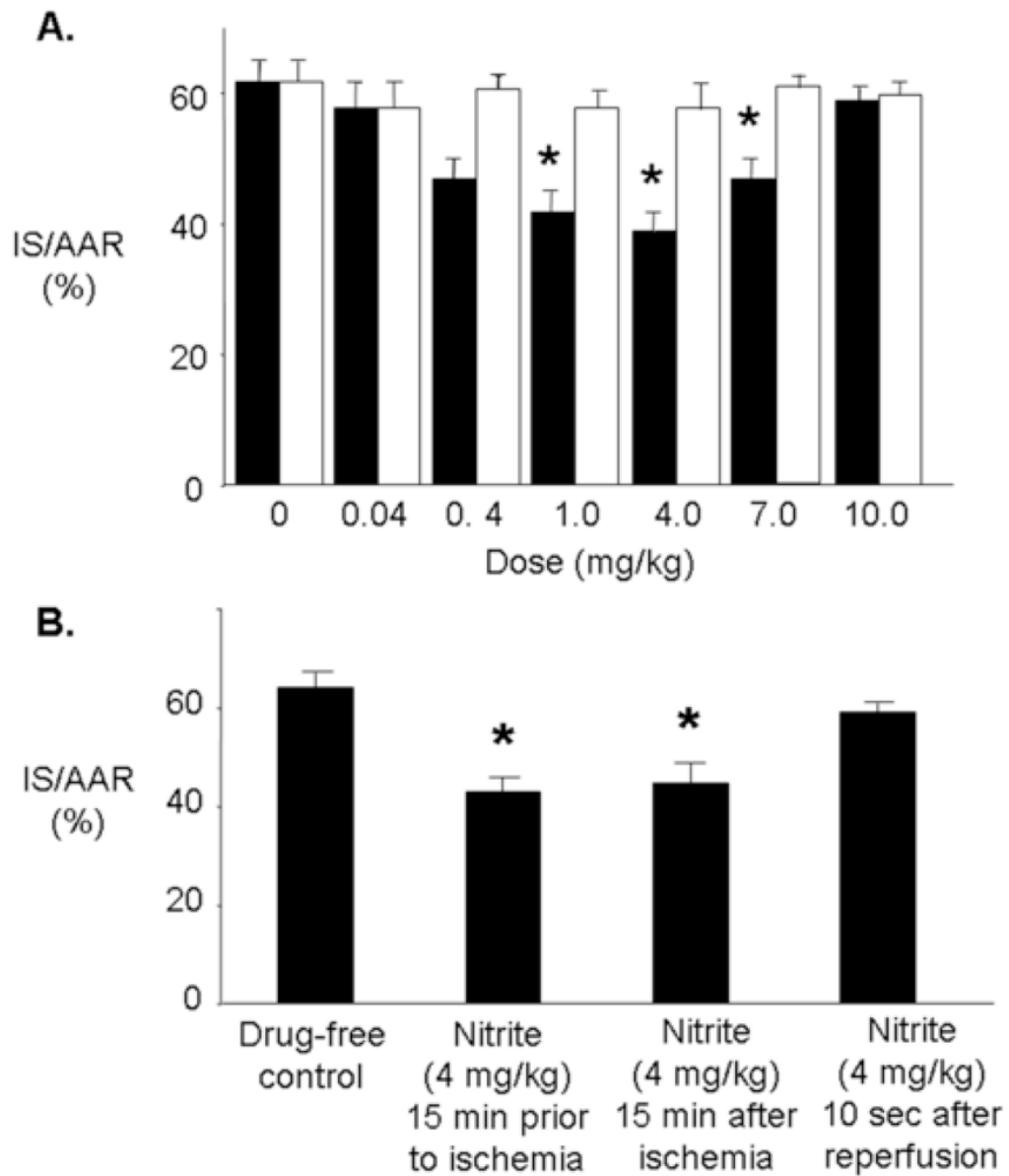
Figure 1.
Nitrite-induced cardioprotection *in vivo*. (**A.**) Dose-response study. Rats were treated intravenously with either saline or increasing doses of nitrite (■) or nitrate (□) administered as an IV bolus at 15 minutes prior to ischemia, followed by 30 minutes regional ischemia and 120 minutes reperfusion. Infarct size was determined at the end of 120 minutes of reperfusion. (**B.**) Phase of action of nitrite. Infarct size was determined after 30 minutes of regional ischemia and 120 minutes of reperfusion. Data are mean ± SD, n=6/group. IS, infarct size * = p<0.05, nitrite vs. drug-free control.
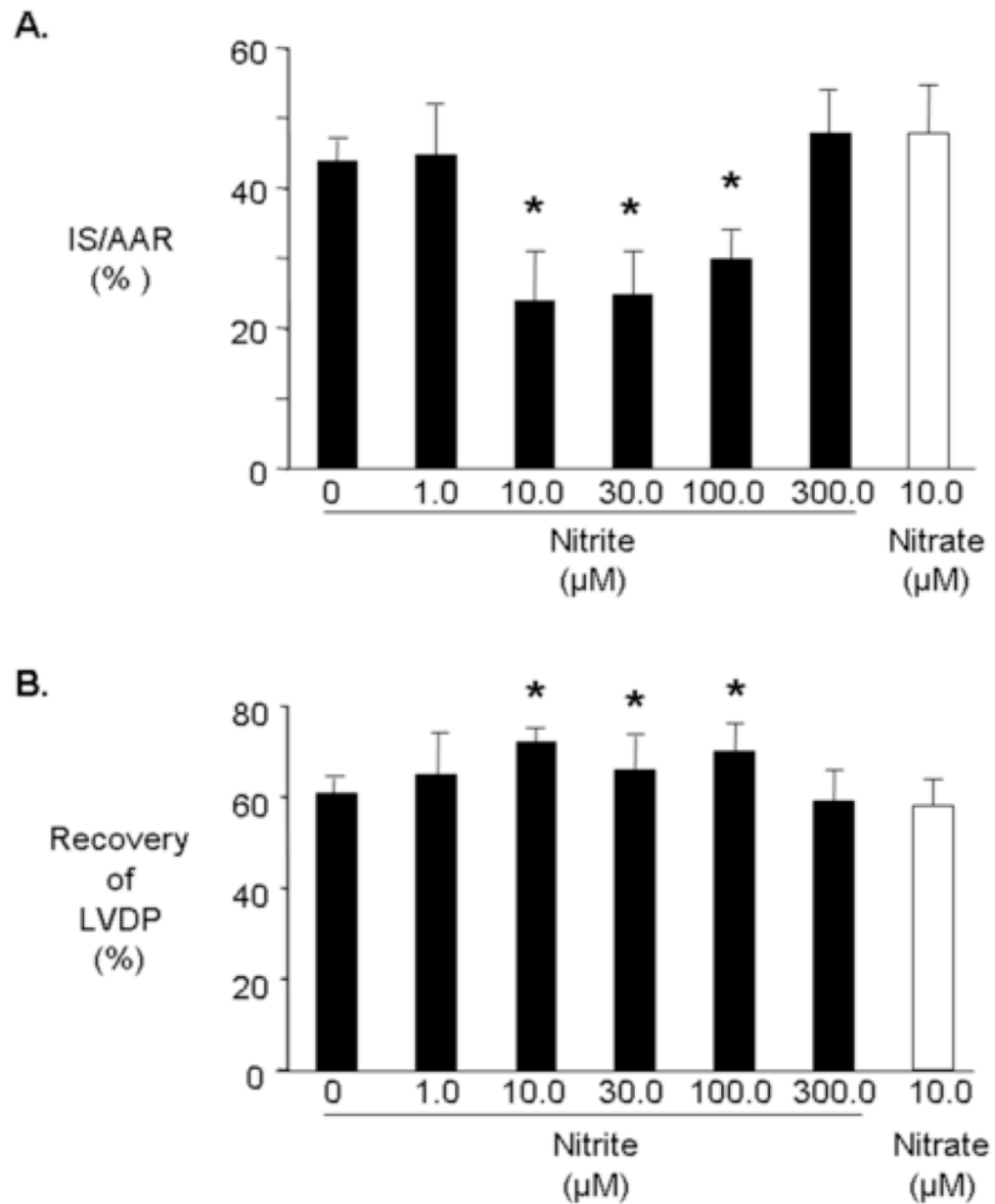
Figure 2.
Nitrite and cardioprotection *in vitro*. Rats were perfused for 15 minutes with nitrite (■) or nitrate (□) prior to 30 minutes regional ischemia and 180 minutes reperfusion. Infarct size was determined at the end of 180 minutes of reperfusion (**A**). Percent infarction after treatment with nitrite or nitrate (**B**). Recovery of left ventricular developed pressure (LVDP). Data are mean ± SD, n=6/group. * = p<0.05, nitrite vs. drug-free control.
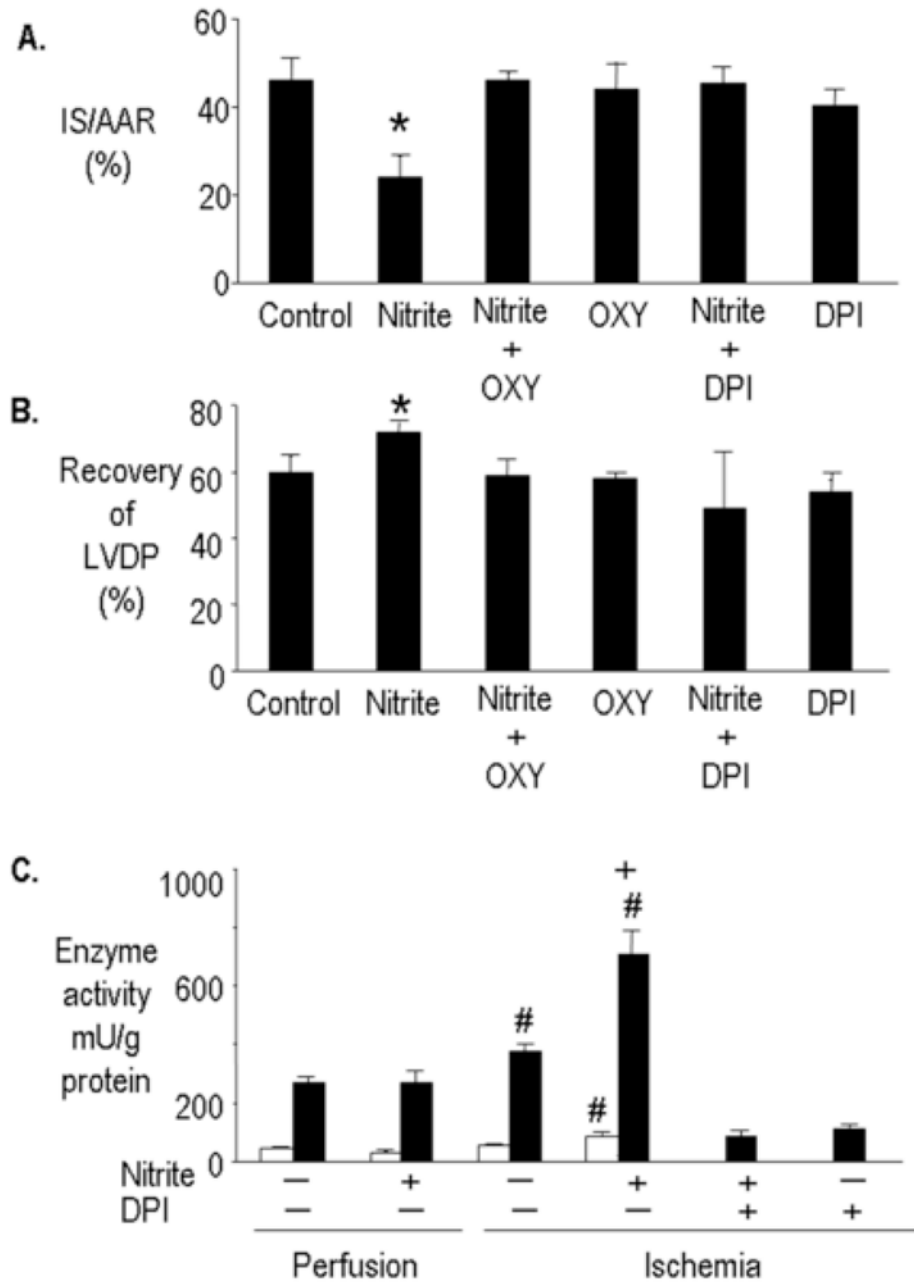
Figure 3.
Inhibition of molybdenum and FAD site in xanthine oxidoreductase prevents nitrite-induced cardioprotection. Rat hearts were perfused with and without nitrite (10 μM) for 15 minutes prior to 30 minutes regional ischemia and 180 minutes of reperfusion. Oxypurinol (OXY) (10 μM) or DPI (2 μM) were present in the coronary perfusate for 30 minutes prior to ischemia (**A**). Percent infarction (**B**). Recovery of left ventricular developed pressure (LVDP). Data are mean ± SD, n=6/group. * = p<0.05, vs. control. (**C**) Ischemia is required for nitrite to increase xanthine oxidase and xanthine dehydrogenase activity. Rat hearts were perfused with nitrite (10 μM) for 15 minutes and subjected to 30 minutes regional ischemia with and without DPI (2μM). Xanthine oxidase (□) and xanthine dehydrogenase (■) activity was then determined.

Data are mean ± SD, n=6/group. # = p<0.05, vs. perfusion. + = p<0.05 ischemia plus nitrite, vs. ischemia.
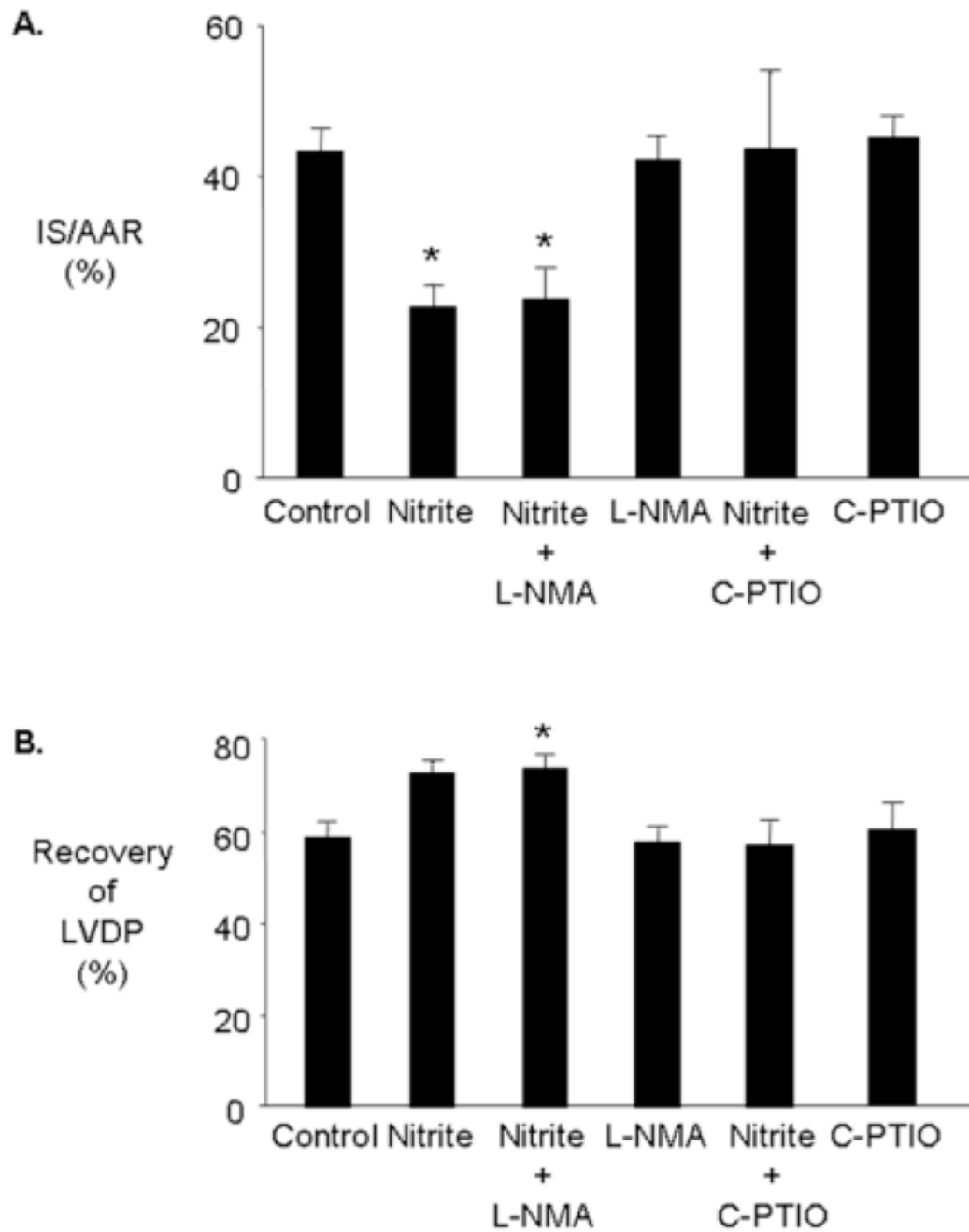
Figure 4.
Mechanisms underlying nitrite-induced cardioprotection. Rat hearts were perfused with and without nitrite (10 μM) and either L-NMA (100 μM) or CPTIO (100 μM) for 15 minutes prior to 30 minutes regional ischemia and 180 minutes of reperfusion. (**A**) Percent infarction (**B**) Recovery of left ventricular developed pressure (LVDP). Data are mean ± SD, n=6/group. * = $p<0.05$, vs. control.
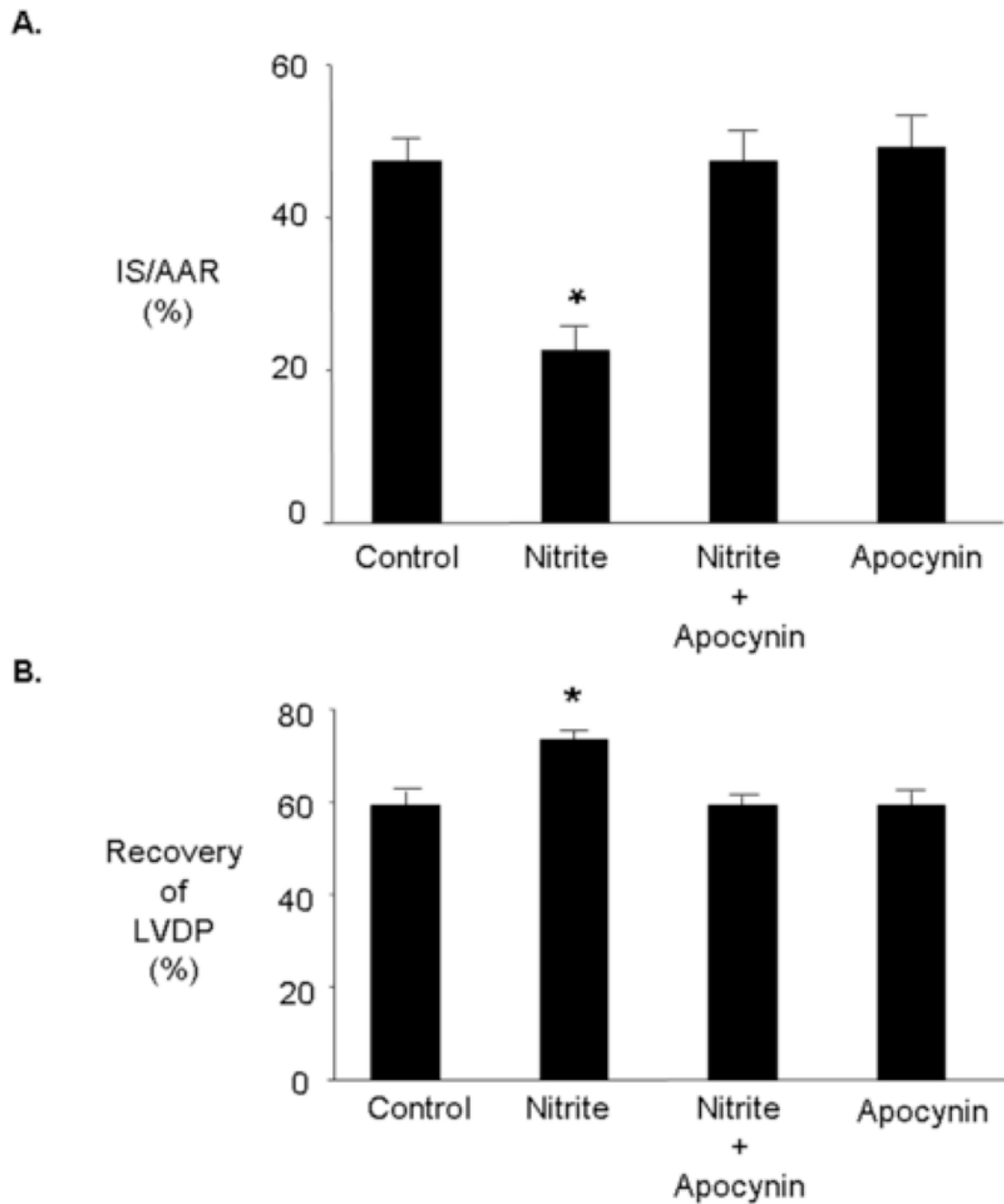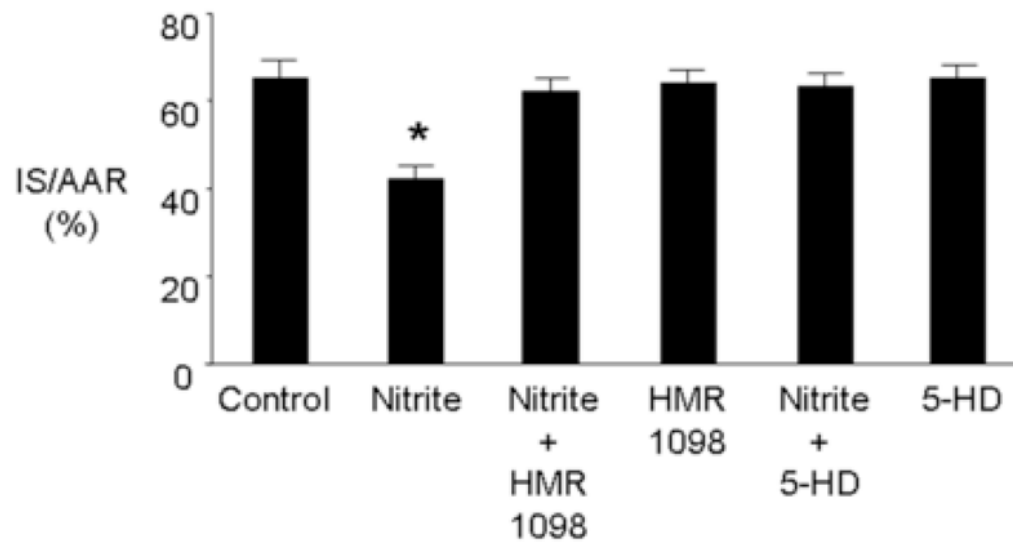
A.

B.

Figure 5.
Nitrite-induced cardioprotection is mediated by NADPH oxidase. Rat hearts were perfused with and without nitrite (10 μM) for 15 minutes prior to 30 minutes regional ischemia and 180 minutes of reperfusion. Apocynin (30 μM) was present in the coronary perfusate for 30 minutes prior to ischemia. (**A**) Percent infarction (**B**) Recovery of left ventricular developed pressure (LVDP). Data are mean ± SD, n=6/group. * = p<0.05, vs. control.

A. HMR 1098 AND 5-HD GIVEN PRIOR TO ISCHEMIA

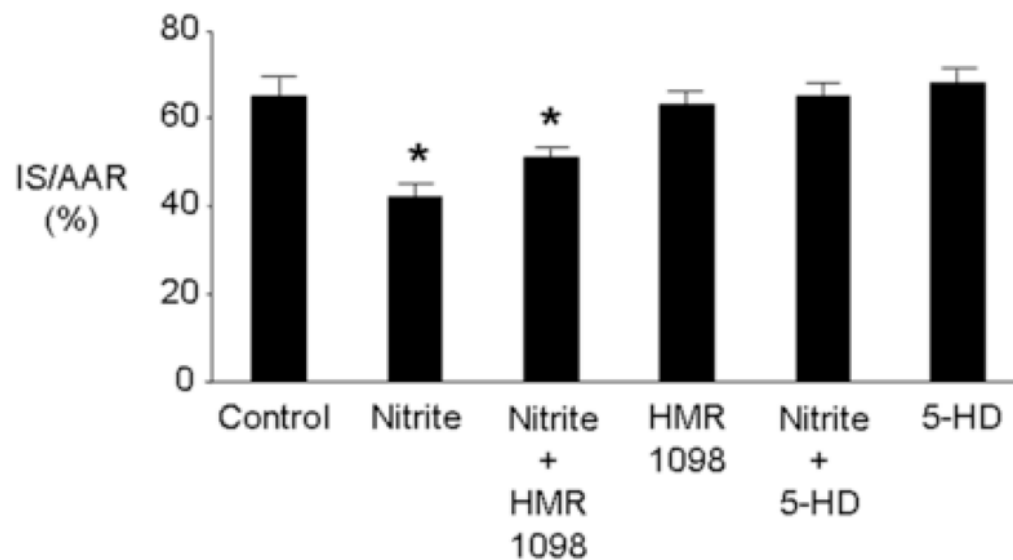## B. HMR 1098 AND 5-HD GIVEN PRIOR TO REPERFUSION

Figure 6.
Nitrite-induced cardioprotection is mediated by K_{ATP} channels. Rats were treated with and without nitrite (4 mg/kg) 15 minutes prior to 30 minutes regional ischemia and 180 minutes of reperfusion. HMR 1098 (3 mg/kg) or 5-HD (10 mg/kg) were administered either 20 minutes prior to ischemia or 5 minutes prior to reperfusion. Data are mean $\pm$ SD, n=6/group. * = p<0.05, vs. control.

Table 1

Hemodynamic values for nitrite acute dose-response studies *in vivo*.

	BASELINE			15min ISCHEMIA			3hr REPERFUSION		
	Heart rate, beats/min	MAP, mmHg	RPP mmHg /sec	Heart rate, beats/min	MAP, mmHg	RPP mmHg /sec	Heart rate, beats /min	MAP, mmHg	RPP mmHg /sec
Drug-free control	378±15	124±6	42±4	360±14	94±11	29±5	370±14	66±9	20±4
Nitrite (0.04mg/kg)	390±12	124±5	49±3	390±12	97±5	38±3	373±7	67±3	25±3
Nitrite (0.4 mg/kg)	380±12	119±4	45±13	377±11	103±5	38±11	353±13	70±11	25±8
Nitrite (1.0 mg/kg)	395±6	136±9	48±4	380±8	105±9	40±9	333±10	94±8	31±8[*]
Nitrite (4.0 mg/kg)	368±12	123±5	44±3	365±13	97±9	36±4	335±12	74±3	25±3
Nitrite (7.0 mg/kg)	368±10	130±7	47±6	375±12	101±6	38±3	358±11	78±8	27±3
Nitrite (10.0 mg/kg)	370±10	122±4	45±2	370±13	98±8	37±6	353±9	66±6	23±2

Data are mean ± SE, n = 6/group,

[*]
= $P < 0.05$, nitrite plus ischemia vs ischemia alone. MAP = mean arterial pressure, RPP = rate pressure product.

Table 2

Hemodynamic values for nitrite concentration-response studies *in vitro*.

Groups	PRE DRUG			POST DRUG			REPERFUSION		
	Heart rate (beats/min)	Coronary flow rate (ml/min/g)	Left ventricle developed pressure (mmHg)	Heart rate (beats/min)	Coronary flow rate (ml/min/g)	Left ventricle developed pressure (mmHg)	Heart rate (beats/min)	Coronary flow rate (ml/min/g)	Left ventricle developed pressure (mmHg)
Drug-free control	274±13	6±1	124±4	---	---	--(70)	193±23	3±1	73±5
Nitrite (1.0μM)	308±15	6±1	135±5	287±16	6±1	136±4	235±22	4±1	85±7
Nitrite (10.0μM)	238±23	5±1	138±6	208±14	5±1	149±7	155±17	3±1	100±7[*]
Nitrite (30.0μM)	253±12	5±1	126±6	235±13	5±1	134±7	175±16	3±1	83±7[*]
Nitrite (100μM)	248±13	5±1	128±6	230±12	5±1	136±7	169±12	3±1	85±6[*]
Nitrite (300μM)	256±19	5±1	121±7	224±24	5±1	132±6	186±21	3±1	71±6

Data are mean ± SE, n = 6/group,

[*] = $P<0.05$, Nitrite vs. drug-free control.