Blockade of electron transport before ischemia protects mitochondria and decreases myocardial injury during reperfusion in aged rat hearts

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Abstract

Myocardial injury is increased in the aged heart following ischemia and reperfusion (I-R) in both humans and experimental models. Hearts from aged 24 mo. old Fischer 344 rats sustain greater cell death and decreased contractile recovery after I-R compared to 6 mo. adult controls. Cardiac mitochondria incur damage during I-R contributing to cell death. Aged rats have a defect in complex III of the mitochondrial electron transport chain (ETC) localized to the interfibrillar population of cardiac mitochondria (IFM), situated in the interior of the cardiomyocyte among the myofibrils. The defect involves the quinol oxidation site (Qo) and increases the production of reactive oxygen species (ROS) in the baseline state. Ischemia further decreases complex III activity via functional inactivation of the iron-sulfur subunit. We studied the contribution of ischemia-induced defects in complex III to the increased cardiac injury in the aged heart. The reversible blockade of the ETC proximal to complex III during ischemia using amobarbital protects mitochondria against ischemic damage, removing the ischemia component of mitochondrial dysfunction. Reperfusion of the aged heart in the absence of ischemic mitochondrial damage decreases net ROS production from mitochondria and reduces cell death. Thus, even despite the persistence of the age-related defects in electron transport, protection against ischemic damage to mitochondria can reduce injury in the aged heart. The direct therapeutic targeting of mitochondria protects against ischemic damage and decreases cardiac injury during reperfusion in the high risk elderly heart.

Keywords

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INTRODUCTION

The aged heart sustains greater injury during ischemia and reperfusion in experimental animal models [1] as well as in elderly patients.[2] Myocardial cell death is increased during ischemia and reperfusion in the aged heart compared to adult hearts. Furthermore, the activation of cytoprotective signal transduction systems to protect the aged heart, either via preconditioning before ischemia[3] or postconditioning at the onset of reperfusion[3; 4] are far less effective in the elderly heart compared to the young heart. Thus, the aged heart suffers not only greater cell death, but has a decreased innate capacity for cytoprotection. New, direct therapeutic approaches to decrease ischemia-reperfusion injury in the aged heart are needed.

Cardiac mitochondria contribute a critical role in the genesis of injury during ischemia and reperfusion,[5] and exist in two populations: subsarcolemmal mitochondria (SSM), located beneath the plasma membrane, and interfibrillar mitochondria (IFM) present between the myofibrils.[6] Aging decreases oxidative phosphorylation and the activity of complexes III and IV only in interfibrillar mitochondria (IFM) that reside among the myofibrils, whereas subsarcolemmal mitochondria (SSM), located beneath the plasma membrane, remain unaltered.[7; 8] The peptide subunit composition of complexes III[7; 9] and IV[10] remains preserved in IFM during aging. The aging-defect in complex IV is due to the altered inner membrane lipid environment.[10; 11] The defect in complex III is within the ubiquinol binding site of the cytochrome b subunit and increases the production of reactive oxygen species even in the baseline state.[12]

Ischemia damages the mitochondrial electron transport chain in both the adult and aged heart, leading to decreased complex III activity,[7] diminished cytochrome c content,[7; 13] and reduced cytochrome oxidase activity.[7] Ischemia inactivates the iron-sulfur peptide subunit in complex III.[7] Ischemia-mediated damage of the electron transport chain persists during reperfusion.[14; 15] The presence of damaged mitochondria during reperfusion decreases energy production,[5; 16] increases the generation of reactive oxygen species (ROS),[17] and favors the release of cytochrome c from mitochondria to trigger myocyte cell death during reperfusion.[5]

We propose that both age-related and ischemia-induced defects in mitochondria contribute to the enhanced age-induced damage in the elderly heart evident following ischemia and reperfusion. Both aging and ischemia lead to defects within the distal electron transport chain. We tested the hypothesis that blockade of electron flow into the distal electron transport chain during the period of ischemia would attenuate mitochondrial damage and subsequent cardiac injury in the aged heart.

The electron transport chain itself is the source of ischemic damage to mitochondria that leads to mitochondrial-derived injury during reperfusion in the adult heart.[15; 18] The blockade of electron transport during ischemia protects cardiac mitochondria[15; 19] indicating that the electron transport chain itself contributes to the mitochondrial chain damage. The reversible blockade of electron transport before ischemia with amobarbital protects mitochondria and decreases myocardial injury during reperfusion in the adult heart, supporting that ischemic mitochondrial damage contributes to myocardial injury during reperfusion.[15; 19]

Amobarbital (AMO) is a short-acting barbiturate that reversibly inhibits at the rotenone-site of complex I.[20] Administration of AMO (2.5 mM) before ischemia resulted in an significant increase of NADH fluorescence in buffer perfused hearts, supporting that AMO blocks complex I in situ.[21] In AMO-treated hearts, cardiac contractility recovered within 30 seconds of reperfusion[21], indicating that AMO is a reversible complex I inhibitor and can

wash out efficiently within a half minute of perfusion. Amobarbital (2.5 mM) given immediately before ischemia preserves oxidative phosphorylation in the isolated adult rat heart,[15] performed as a preliminary dose-ranging study for the current investigation. In the present study, we tested if reversible blockade of electron transport with amobarbital before ischemia could attenuate the ischemic component of damage present in the aged heart. More importantly, the impact of potential mitochondrial protection was assessed to evaluate if a decrease in ischemic mitochondrial damage would translate into reduced myocardial injury following ischemia and reperfusion in the aged heart. Despite the presence of age-related defects in electron transport, the reversible blockade of electron flow during ischemia does indeed protect the aged heart. Thus, the aged heart can still be protected despite pre-existing aging-induced defects in respiration. The direct modulation of mitochondrial electron transport during ischemia can attenuate the damage from age-related respiratory defects and provides a key option to protect the high risk aged heart during ischemia and reperfusion.

METHODS

Preparation of rat hearts for perfusion

The Animal Care and Use Committees of the Louis Stokes Cleveland VA Medical Center and Case Western Reserve University approved the protocol. Male Fisher 344 24 months rats (410 – 440 g) were anesthetized with pentobarbital sodium (100 mg/kg i.p.) and anti-coagulated with heparin (1000 IU/kg i.p.). Hearts were excised and perfused retrograde via the aorta with modified Krebs-Henseleit (K-H) buffer oxygenated with 95% O_2/5% CO_2 as previously described.[13] Left ventricular developed pressure (LVDP) was measured with a balloon inserted into left ventricle. In the untreated ischemia-reperfusion group (IR), hearts initially were perfused for 15 min. followed by 25 min. global ischemia (37°C) and 30 min. reperfusion. In the amobarbital-treated group (AMO), hearts followed the same perfusion protocol except that amobarbital (2.5 mM) was perfused in identical K-H buffer for one min. immediately before ischemia.[13] Hearts in the time control group (TC) were perfused for 71 min without any treatment. Hearts were paced at 300 beats per min during the 15 min. equilibration period and after 10 min. reperfusion. During amobarbital perfusion, pacing was stopped. The release of lactate dehydrogenase (LDH) from myocardium was assayed in coronary effluent by the fluorometric measurement of NADH consumption during the conversion of lactate to pyruvate.[15]

Isolation of subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria

At the end of perfusion, hearts were removed from the cannula and placed into buffer A [(mM) 100 KCl, 50 MOPS, 1 EGTA [ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid], 5 $MgSO_4 \bullet 7 H_2O$, and 1 ATP; pH 7.4] at 4°C. Cardiac mitochondria were isolated using the procedure of Palmer[22] except that trypsin was used as the protease.[13] Cardiac tissue was finely minced and placed in buffer A containing 0.2% bovine serum album and homogenized with a polytron tissue processor (Brinkman Instruments, Westbury, NY) for 2.5 s at a rheostat setting of 6.0. The polytron homogenate was centrifuged at 500 g, the supernatant saved for isolation of SSM, and the pellet washed. The combined supernatants were centrifuged at 3,000 g to sediment SSM. IFM were isolated by incubation of skinned myofibers, obtained following polytron treatment, with 5mg/g (wet weight) trypsin for 10 min at 4 °C. SSM and IFM were washed twice and then suspended in KME (80 mM KCl, 50 mM MOPS, and 0.5 mM EGTA). Mitochondrial protein concentration was measured by the Lowry method, using bovine serum album as a standard.[23]

Mitochondrial oxidative phosphorylation

Oxygen consumption by mitochondria was measured using a Clark-type oxygen electrode at 30°C.[13] Mitochondria were incubated in 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM

KH_2PO_4, and 1 mg defatted, dialyzed bovine serum albumin/ml BSA at pH 7.4. Glutamate (complex I substrate, 20 mM), succinate (complex II substrate, 20 mM), duroquinol (complex III substrate, 5 mM), and TMPD-ascorbate (complex IV substrate, 10 mM) were used and state 3 (0.2 mM ADP-stimulated), state 4 (ADP-limited) respiration, respiratory control ratio, maximal ADP-stimulated respiration (2 mM ADP), and the ADP/O ratio were determined. Rotenone (5 μM) was used with non-complex I substrates. Mitochondria always were used within 6 hr. after isolation from tissue. Endogenous substrates were depleted by addition of 0.1 mM ADP when glutamate was the substrate.

Citrate synthase enzyme activities

Citrate synthase activity was measured in detergent-solubilized, isolated SSM and IFM using previously described methods.[24]

Detection of H_2O_2 production

H_2O_2 production from intact mitochondria was measured using the oxidation of the fluorogenic indicator amplex red in the presence of horseradish peroxidase (HRP).[25]

Measurement of cytochrome content

Cytochrome contents were determined in mitochondria solubilized in 2% deoxycholate in 10 mM sodium phosphate buffer using the difference of sodium dithionite reduced and air-oxidized spectra.[7]

Statistical Analysis

[26] Data are expressed as the mean ± standard error of the mean. Differences among groups were compared by one-way analysis of variance with post-hoc comparisons performed using the Student-Newman-Keuls test of multiple comparisons. A paired t-test was used to analyze the difference between SSM and corresponding IFM in oxidative phosphorylation and H_2O_2 generation experiments. A difference of $p<0.05$ was considered significant.

RESULTS

Reversible blockade of electron transport during ischemia decreases myocardial injury measured following reperfusion

There were no differences in left ventricle developed pressure (LVDP) and diastolic pressure between groups (TC, IR, AMO) at the end of the equilibration perfusion (15 min.) immediately before the administration of amobarbital or vehicle (Figure 1, Panel A & B). Ischemia markedly increased diastolic pressure in untreated hearts, whereas blockade of electron transport with amobarbital prevented this increase (Figure 1, Panel B). The recovery of left ventricular systolic function (Figure 1&2, Panel A: LVDP and dp/dt_{max}) and diastolic function (Figure 1&2, Panel B: diastolic pressure and dp/dt_{min}) during reperfusion were substantially improved by reversible blockade of electron transport during ischemia. Reversible blockade of electron transport during ischemia significantly attenuated myocardial injury as shown by the decreased LDH release during reperfusion (Figure 3).

Reversible blockade of electron transport during ischemia preserves mitochondrial function following reperfusion

The protein yield of SSM and IFM was not altered by ischemia-reperfusion or amobarbital treatment (Table 1). The activity of citrate synthase, a mitochondrial matrix marker enzyme, was also similar in all groups, confirming a similar relative purity of mitochondrial isolates in all groups (Table 1). In time control and amobarbital treated hearts, the rate of oxidative

phosphorylation in IFM is higher than that in corresponding SSM, consistent with our previous findings (Table 2 and Figure 4) [10].

With glutamate as complex I substrate, ischemia-reperfusion decreased the rate of ADP-stimulated state 3 and dinitrophenol-uncoupled respiration in both SSM and IFM compared to the time control group (Table 2, Figure 4 Panel A). Amobarbital treatment only during ischemia markedly improved state 3 and uncoupled respiration measured following 30 min. reperfusion compared to the untreated ischemia-reperfusion group (Table 2). Ischemia-reperfusion slightly increased the rate of ADP-limited state 4 respiration compared to time control in SSM (Table 2). The preserved rate of state 3 respiration improved the coupling of respiration, reflected in the respiratory control ratio (Table 2).

Ischemia-reperfusion decreased the rate of oxidative phosphorylation with succinate (complex II), duroquinol (complex III), and complex IV substrates in both SSM and IFM, whereas amobarbital treatment preserved oxidative phosphorylation in the two populations of mitochondria with all substrates (Figure 4, Panel B, C, and D). Thus, the reversible blockade of proximal electron transport only during ischemia prevented damage to distal part of electron transport chain during the subsequent reperfusion period.

Reversible blockade of electron transport during ischemia preserves cytochrome c content following reperfusion

Ischemia-reperfusion caused cytochrome c loss from both SSM and IFM (Table 3). Blockade of electron transport with amobarbital before ischemia preserved cytochrome c content in both SSM and IFM that were isolated at the end of reperfusion (Table 3). There were no differences in contents of cytochromes c_1, b, and aa_3 between time control, ischemia-reperfusion, and amobarbital treated ischemia-reperfusion groups (Table 3).

Reversible blockade of electron transport during ischemia decreases net H_2O_2 production from SSM and IFM following reperfusion

Ischemia-reperfusion damaged the mitochondrial electron transport chain and increased the net release of H_2O_2 from isolated, intact mitochondria compared to time control with glutamate as complex I substrate (Figure 5, Panel A). With succinate as the complex II substrate in the presence of rotenone to prevent reverse electron flow, net H_2O_2 release was also markedly increased in SSM and IFM following ischemia-reperfusion (Figure 5, Panel B). Amobarbital inhibition protected the electron transport chain and decreased net H_2O_2 release in both SSM and IFM in the presence of complex I and complex II substrates (Figure 5), suggesting that the damaged electron transport chain is the source for the increased H_2O_2 generation. Ischemia-damaged IFM generated more H_2O_2 compared to their corresponding SSM when glutamate and succinate + rotenone were used as complex I and complex II substrates (Figure 5). Thus, the superimposed damage in aged IFM may augment myocardial injury by increasing ROS generation.

DISCUSSION

Myocardial injury is increased in the aged heart following ischemia-reperfusion.[1] Ischemic preconditioning, a powerful endogenous protective mechanism, is unable to protect the aged heart during ischemia-reperfusion.[3] In the present study, we found direct, mitochondrial-targeted therapy using the reversible blockade of electron transport during ischemia with amobarbital decreased myocardial injury assessed following reperfusion in aged rat hearts. This protection is related to a decrease in ischemia-mediated damage to the electron transport chain. During reperfusion, the protected mitochondria exhibit improved oxidative phosphorylation, decreased production of reactive oxygen species and improved retention of

cytochrome c. These salubrious effects all favor a decrease in myocardial injury during reperfusion. Thus, the direct modulation of mitochondrial respiration protects the aged heart and provides a potential strategy to mitigate aged-enhanced cardiac injury despite the presence of persistent aging-induced defects in mitochondrial respiration.

The aging process decreases mitochondrial respiration.[27] Mitochondrial dysfunction is associated with a pro-apoptotic cellular environment in senescent cardiac muscle, especially in IFM in aged Fisher 344 rats.[28; 29] The decrease in oxidative phosphorylation in IFM from aged hearts is due to the decreased activities of complex III[12] and cytochrome oxidase.[28] The age-related defect in complex III in IFM increases ROS generation in the baseline state from complex III [12] leading to increased net production from intact mitochondria.[30] These age-related defects in metabolism contribute to the increased injury observed in the aged heart. Acetylcarnitine treatment administered to the animal restores the activities of complex III and complex IV in IFM isolated from the hearts of elderly 24 month F344 rats.[31] Hearts from acetylcarnitine-treated 24 month Fischer 344 rats exhibited less LDH release and improved functional recovery following ischemia and reperfusion compared to untreated elderly hearts, supporting that notion that pre-existing age-related defects in complex III contribute to greater cardiac damage following ischemia and reperfusion.[31]

In the present study, the contribution of mitochondrial damage caused by ischemia to cardiac injury in the aged heart was directly addressed. Could blockade of electron transport during ischemia protect the aged heart that already contained defects in oxidative phosphorylation? The reversible blockade of electron transport only during ischemia using amobarbital protects adult heart mitochondria during ischemia.[15] When adult hearts that contain mitochondria lacking ischemic damage are reperfused without additional treatment, mitochondria do not sustain additional damage during reperfusion and myocardial injury is decreased.[15] In the present study, amobarbital blockade only during ischemia in the aged heart results in rates of oxidative phosphorylation similar to elderly non-ischemic time controls (Figure 4, Table 2), supporting the notion that mitochondria protected during ischemia do not sustain significant additional damage during reperfusion in the aged heart. In the presence of preserved mitochondrial function during early reperfusion, myocardial injury is decreased, even in the aged heart (Figure 1). Amobarbital treatment before ischemia decreased LDH release about 40% in adult rat hearts following 25 min ischemia and 30 min reperfusion.[15] In the current study, amobarbital treatment before ischemia decreased LDH release by approximately 30% in aged hearts during reperfusion. Thus, blockade of electron transport using amobarbital provides a comparable extent of protection with the adult heart during ischemia-reperfusion.

The observation that reversible blockade of respiration during ischemia can decrease myocardial injury in the aged heart suggests that aging-impaired mitochondria can nonetheless still be manipulated at the proximal electron transport chain in order to decrease cardiac injury. In contrast to the direct inhibition of amobarbital, indirect modulation of mitochondrial function by ischemic preconditioning or postconditioning is less effective in the aged heart as an intervention to reduce cardiac injury.[32] Thus, direct modulation of mitochondrial respiration could be considered to protect the aged heart despite impaired endogenous protection.

Increased ROS generation from the electron transport chain contributes to cardiac injury.[33] Ischemic damage to the electron transport chain increases ROS generation during re-oxygenation.[18; 21; 33] Protection of mitochondria during ischemia decreases ROS generation during reperfusion in the adult heart.[15] In the current study, amobarbital treatment prevented the ischemic component of damage to the electron transport chain. In the absence of ischemic damage to electron transport, there was less ROS generation during reperfusion,

supporting that despite the continued presence of aging defects in electron transport, that prevention of the ischemic component of damage decreased the ROS production during reperfusion.

Cytochrome *c* contributes a critical role in electron transport under physiological conditions, whereas cytochrome *c* release from mitochondria triggers programmed cell death.[34; 35] Blockade of electron transport at complex I prevents cytochrome *c* release in adult hearts during ischemia[13; 19] with protection that persists into reperfusion.[15] Blockade of electron transport only during ischemia preserved cytochrome *c* content in both SSM and IFM in aged hearts measured after reperfusion, suggesting that electron transport mediated damage during ischemia remains a major contributor to cytochrome *c* release during reperfusion in the aged heart. Preservation of cytochrome *c* content is a key mechanism in the protection of myocardium during ischemia and reperfusion.[15; 16; 19; 34]

Ischemic preconditioning protects mitochondria and decreases myocardial injury in adult hearts.[36; 37] Unfortunately, ischemic preconditioning does not protect aged hearts during ischemia and reperfusion as shown by multiple investigators.[38] The cardioprotection of preconditioning is mediated via the activation of cytoprotective signaling cascades that converge on mitochondria to attenuate mitochondrial-driven cardiomyocyte injury. Cytoprotective cascades include the activation of PKC epsilon[39] to modulate the mitochondrial K_{ATP} channel [40] and the activation of the "RISK" kinase pathway that includes Akt to phosphorylate GSK3beta to block mitochondrial permeability transition.[41] Although it not entirely clear, it appears that the defect that limits signal transduction mediated cytoprotection lies upstream of the mitochondria.[42] The endogenous protective mechanisms can be restored in aged hearts by longer term treatments including caloric restriction,[43] an exercise program,[44] or a pharmacologic strategy to inhibit protein phosphatase 2A activity.[45]

Mitochondria in the aged heart remain responsive to direct pharmacologic modulation. In addition to the robust cardioprotection derived from direct targeting of mitochondria to modulate electron transport in the present study, mitochondria from aged hearts remain responsive to direct manipulation of the K_{ATP} channel by nicorandil[46] or diazoxide treatment.[47] Direct modulation of permeability transition pore susceptibility[48–50] also limits damage in the aged heart. Alternative approaches to modulate electron transport also exist, including the use of chronic treatment with nitrate to attenuate oxidative damage from mitochondria,[51] the use more acutely of nitrite to partially block complex I[52] and perhaps hydrogen sulfide generation within the myocardium.[53] Thus, when the presence of comorbid conditions such as aging[3] or diabetes[4] blunt the effectiveness of classic preconditioning, direct targeting of the effector of cardiac injury, the mitochondria, fortunately remains an effective alternative therapeutic strategy.

The protected mitochondria during ischemia decrease myocardial injury during reperfusion in aged heart, providing strong support that the resumption of aerobic metabolism by mitochondria with ischemic damage to the electron transport chain augments cardiac injury during reperfusion. If the ischemia-damaged electron transport chain is the cause, then the transient modulation of electron transport at the onset of reperfusion should reduce cardiac injury. Ischemic postconditioning, the use of brief, transient recurrent ischemia during early reperfusion, reduces injury in the adult heart[54] but is again ineffective in the elderly heart.[3] Since postconditioning and preconditioning and share overlapping mechanisms of protection[55] and overlapping extents of cardioprotection,[56] it is not surprising that kinase dependent cytoprotection applied at the onset of reperfusion is also ineffective in the aged heart. The brief, transient blockade of electron transport by amobarbital at the onset of reperfusion decreases myocardial injury in adult rat heart[15; 57] and rabbit heart.[57; 58] Initial

results in the aged heart[59] suggest that the modulation of electron transport for a brief period at the onset of reperfusion reduces injury consistent with the important principal advanced in the present study.

Thus, even in a setting where the presence of comorbid conditions, in this case aging, enhance myocardial injury[2] and reduce the effectiveness of endogenous cytoprotective mechanism,[3] transient blockade of electron transport remains an effective intervention to protect mitochondria and myocardium against injury. The robust protection observed by the reversible blockade of electron transport during ischemia highlights the contributions of age-related defects at complex III and complex IV in the distal electron transport chain to cardiac injury in the aged heart. The present study provides key evidence that direct modulation of mitochondrial function despite the presence of important preexisting defects in respiratory function, can nonetheless attenuate damage to mitochondria and translate into myocardial protection, even if activation of upstream signaling systems that converge upon the mitochondria are ineffective. The present study provides potential insight into future strategies for protection of the elderly [3] and possibly diabetic hearts.[4]

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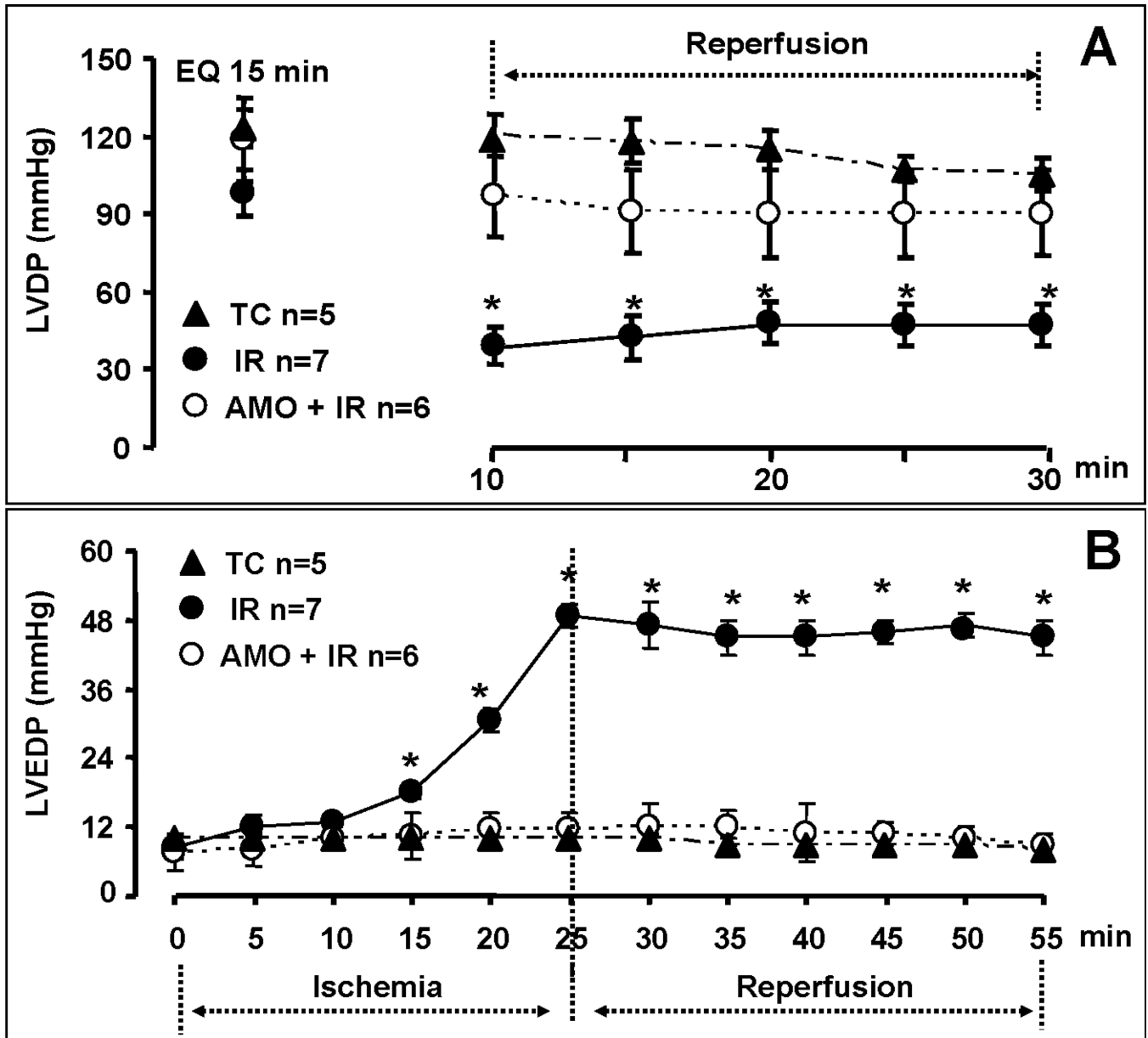
Figure 1. Hemodynamic performance during ischemia-reperfusion (IR) with and without amobarbital (AMO) treatment
AMO treatment immediately before ischemia improved the recovery of myocardial contractile function during reperfusion shown by the improvement in left ventricular developed pressure (LVDP, Panel A). AMO also prevented ischemic contracture during 25 min. of ischemia and decreased diastolic pressure during reperfusion (Panel B). Data are expressed as mean ± SEM; * p<0.05 vs. non-ischemic time control (TC); † p<0.05 vs. untreated ischemia and reperfusion (IR).
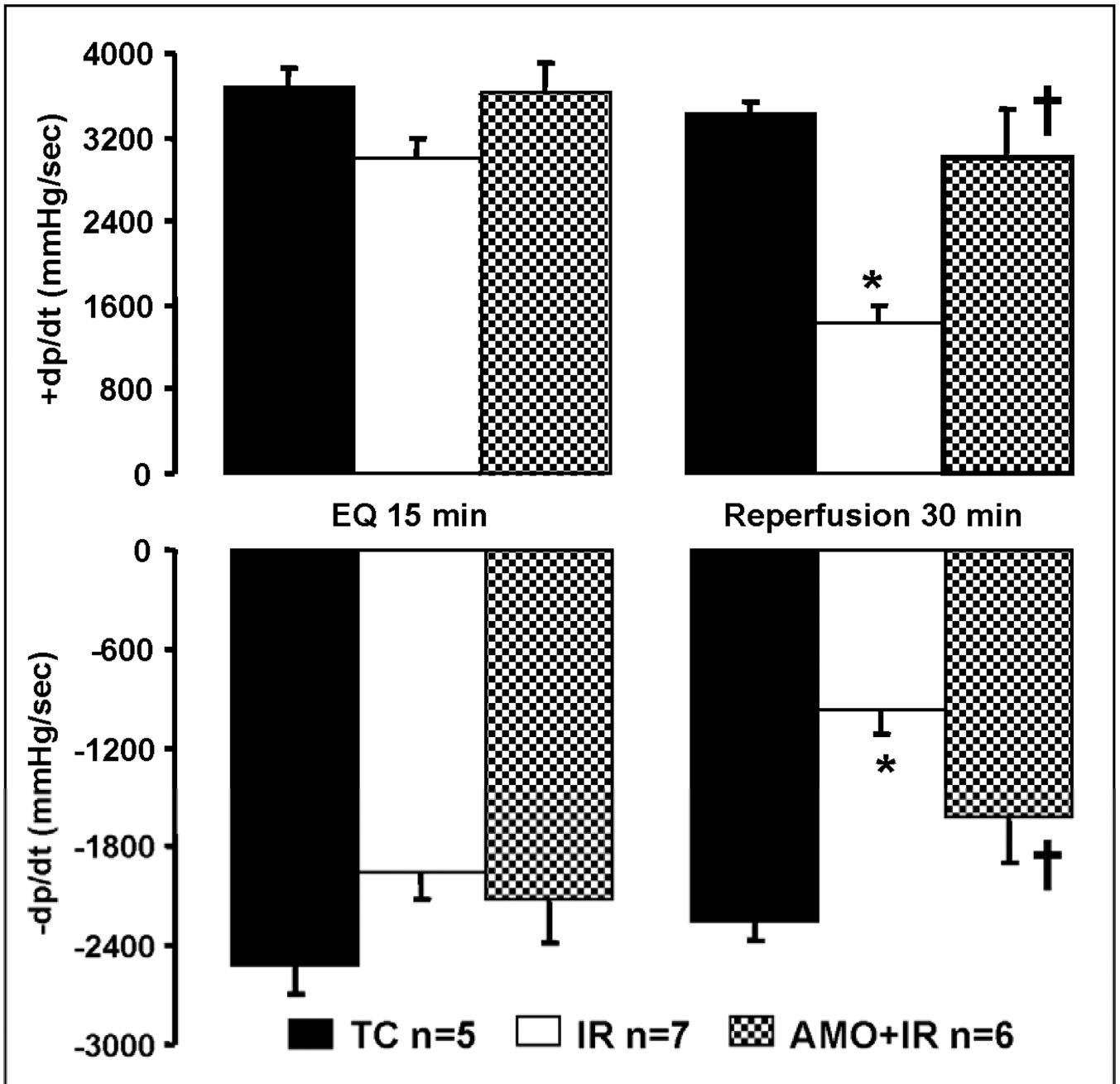
**Figure 2. Left ventricular contractile function during ischemia-reperfusion**
AMO treatment immediately before ischemia improved the recovery of myocardial contractility during reperfusion shown by the improvement in +dp/dt compared to untreated ischemia-reperfusion hearts (Panel A). AMO also significantly improved myocardial relaxation during reperfusion with improved −dp/dt (Panel B). Data are expressed as mean ± SEM; * $p < 0.05$ vs. non-ischemic time control (TC); † $p < 0.05$ vs. untreated ischemia and reperfusion.
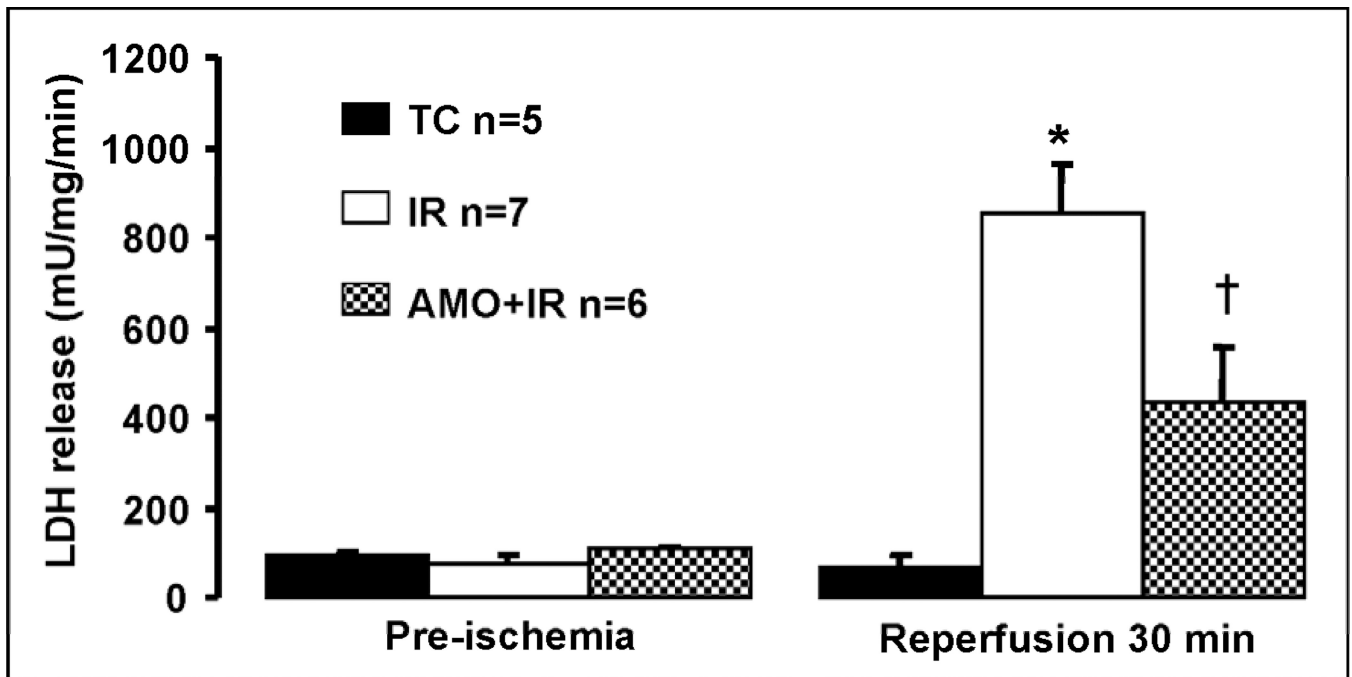
Figure 3. Amobarbital treatment decreases myocardial injury during reperfusion
LDH release into coronary effluent was measured as an index of myocardial injury. Baseline release of LDH during the equilibration period was minimal in all groups as expected (Pre-ischemia value). Ischemia-reperfusion led to marked increase in LDH release during the 30 min reperfusion period as expected. In contrast, amobarbital treatment immediately before ischemia significantly decreased LDH release during reperfusion, indicating less myocardial injury. Data are expressed as mean ± SEM; * $p < 0.05$ vs. non-ischemic time control (TC); † $p < 0.05$ vs. untreated ischemia and reperfusion.
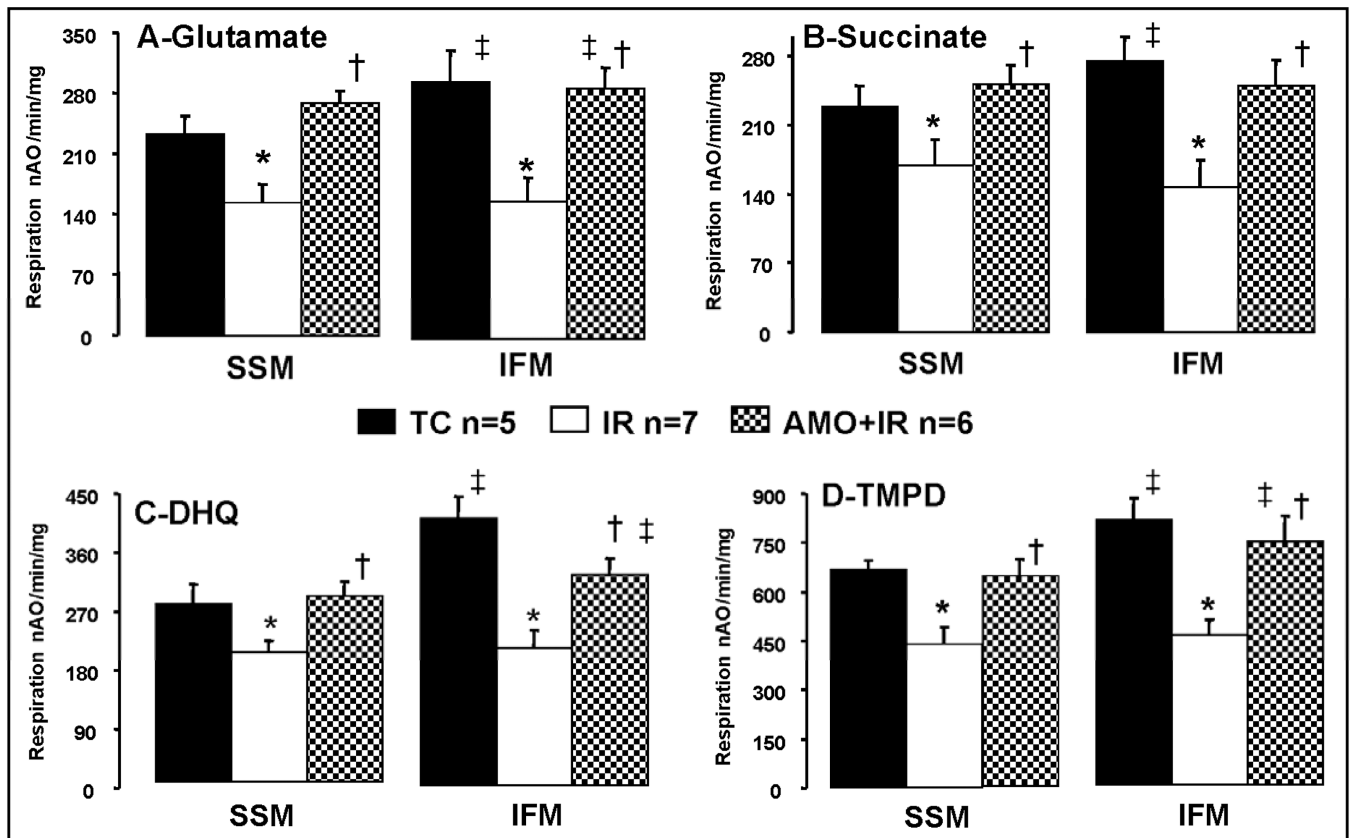
Figure 4. Maximal rates of state 3 (ADP-stimulated) respiration in subsarcolemmal (SSM) and interfibrillar (IFM) cardiac mitochondria following ischemia-reperfusion
Cardiac ischemia-reperfusion decreased the ADP stimulated respiration in both SSM and IFM oxidizing glutamate (Panel A), succinate (Panel B), DHQ (Panel C), and TMPD-ascorbate (Panel D) as complex I, complex II, complex III, and complex IV substrates, respectively. Oxidative phosphorylation was significantly improved in mitochondria isolated from amobarbital treated hearts following ischemia and reperfusion compared to untreated hearts. Data are expressed as mean ± SEM; * $p < 0.05$ vs. time control; † $p < 0.05$ vs. untreated ischemia and reperfusion; ‡ $p < 0.05$ vs. corresponding SSM; nAO: natom oxygen.
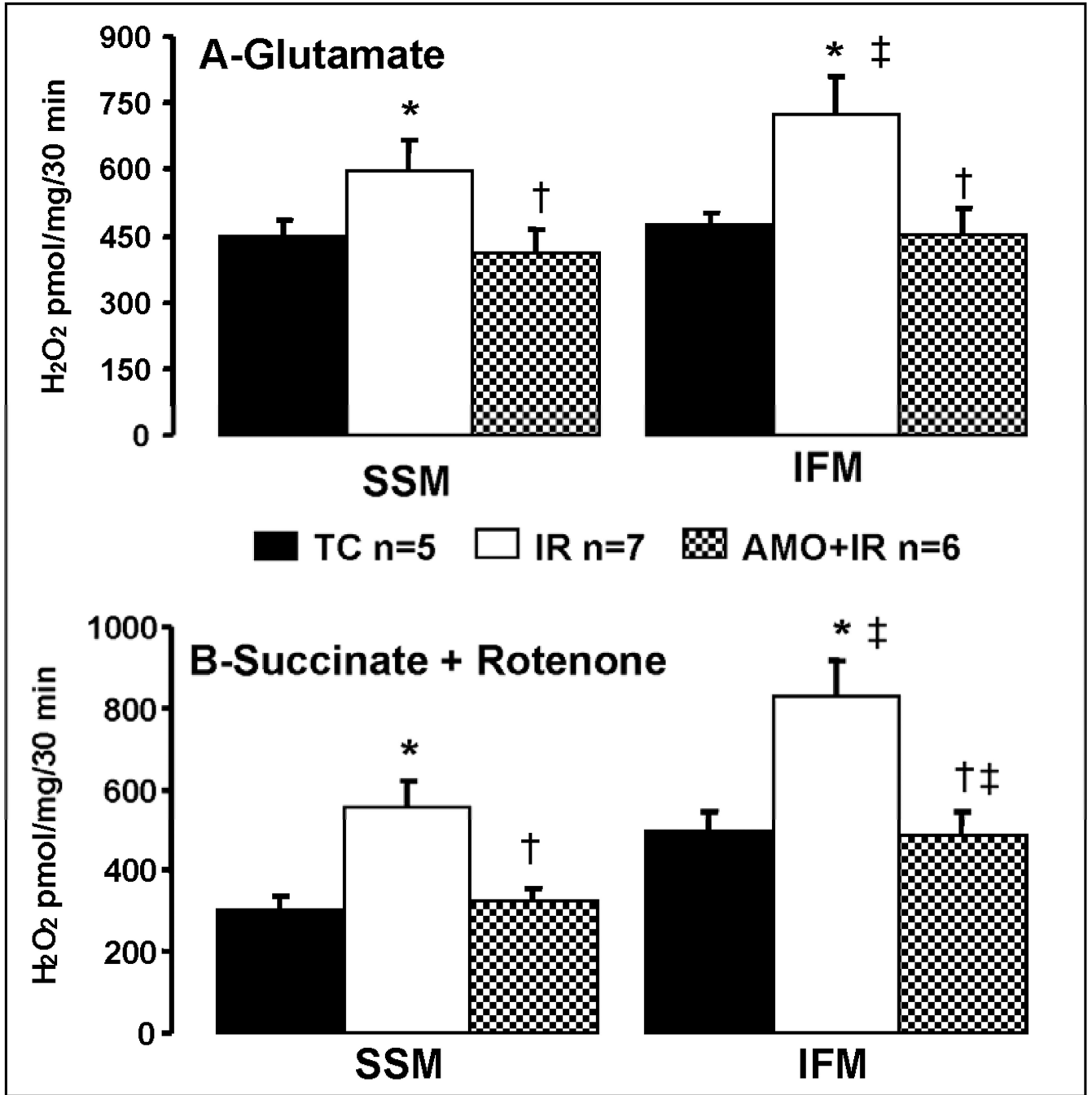
Figure 5.
Net H_2O_2 production from cardiac mitochondria following ischemia-reperfusion. The production of H_2O_2 from subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria following ischemia-reperfusion was markedly increased compared to mitochondria from time control hearts. Glutamate was used as a complex I substrate and succinate (with rotenone) as a complex II substrate. Amobarbital treatment before ischemia significantly decreased H_2O_2 release from SSM and IFM oxidizing both substrates compared to mitochondria from untreated hearts following ischemia-reperfusion. Data are expressed as mean ± SEM; * $p<0.05$ vs. time control; † $p<0.05$ vs. untreated ischemia and reperfusion, ‡ $p<0.05$ vs. corresponding SSM.

Table 1

Mitochondrial protein yield and citrate synthase activity in SSM and IFM following ischemia and reperfusion

	SSM			IFM		
	TC	IR	AMO	TC	IR	AMO
Protein	11.6±0.6	10.4 ± 0.4	10.5 ± 0.5	5.3± 0.5	5.2± 0.4	7.6±0.3
CS	2800±822	3194 ± 172	2731±233	3151±1225	3387±116	2953±292

Mean ± SEM. Ischemia and reperfusion does not change the protein yield (mg/g tissue) and the activity of citrate synthase (CS). TC, time control; IR, ischemia-reperfusion; AMO, amobarbital.

Table 2

Oxidative phosphorylation in SSM and IFM with glutamate as complex I substrate

	TC	SSM IR	AMO	TC	IR IFM	AMO
State 3	229 ± 22	$132 \pm 13^{*}$	$244 \pm 9^{\dagger}$	$288 \pm 21^{\ddagger}$	$149 \pm 17^{*}$	$270 \pm 14^{\dagger\ddagger}$
State 4	27 ± 4	$41 \pm 5^{*}$	$40 \pm 4^{*}$	37 ± 7	46 ± 6	42 ± 3
RCR	9.1 ± 1.3	$3.5 \pm 0.4^{*}$	$6.6 \pm 0.9^{\dagger}$	8.8 ± 1.1	$3.5 \pm 0.5^{*}$	$6.8 \pm 0.8^{\dagger}$
DNP	233 ± 21	$154 \pm 21^{*}$	$269 \pm 14^{\dagger}$	$294 \pm 32^{\ddagger}$	$159 \pm 26^{*}$	$286 \pm 25^{\dagger\ddagger}$

Mean ± SEM;

*
$p<0.05$ vs. TC-(time control, n=5);

†
$p<0.05$ vs. IR (untreated, n=7);

‡
$p<0.05$ vs. corresponding SSM, AMO (amobarbital, n=6); DNP (0.2 mM), dinitrophenol; TC, time control; IR, ischemia-reperfusion; AMO, amobarbital.

Table 3

Cytochrome contents in SSM and IFM following ischemia and reperfusion

	SSM			IFM		
	TC	IR	AMO	TC	IR	AMO
c	0.294±0.016	0.249±.024 *	0.327±0.009	0.353±0.041	0.208±0.030 *	0.355±0.250 †
c_1	0.169±0.012	0.177±0.140	0.217±0.007	0.115±0.044	0.068±0.035	0.141±0.039
b	0.235±0.014	0.275±0.010	0.219±0.016	0.301±0.103	0.382±0.058	0.279±0.017
aa_3	0.645±0.054	0.696±0.039	0.549±0.058	0.893±0.286	1.070±0.201	0.790±0.097

Mean ± SEM;

*
$p < 0.05$ vs. time control (n=5);

†
$p < 0.05$ vs. untreated (IR, n=7); amobarbital (AMO, n=6); TC, time control; IR, ischemia-reperfusion; AMO, amobarbital.