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The Red Wine Antioxidant Resveratrol Prevents Cardiomyocyte Injury Following Ischemia-Reperfusion Via Multiple Sites and Mechanisms

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ABSTRACT

The objective was a comprehensive investigation of the mechanisms and sites of resveratrol cardioprotection during and following ischemia–reperfusion (I–R) injury, and to determine whether direct preservation of cardiomyocytes is an important site of cardioprotection. We now provide the first definitive evidence that resveratrol specifically protects cardiomyocytes from I–R injury via a combination of suppression of superoxide levels and activation of potassium channels. This protection is apparent whether resveratrol is present for the full duration of the insult or only on recovery. In addition, resveratrol improved postischemic recovery of left ventricular contractile function, attenuated myocardial injury, and increased myocardial activation of the survival kinase Akt in the intact heart. Furthermore, resveratrol elicited direct concentration-dependent protective actions on the vasculature (vasorelaxation, superoxide suppression) and enhanced endothelium-dependent vasodilatation. Resveratrol thus targets a number of consequences of myocardial I–R, including release of reactive oxygen species, loss of recovery of contractile function, and impaired endothelium-dependent vasodilatation. Previous evidence indicates that resveratrol elicits potent preconditioning in the heart. Given that myocardial ischemic events are often unpredictable in humans, the findings that resveratrol protection is also evident when administered during and/or after the insult adds new dimensions to the clinical potential of resveratrol. *Antioxid. Redox Signal.* 9, 101–113.

INTRODUCTION

ISCHEMIC MYOCARDIAL INJURY is a major cause of morbidity and mortality in developed nations. Release of reactive oxygen species (ROS), loss of recovery of contractile function, and impaired endothelium-dependent vasodilatation are all adverse outcomes of myocardial damage evident following prolonged interruption in coronary blood flow (28, 35). Development of novel treatment strategies that protect against multiple mechanisms of ischemia–reperfusion (I–R) injury will have major clinical impact. Resveratrol (3,4,5′-trihydroxystilbene) is an antioxidant abundant in red wine that is a likely contributing factor to the cardiovascular benefits of moderate red wine consumption (11, 27, 29). In recent years, several laboratories have described the powerful preconditioning effect of resveratrol. Transient pretreatment of the heart with resveratrol, followed by a washout period prior to I–R, is cardioprotective (3, 8, 9, 19–21). Adenosine, the cell survival kinase Akt and p38 mitogen-activated protein kinase, known mediators of cardioprotection, are implicated in this effect (8, 9). This early time point of cardioprotection strongly favors use of resveratrol as a preventative approach, for example, as a dietary additive. In contrast, the protective actions of resveratrol treatment during and/or following an is-

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chemic insult (also of clinical relevance given that the timing of myocardial ischemia and infarction is unpredictable) remain to be fully characterized. Specifically, whether resveratrol directly protects cardiomyocytes from I–R injury, and the mechanisms of this cardioprotection, have not previously been determined.

The objective of the present study was to comprehensively investigate the mechanisms and sites of resveratrol cardioprotection during I–R injury. Although the potent vasodilatory actions of resveratrol (3, 12–14) likely limit the no-reflow phenomenon, direct preservation of cardiomyocytes is an important site of cardioprotection. We now test the hypotheses that (a) resveratrol prevents cardiomyocyte I–R injury independently of any vascular activity; (b) protection is evident even when administered during recovery from the ischemic insult; and (c) preservation of endothelial function accompanies myocardial protection. We now demonstrate resveratrol causes cardiomyocyte protection via antioxidant actions as well as by activation of mitochondrial ATP-sensitive (K_{ATP}) and calcium-activated (BK_{Ca}) potassium channels. Many of these protective actions were evident even when resveratrol was administered only during recovery from the insult, a clinically important time point. Resveratrol also improved recovery of myocardial function, enhanced Akt activation, and augmented endothelium-dependent vasodilatation. Thus, resveratrol targets a number of outcomes of myocardial I–R injury (28, 35), including release of ROS, loss of recovery of contractile function, and impaired endothelium-dependent vasodilatation.

MATERIALS AND METHODS

This investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the United States National Institutes of Health (NIH Publications No. 85-23, revised 1996) and the National Health and Medical Research Council of Australia guidelines, and was approved by the Animal Ethics Committee of the Baker Heart Research Institute (isolated cardiomyocytes, whole heart studies) and the University of Melbourne's Animal Experimentation Ethics Committee (vascular studies).

Ischemia–reperfusion in adult rat cardiomyocytes

Adult male Sprague–Dawley rats (200–300 g) were anesthetized (intraperitoneal ketamine hydrochloride 100 mg/kg + xylazine 12 mg/kg, Troy Laboratories, NSW, Australia) for isolation of primary cardiomyocytes, as previously described (15, 31). Cardiomyocytes plated at a density of 10^4 cells/cm^2 were incubated in modified serum-free medium 199 (JRH Biosciences Pty Ltd, Brooklyn, Australia) prior to hypoxia (95% N_2–5% CO_2, using a hypoxic chamber from QNA International, Australia) and subsequent reoxygenation, all at 37°C. The optimal time period of hypoxia (over 30–120 min, all followed by 2.5 h reoxygenation), and the optimal concentrations of both resveratrol (over 1–100 μ*M*, Sigma-Aldrich, St. Louis, MO) and the antioxidant tempol (over 0.01–1 m*M*, Sigma-Aldrich) in hypoxic cardiomyocytes were determined initially in pilot studies; 90 min hypoxia and 10 μ*M* of either agent were demonstrated most effective and were used in all subsequent cardiomyocyte studies (Table 1). Resveratrol or the antioxidant tempol were added at two different time points, either 5 min prior to (and for the full duration of) hypoxia-reoxygenation, or present only during reoxygenation. The role of BK_{Ca} and mitochondrial K_{ATP} channels in resveratrol actions was investigated using tetraethylammonium bromide (TEA, 1 m*M*, Sigma-Aldrich) and 5-hydroxydecanoate (5-HD, 500 μ*M*, Sigma-Aldrich), respectively, at concentrations previously shown to prevent cardioprotective actions without any direct effects themselves on cardiomyocyte LDH release (15, 31). Inhibitors were added concomitantly with resveratrol. LDH activity in the culture medium and loss of characteristic rod-shaped striated cardiomyocyte morphology (light microscopy) was determined as markers of cardiomyocyte injury. Cardiomyocyte levels of the ROS superoxide were also determined in real time over the full 2.5 h of reoxygenation, using lucigenin (5 μ*M*)-enhanced chemiluminescence (6), following replacement of culture medium with Krebs buffer. Resveratrol or the antioxidant tempol were added at two different time points, either 5 min prior to, and for the full duration of, hypoxia (but not for reoxygenation), or added only during reoxygenation, to specifically dissect out resveratrol suppression of triggers of ROS production activated during hypoxia, independent of any effect it has on

TABLE 1. PILOT STUDIES DETERMINED A: THE OPTIMAL TIME PERIOD OF HYPOXIA (PRIOR TO 2.5 H REOXYGENATION) AND B: THE OPTIMAL CONCENTRATIONS OF RESVERATROL AND TEMPOL

A: Optimal Time Period of Hypoxia

	Cardiomyocyte injury (LDH, U/L)			
Period of hypoxia (min, n = 6)	30 min 11 ± 6 U/L	60 min 15 ± 9 U/L	90 min 55 ± 19 U/L*	120 min 20 ± 7 U/L

B: Optimal Drug Treatment Concentrations

	Cardiomyocyte injury (LDH, U/L)		
Resveratrol, μ*M* (n = 6)	1 μ*M* 35 ± 6 U/L	10 μ*M* 20 ± 4 U/L*	100 μ*M* 39 ± 8 U/L
Tempol, m*M* (n = 6)	0.01 m*M* 12 ± 3 U/L*	0.10 m*M* 15 ± 6 U/L	1.00 m*M* 20 ± 8 U/L

Asterisk (*) indicates optimum point chosen.

directly scavenging ROS produced on reoxygenation. The area-under-the-curve was then calculated for superoxide accumulation as a function of time post onset of reoxygenation. Cardiomyocytes isolated from each heart were exposed to different treatments to allow direct paired comparisons between treatment groups. An additional vehicle control (0.04% dimethylsulfoxide, DMSO) was also included in all cardiomyocyte studies using resveratrol.

Ischemia–reperfusion in isolated Langendorff-perfused rat hearts

Hearts isolated from adult male Sprague–Dawley rats (380–440 g) were perfused at constant flow and heart rate (10 ml/min, 300 beats/min) as previously described (40). Under these constant flow conditions, perfusion pressure as measured at the aorta, was approximately 75 mm Hg. Following equilibration, hearts were subjected to 45 min global, no-flow ischemia with 20 min reperfusion. Isovolumic LV function (intraventricular fluid-filled balloon catheter) and myocardial injury (lactate dehydrogenase activity, LDH) were continuously assessed. Resveratrol (10 μ*M*) was present in the perfusion buffer from 10 min prior to ischemia until the end of reperfusion. Results were compared to drug-free hearts subjected to I–R, and to sham (time control) normoxic hearts. DMSO (0.04%) was included in the perfusion buffer for all hearts to control for vehicle. At the end of each experiment, a small cube of left ventricular (LV) free wall was rapidly dissected and fixed for electron microscopy. LV activation of Akt and p38 mitogen-activated protein kinase (p38MAPK), as well as inactivation of glycogen synthase kinase-3β (GSK-3β), following I–R was also determined, using phospho-specific antibodies (Cell Signaling Technology, Beverly, MA). Results for kinase (in)activation were expressed as the ratio of phosphorylated to total kinase, normalized to normoxic sham controls, for each sample from densitometric quantitation. Protein expression of the constitutive (eNOS) and inducible (iNOS) isoforms of nitric oxide synthase using selective antibodies (BD Biosciences, Bedford, MA), was determined as previously described (32).

Vascular function and superoxide generation

Endothelium-dependent and -independent function of male Sprague–Dawley rat (200–450 g) isolated, endothelium-intact carotid arterial rings was determined using cumulative concentration–response curves to acetylcholine (BDH Chemicals, Kilsyth, Australia) and sodium nitroprusside (Sigma-Aldrich) respectively, in 5 ml myograph chambers as previously described (6), in the absence or presence of resveratrol (10 μ*M*, added 20 min prior to phenylephrine preconstriction, Sigma-Aldrich). The impact of the auto-oxidant pyrogallol (100 μ*M*, Sigma-Aldrich, added 20 min prior to preconstriction) ± resveratrol on endothelial function was then determined. The direct vasorelaxant actions of resveratrol (0.01–100 μ*M*) were also determined in preconstricted, endothelium-intact rat carotid arteries. Relaxation responses were expressed as a percent of the preconstricted level of tone. Vascular superoxide levels in response to vehicle or resveratrol (0.1–100 μ*M*) was determined in rat aortic rings as previously described (6), using lucigenin (5 μ*M*, Sigma-Aldrich)-enhanced chemiluminescence normalized to dry tissue weight. DMSO (0.04%) was included in the incubation buffer for all vessels to control for vehicle.

Statistical analysis

Results were expressed as mean ± SE, with *n* representing the number of animals per treatment group in the heart and vascular studies, or the number of cardiomyocyte preparations studied, respectively. Statistical evaluation used two-way repeated measures analysis of variance (ANOVA) for changes in LDH and LV function in isolated perfused hearts over time. Student's unpaired *t* tests, paired *t*-tests, one-way ANOVA, or one-way repeated measures ANOVA were used as appropriate. The Student–Newman–Keuls (SNK) method for multiple comparisons was applied where appropriate. $p < 0.05$ was accepted as significant.

RESULTS

Resveratrol prevents hypoxia-induced cardiomyocyte injury: role of potassium channels

Hypoxia-reoxygenation significantly increased LDH activity, from 27 ± 2 to 64 ± 7 U/L, and increased loss of rod-shaped striated cardiomyocytes by 22 ± 3% (all $n = 21$, $p < 0.001$, paired *t*-test, compared to paired control normoxic cardiomyocytes). This period of hypoxia (90 min) induced the greatest cardiomyocyte injury in pilot studies comparing 30–120 min hypoxia, and was used for all subsequent experiments (see Table 1).

Cardiomyocyte LDH release was largely prevented when resveratrol was present from 5 min prior to and for the duration of hypoxia–reoxygenation (10 μ*M*, Fig. 1A, $n = 21$ cardiomyocyte preparations, $p < 0.001$, one-way ANOVA). Hypoxia-induced loss of rod-shaped striated cardiomyocytes was similarly prevented by 10 μ*M* resveratrol (restored to 106 ± 3% of normoxic cardiomyocytes, $n = 21$). Lower concentrations of resveratrol also tended to decrease LDH activity (by 38 ± 11% $n = 6$, $p < 0.05$ paired *t*-test for 1 μ*M* resveratrol); higher concentrations of resveratrol (100 μ*M*) did not elicit further reduction of LDH activity (see Table 1). Although the vehicle control (0.04% DMSO) modestly reduced cellular injury, this was significantly less protective than resveratrol (Fig. 1A). Tempol elicited cardiomyocyte protection similar to resveratrol. When added 5 min prior to hypoxia–reoxygenation, tempol (10 μ*M*) significantly reduced LDH activity compared to that observed in untreated hypoxic myocytes ($n = 6$ cardiomyocyte preparations, $p < 0.05$, Fig. 1B), and prevented hypoxia-induced loss of rod-shaped striated cardiomyocytes (restored to 97 ± 4% of normoxic cardiomyocytes, $n = 6$). The tempol effect was comparable at all concentrations studied (10–1000 μ*M*, Table 1).

The potential mechanism(s) of resveratrol cardiomyocyte protection were further investigated, using 5-hydroxydecanoate (500 μ*M*, $n = 9$ cardiomyocyte preparations, $p < 0.05$, Fig. 1C) and tetraethylammonium bromide (1 m*M*, $n = 9$

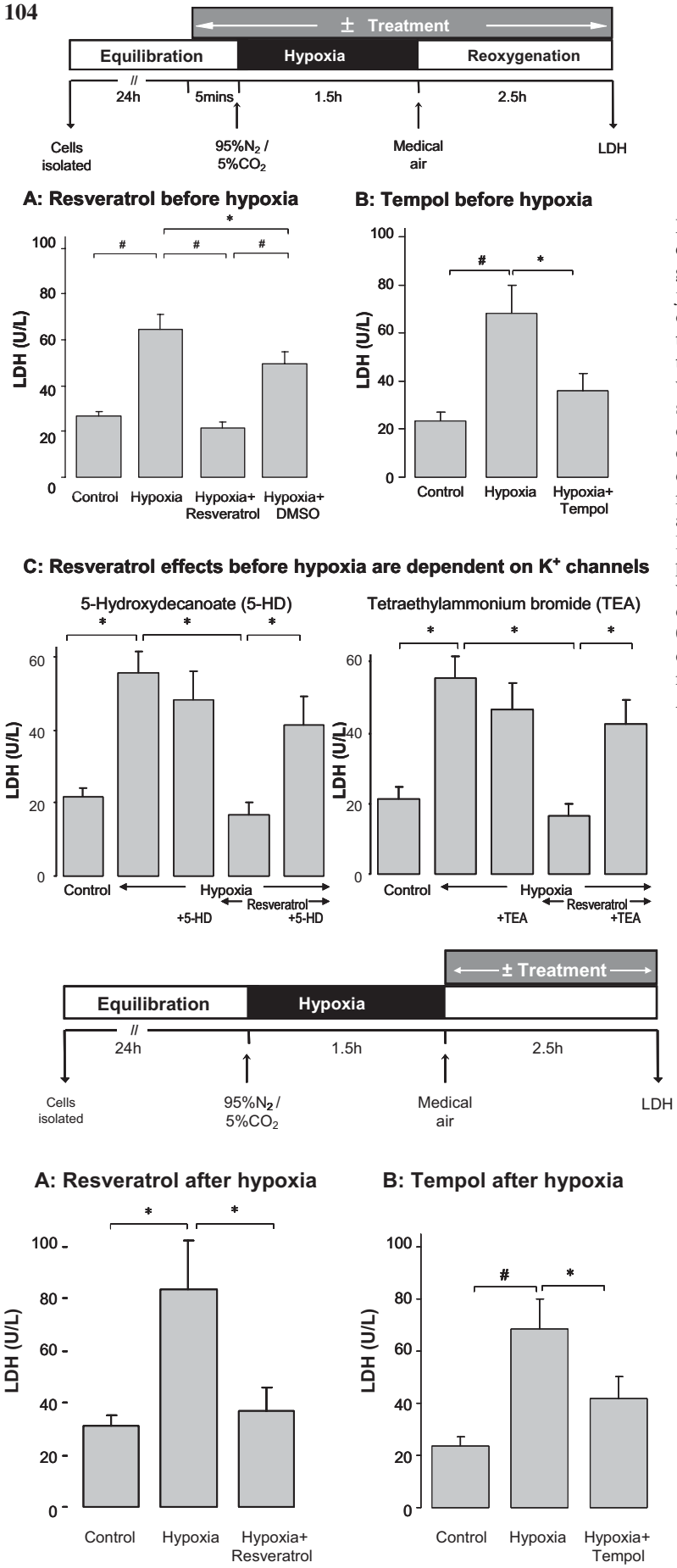


FIG. 1. Effect of resveratrol on adult rat cardiomyocytes subjected to hypoxia-reoxygenation injury. Cardiomyocytes were subjected to 90-min hypoxia followed by 2.5 h reoxygenation. Treatments (resveratrol 10 μ*M*, or tempol 10 μ*M*) were present for the full duration of hypoxia and reoxygenation. Cell injury was assessed using LDH. The role of potassium channels in cardiomyocyte protection was determined using selective inhibitors of mitochondrial K_{ATP} and BK_{Ca} channels, 5-hydroxydecanoate (5-HD, 500 μ*M*), and tetraethylammonium bromide (TEA, 1 m*M*) respectively, added concomitantly with resveratrol. (**A**) Resveratrol present for the full duration of hypoxia-reoxygenation ($n = 21$, $p < 0.001$, one-way ANOVA). (**B**) Tempol present for the full duration of hypoxia-reoxygenation ($n = 6$, $p < 0.001$ on one-way ANOVA). (**C**) Potassium channel inhibitors added concomitantly with resveratrol (both $n = 9$, $p < 0.001$ on one-way ANOVA). $^{*}p < 0.05$ and $^{\#}p < 0.001$, on SNK.

FIG. 2. Effect of resveratrol on adult rat cardiomyocytes subjected to hypoxia-reoxygenation injury. Cardiomyocytes were subjected to 90-min hypoxia followed by 2.5 h reoxygenation. Treatments (resveratrol 10 μ*M*, or tempol 10 μ*M*) were only present during post-hypoxic reoxygenation. Cell injury was assessed using LDH. (**A**) Resveratrol only present during post-hypoxic reoxygenation ($n = 6$, $p < 0.05$, one-way ANOVA). (**B**) Tempol only present during post-hypoxic reoxygenation ($n = 6$, $p < 0.001$ on one-way ANOVA). $^{*}p < 0.05$ and $^{\#}p < 0.001$, on SNK.

cardiomyocyte preparations, $p < 0.05$, Fig. 1C); both inhibitors abolished the protective effects of resveratrol against hypoxia-induced cardiomyocyte injury. Tempol, resveratrol and DMSO vehicle had no effect alone on LDH released from normoxic cardiomyocytes, or on cardiomyocyte morphology (data not shown). In addition, neither K^+ channel inhibitor alone affected LDH release.

Resveratrol treatment following hypoxia prevents cardiomyocyte injury

Resveratrol (10 μ*M*) also protected isolated cardiomyocytes against hypoxia-induced cardiomyocyte injury when only present during reoxygenation (Fig. 2A, $n = 6$ cardiomyocyte preparations, $p < 0.05$). Loss of rod-shaped striated cardiomyocytes was similarly prevented (restored to 97 ± 10% of that observed in normoxic cardiomyocytes, $n = 6$). Tempol (10 μ*M*) added at the onset of reoxygenation also protected cardiomyocytes from both increased LDH ($n = 6$ cardiomyocyte preparations, $p < 0.05$, Fig. 2B) and loss of rod-shaped striated cardiomyocytes (restored to 85 ± 6% of that in normoxic cardiomyocytes, $n = 6$).

Resveratrol reduces hypoxia-induced cardiomyocyte superoxide levels

As shown in Fig. 3, hypoxia–reoxygenation increased cardiomyocyte superoxide levels; this effect was maximal by ~20 min reoxygenation. At this time point, superoxide levels were increased from 44 ± 4 in normoxic cardiomyocytes to 65 ± 5 RLU/s in paired post-hypoxic cardiomyocytes ($n = 6$ cardiomyocyte preparations); resveratrol (10 μ*M*) reduced this to 54 ± 5 and to 42 ± 5 RLU/s when present only during hypoxia, or only during reoxygenation, respectively (Fig. 3A). Tempol (10 μ*M*) similarly attenuated the peak of superoxide accumulation, to 53 ± 4 and to 48 ± 3 RLU/s when present either only during hypoxia, or only during reoxygenation, respectively (Fig. 3A).

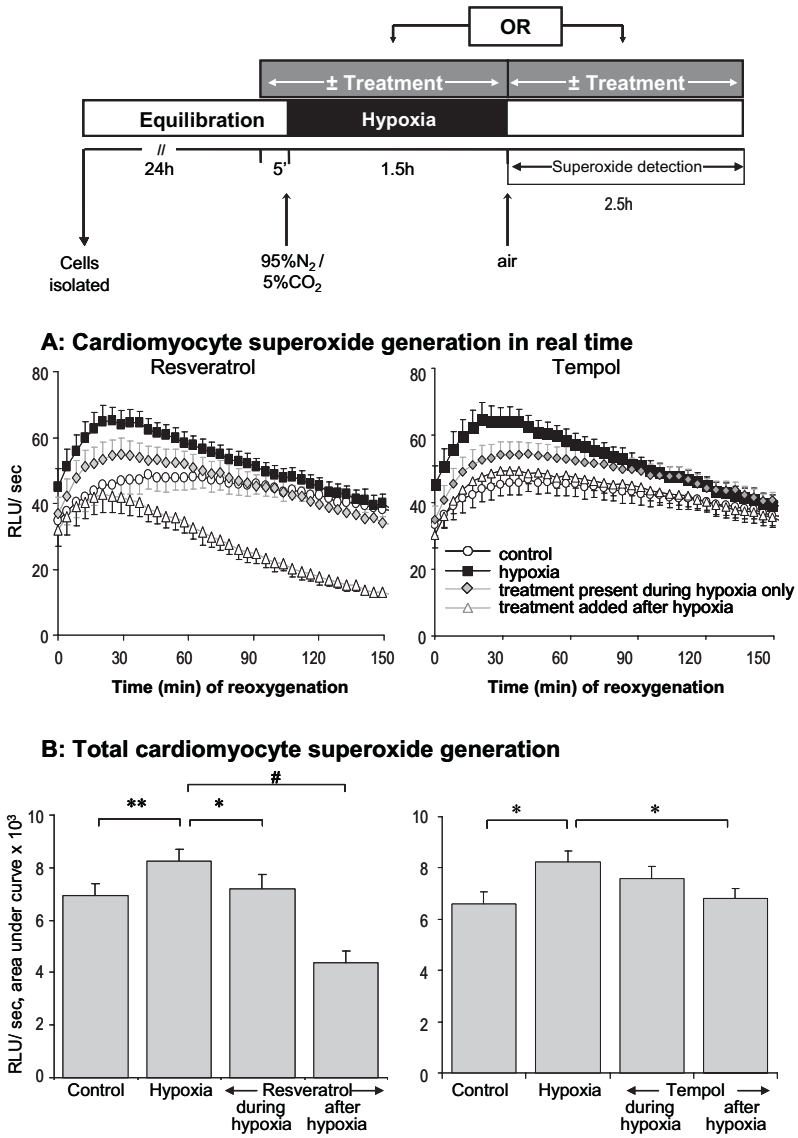
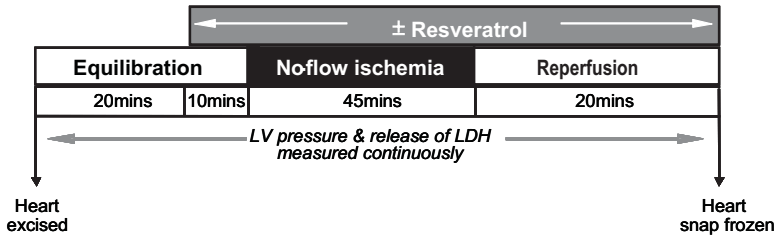


FIG. 3. Effect of resveratrol on adult rat cardiomyocyte superoxide levels during 2.5 h post-hypoxic reoxygenation. Cardiomyocytes were subjected to 90-min hypoxia followed by 2.5 h reoxygenation. Treatments (resveratrol 10 μ*M*, or tempol 10 μ*M*) were present either during hypoxia or only during reoxygenation. Superoxide levels were determined using lucigenin-enhanced chemiluminescence. (**A**) Cardiomyocyte superoxide levels in real time during 2.5 h post-hypoxic reoxygenation in the presence and absence of resveratrol or tempol (all $n = 6$). (**B**) Area-under-curve analysis of superoxide generation over 2.5 h (all $n = 6$). Untreated normoxic and hypoxic cardiomyocytes are shown in open circle and closed square symbols, respectively. Cardiomyocyte treatments present only during hypoxia or only during rexoygenation represented by grey diamond and triangle symbols, respectively. $^{\#}p < 0.001$, $^{**}p < 0.005$, and $^{*}p < 0.05$, on SNK.

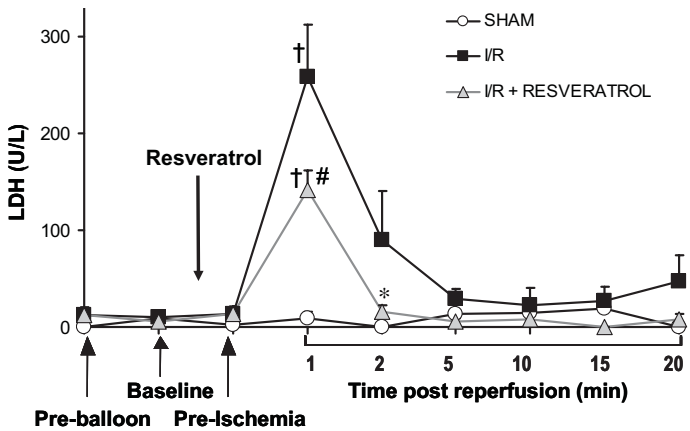
The area-under-the-curve of cardiomyocyte superoxide for the full 2.5 h reoxygenation period was calculated to indicate the total superoxide generated. Hypoxia significantly increased total cardiomyocyte superoxide accumulation (by 21 ± 7% on area-under-curve analysis, $n = 6$ cardiomyocyte preparations, $p < 0.005$). This was significantly reduced by resveratrol (by 13 ± 4%, $n = 6$, $p < 0.05$) during the hypoxic period, as well as only during reoxygenation (by 47 ± 5%, $n = 6$, $p < 0.001$, Fig. 3B). DMSO vehicle did not affect total superoxide accumulation, whether added during hypoxia or during reoxygenation (96 ± 1% and 96 ± 3% of drug-free hypoxic cardiomyocytes, respectively, both $n = 6$). Tempol also tended to attenuate total cardiomyocyte superoxide accumulation, whether present only during the hypoxic period (by 8 ± 4%, $n = 6$, cardiomyocyte preparations), or when present only during reoxygenation (by 17 ± 4%, $n = 6$, $p < 0.05$, Fig. 3B). Resveratrol and tempol did not affect basal superoxide levels (93 ± 6% and 97 ± 2%, versus paired control respectively, both $n = 6$) in normoxic cardiomyocytes when present during the 90 min prior to lucigenin exposure; however, the addition of resveratrol during the 2.5 h period of superoxide detection following 1.5 h normoxia (the analogous time point to reoxygenation) significantly reduced basal superoxide (by 44 ± 4% of drug-free normoxic cardiomyocytes, $n = 6$ cardiomyocyte preparations, $p < 0.001$), suggesting resveratrol has direct superoxide scavenging actions. Having fully characterized resveratol cardiomyocyte protection, our next aims were to elucidate whether resveratrol preservation of both myocardial and endothelial function accompanied cardiomyocyte protection.

Resveratrol prevents ischemia–reperfusion injury in rat isolated hearts

LDH release at the onset of reperfusion was significantly increased above baseline, from 10 ± 6 to 258 ± 54 U/L ($n = 9$ hearts/group, $p < 0.001$, Fig, 4A). By 10 min reperfusion, LDH had returned close to baseline (22 ± 17 U/L), and showed no further decline. The switch from drug-free to resveratrol (10 μ*M*)-containing perfusion buffer after 20 min equilibration did not influence release of LDH into the coronary perfusate (LDH was 6 ± 3 and 13 ± 4 U/L respectively, $n = 8$ hearts/group, p = NS). Resveratrol significantly reduced reperfusion-induced LDH release, however, to 142 ± 20 U/L



A: LV injury (LDH)



B: LV injury (Electron microscopy)

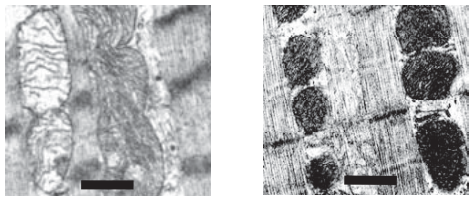


FIG. 4. Effect of resveratrol on adult rat hearts subjected to ischemia-reperfusion (I-R) injury. Hearts were subjected to 45-min ischemia followed by 20-min reperfusion. Resveratrol (10 μ*M*) was present from 10 min prior to, and for the full duration of I-R. LV injury was assessed using LDH release into coronary perfusate and on electron microscopy. (**A**) LV injury on LDH release. Time control hearts, untreated and resveratrol-treated hearts subjected to I-R shown in open circle, closed square, and gray triangle symbols, respectively. $^{*}p < 0.05$ and $^{\#}p < 0.001$ versus I-R at the same time-point, and $^{\dagger}p < 0.001$ versus baseline (all on SNK). (**B**) LV injury on electron microscopy (bar = 0.5 μm).

($p < 0.001$ versus drug-free reperfusion at the same time point, Fig. 4A), and LDH values were returned to baseline within 2 min of reperfusion (16 ± 3 U/L). In time control (sham) hearts, LDH release at baseline was 2 ± 2 U/L ($n = 3$ hearts per group), and did not change over the 95 min of normoxic perfusion at 10 ml/min. Myocardial injury assessed by electron microscopy showed that I–R induced marked mitochondrial swelling and disruption, and early onset of myofilament contracture in LV fibers and adjacent mitochondria (Fig. 4B). In contrast, only modest postischemic structural perturbations were observed in resveratrol-treated myocardium.

Resveratrol also significantly improved postischemic recovery of LV function. In untreated hearts ($n = 9$), each of LV systolic pressure (LVSP, from 115 ± 6 to 68 ± 4 mm Hg, $p < 0.001$, Fig. 5C), LV + dP/dt_{max} (from 3100 ± 190 to 1980 ± 170 mm Hg/s, $p < 0.001$, Fig. 5E) and LV − dP/dt_{min} (from 1830 ± 120 to 1350 ± 90 mm Hg/s, $p < 0.05$, Fig. 5F) and LVDP (from 111 ± 6 to 50 ± 5 mm Hg, $p < 0.001$, Fig. 5G), were significantly decreased below baseline during early reperfusion. Concomitantly LV end-diastolic pressure was increased (LVEDP, from 4.3 ± 0.3 to 18.3 ± 2.3 mm Hg, $p < 0.001$, Fig. 5D). All parameters of LV function were still impaired after 20 min reperfusion. There was no sustained effect on LV function at the commencement of resveratrol perfusion (10 μ*M*, $n = 8$ hearts); LVSP after 10 min normoxic perfusion with resveratrol was 92 ± 2% of the preresveratrol value (p = NS). Resveratrol significantly improved recovery of LV function on reperfusion, on LVSP, LVDP, and LVEDP, evident after 5 min reperfusion. These effects persisted for the duration of reperfusion. Furthermore, LV + dP/dt_{max} and LV − dP/dt_{min} were significantly improved by resveratrol after 10 and 20 min reperfusion compared to untreated hearts at the same time point (Fig. 5). The time to onset of contracture, defined as the ischemic time required to evoke a 5 mm Hg rise in LVEDP, was 23.7 ± 1.5 min in the absence of resveratrol. The addition of resveratrol to the perfusion buffer resulted in later contracture at 31.3 ± 1.6 min of ischemia ($p < 0.005$, Fig. 5H).

Resveratrol cardioprotection was accompanied by myocardial activation of cardioprotective signaling (Fig. 6A). Akt activity increased from 0.94 ± 0.24 to 2.04 ± 0.41 (ratio of phosphorylated to total Akt, relative to sham normoxic

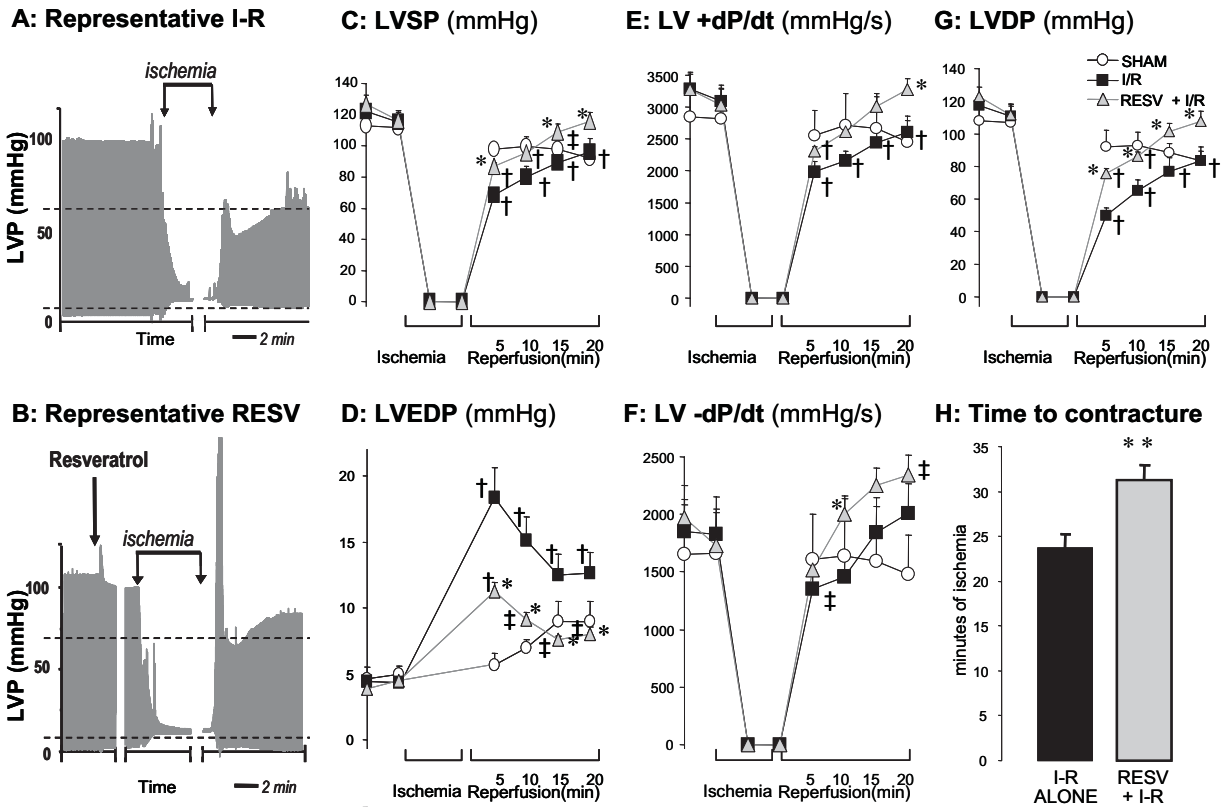


FIG. 5. Effect of resveratrol on post-ischemic LV function in hearts subjected to I-R injury. Hearts were subjected to 45-min ischemia followed by 20-min reperfusion. Resveratrol (10 μ*M*) was present from 10 min prior to, and for the full duration of I-R. (**A**) Representative trace of LV function from an untreated heart subjected to I-R. Bar = 2 min. (**B**) Representative trace of LV function from a resveratrol-treated heart subjected to I-R. Bar = 2 min; dashed lines indicate minimum and maximum LVP in the absence of resveratrol treatment. (**C**) Recovery of LVSP (mm Hg, $p < 0.05$ on two-way RM ANOVA). (**D**) LVEDP (mm Hg, $p < 0.001$ on two-way RM ANOVA). (**E**) LV +dP/dt_{max} (mm Hg/s). **(F)** LV -dP/dt_{min} (mm Hg/s). (**G**) LVDP (mm Hg, $p < 0.001$ on two-way RM ANOVA). (**H**) Time to ischemic contracture in untreated (n = 9) and resveratrol-treated ($n = 8$) hearts subjected to I-R. Pooled data from untreated I-R hearts are represented by closed squares, resveratrol-treated I-R hearts by gray triangles, and sham controls as open circles, except for H, where untreated and resveratrol-treated hearts are shown in black and gray columns, respectively. $^{*}p < 0.05$ and $^{**}p < 0.005$ versus I-R at the same timepoint; $^{‡}p < 0.05$ and $^{†}p < 0.001$ versus baseline (SNK).

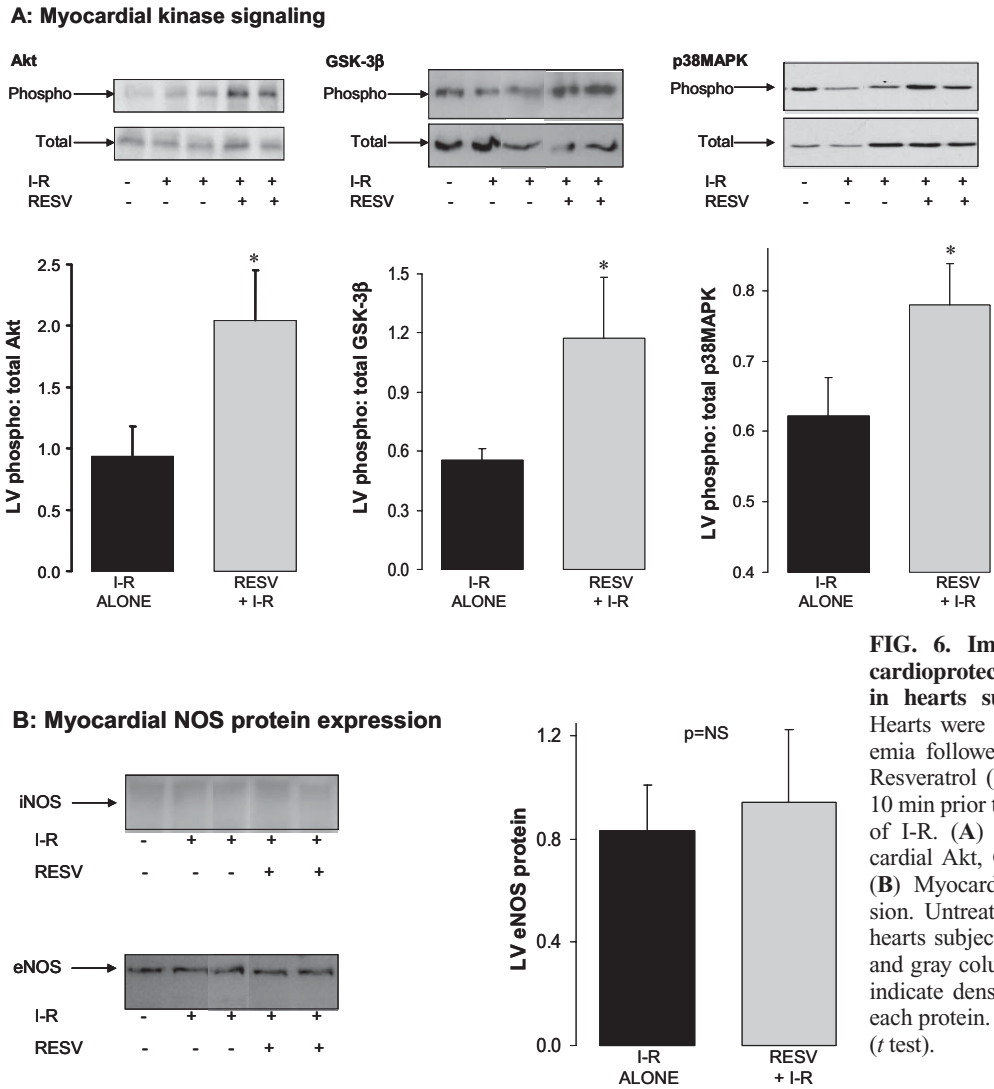


FIG. 6. Impact of resveratrol on cardioprotective signal transduction in hearts subjected to I-R injury. Hearts were subjected to 45-min ischemia followed by 20-min reperfusion. Resveratrol (10 μ*M*) was present from 10 min prior to, and for the full duration of I-R. (**A**) Phosphorylation of myocardial Akt, GSK-3β, and p38MAPK. (**B**) Myocardial NOS protein expression. Untreated and resveratrol-treated hearts subjected to I-R shown in black and gray columns, respectively. Arrows indicate density of expected bands for each protein. $^{*}p < 0.05$ versus I-R alone (*t* test).

hearts, $p < 0.05$). This was associated with increased phosphorylation of the downstream kinase GSK-3β, from 0.55 ± 0.06 to 1.17 ± 0.31 (ratio of phosphorylated to total GSK-3β, relative to sham normoxic hearts, $p < 0.05$), indicative of inactivation of this enzyme. Furthermore, activity of p38MAPK was also modestly increased, from 0.62 ± 0.05 to 0.78 ± 0.06 (ratio of phosphorylated to total p38MAPK, relative to sham normoxic hearts, $p < 0.05$). Conversely, resveratrol failed to alter expression of either isoform in hearts subjected to acute I–R, and iNOS was not detected in any samples obtained (Fig. 6B). Sham hearts were not included in the statistical analysis.

Resveratrol protects vascular function in isolated rat carotid arteries

Resveratrol elicited direct, concentration-dependent vascular responses, including relaxation of phenylephrine-preconstricted carotid artery rings ($n = 7$ animals/group, Fig. 7A) and suppression of aortic superoxide levels ($n = 5$ animals/group, $p < 0.05$ on one-way ANOVA, Fig. 7B) compared to paired vehicle controls. DMSO vehicle did not exert significant vascular actions. At the highest concentration studied (100 μ*M*), resveratrol decreased vascular superoxide levels by 54 ± 8% ($p < 0.05$, one-way ANOVA, SNK). In carotid artery rings preconstricted with phenylephrine in the absence of resveratrol, acetylcholine elicited concentration-dependent relaxation responses ($n = 6$ animals/group, Fig. 8A). The presence of resveratrol (10 μ*M*, added 20 min prior to phenylephrine, $n = 4$ animals/group, significantly shifted the acetylcholine concentration-response curve to the left (pEC_{50} was increased, $p < 0.01$, unpaired *t*-test), and increased the maximal relaxation response to the vasodilator ($p < 0.005$, unpaired *t*-test) in comparison to vehicle. Sodium nitroprusside also induced concentration-dependent relaxation of endothelium-intact arteries (Fig. 8B), but this was not modulated by resveratrol ($n = 4$ animals/group) compared to vehicle ($n = 5$ animals/group). Responses to acetylcholine were also examined in endothelium-intact carotid arteries in the absence and presence of the auto-oxidant pyrogallol (100 μ*M*, added 20 min prior to phenylephrine). Although acetylcholine still elicited concentration-dependent relaxation (all

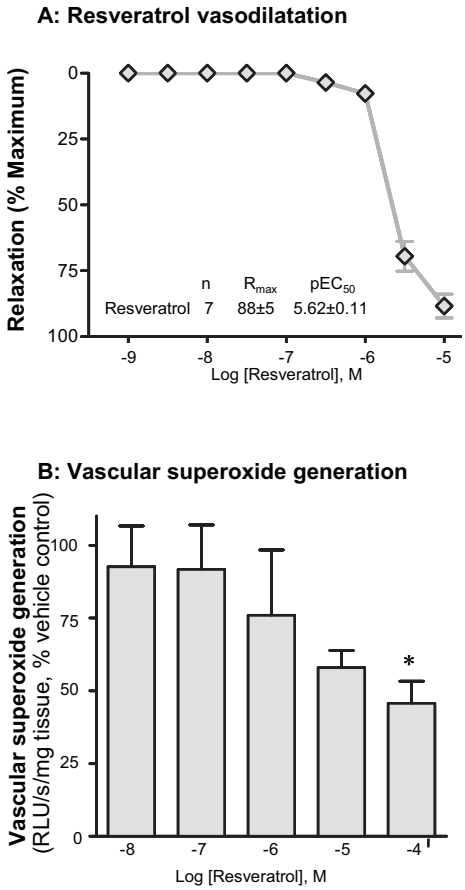


FIG. 7. Direct vascular actions of resveratrol. (**A**) The vasorelaxant actions of resveratrol (0.01–100 μ*M*) were determined in preconstricted, endothelium-intact rat carotid arteries. Relaxation responses were expressed as a percent of the preconstricted level of tone. The number of experiments (*n*), maximum relaxation (R_{max}), and sensitivity (pEC_{50}) is shown. (**B**) Vascular superoxide levels in response to vehicle or resveratrol (0.1–100 μ*M*) was determined in rat aortic rings, using lucigenin-enhanced chemiluminescence normalized to dry tissue weight ($n = 5$). $^{*}p < 0.05$ versus control, one-way ANOVA with SNK.

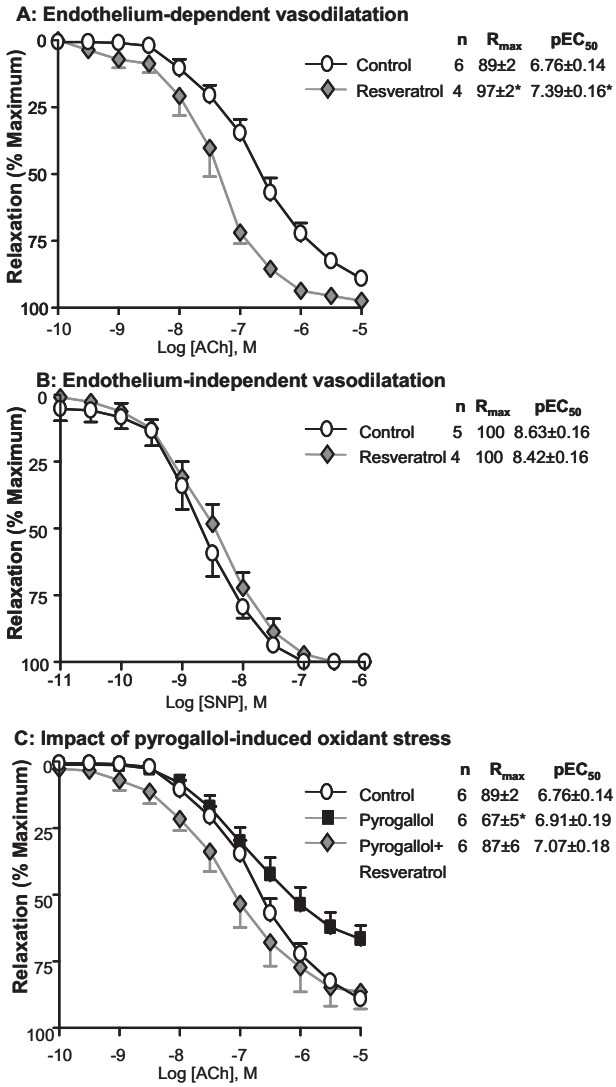


FIG. 8. Impact of resveratrol on endothelium-dependent and -independent vasodilatation in rat carotid arteries. Concentration–response curves to acetylcholine (ACh) and sodium nitroprusside (SNP) were constructed in preconstricted arterial rings, in the absence and presence of resveratrol (10 μ*M*). Relaxation responses were expressed as a percent of the preconstricted level of tone. (**A**) Endothelium-dependent vasodilatation (response to ACh); (**B**) endothelium-independent vasodilatation (response to SNP); and (**C**) endothelium-dependent vasodilatation in the presence and absence of the autooxidant pyrogallol 100 μ*M*, added 20 min prior to preconstriction). Treatments are represented by open circles (control), gray diamonds (resveratrol), closed squares (pyrogallol), and open diamonds (pyrogallol + resveratrol). The number of experiments (*n*), maximum relaxation (R_{max}), and sensitivity (pEC_{50}) for each treatment group are shown. $^{*}p < 0.05$ and $^{\#}p < 0.01$ versus control, one-way ANOVA with SNK.

$n = 6$ animals/group, Fig. 8C), pyrogallol significantly reduced the maximum relaxant response ($p < 0.01$ on one-way ANOVA). Resveratrol, in the presence of pyrogallol, restored the acetylcholine concentration-response comparable to vessels in the absence of pyrogallol; the maximal relaxation response to acetylcholine was significantly improved by resveratrol ($p < 0.01$, SNK) in comparison to pyrogallol alone. No significant changes in pEC_{50} were observed.

DISCUSSION

The key finding was that resveratrol prevents cardiomyocyte injury (evidenced by reduced extracellular LDH release and preserved post-hypoxic cardiomyocyte morphology), via a combination of activation of cardiomyocyte mitochondrial K_{ATP} and BK_{Ca} channels and suppression of cardiomyocyte superoxide levels. Importantly, we provide the first evidence that cardiomyocyte protection is apparent whether resveratrol was present for the full duration of the hypoxia–reoxygenation insult, or only on reoxygenation. Our comprehensive analysis also demonstrated that resveratrol elicits direct protective actions on the vasculature (vasorelaxation, superoxide

suppression) in addition to improvement of endothelium-dependent vasodilatation. Furthermore, resveratrol improved post-ischemic recovery of LV contractile function, attenuated myocardial injury, and increased LV activation of the survival kinases Akt and p38MAPK in the intact heart. Our studies thus strongly indicate that resveratrol targets multiple mechanisms and sites of action of cardioprotection. This, together with our demonstration that protection was evident even when resveratrol was only administered after the insult, indicates promising clinical utility for resveratrol in acute myocardial ischemia.

Resveratrol protection of cardiomyocytes

The potent resveratrol prevention of cardiomyocyte injury was one of the most exciting findings to emerge from this study. Resveratrol markedly reduced both cardiomyocyte LDH release and cardiomyocyte superoxide levels, in addition to preserving post-hypoxic cardiomyocyte morphology. Previously, resveratrol has been shown to limit post-hypoxic cardiomyocyte calcium overload and generation of reactive oxygen species. Resveratrol at higher concentrations (≥ 50 μ*M*) upregulates cardiomyocyte antioxidant proteins thioredoxin and hemoxygenase-1 and antioxidant enzyme activity. Resveratrol prevention of hypoxia-induced cardiomyocyte injury (release of cardiac enzymes such as LDH and/or changes in cardiomyocyte morphology) were, however, not assessed in these previous studies, nor did they control for vehicle effect (5, 12, 23). Both DMSO and ethanol, common vehicles for resveratrol stock solutions, can themselves evoke cardioprotective signaling. Furthermore, the earlier investigations only demonstrated protection when resveratrol was administered at the onset of the hypoxic insult, and no further mechanistic insight was sought. Of clinical importance, the extent of resveratrol cardiomyocyte protection in our hands was comparable whether administered only on reoxygenation or for the full duration of the hypoxia–reoxygenation insult.

Tempol elicited similar effects to resveratrol on both cardiomyocyte injury and cardiomyocyte superoxide levels, suggesting that the antioxidant capacity of resveratrol was important for its cardioprotective actions. Detection of superoxide in the present investigation was determined in the presence of NADPH, to ensure sufficient substrate availability for NADPH oxidase, a major cardiovascular source of ROS (33). Our studies were, however, not designed to distinguish between resveratrol superoxide scavenging actions or resveratrol inhibition of cardiomyocyte NADPH oxidase activity; the latter mechanism may also occur. At the concentration used here, resveratrol consistently inhibits NADPH oxidase activity and upregulates endogenous antioxidants in other cell types (10, 27). In the present study, resveratrol both suppressed ROS production triggered by events occurring during hypoxia as well as directly scavenging ROS production on reoxygenation. This is the first definitive evidence that resveratrol decreases cardiomyocyte superoxide levels in parallel with cardiomyocyte preservation.

In the present study, we demonstrate that activation of cardiomyocyte mitochondrial K_{ATP} and BK_{Ca} channels is an important site of resveratrol cardioprotection from I–R injury. These novel actions in isolated cardiomyocytes indicate that resveratrol cardioprotection goes well beyond coronary vasodilatation. Influx of potassium into mitochondria via K_{ATP} or BK_{Ca} channels is a key trigger of cardioprotection in the intact heart and in cardiomyocytes (4, 14, 15, 17, 22, 39). The present study used both 5-HD and TEA to provide evidence supporting the involvement of both channels in resveratrol cardiomyocyte protection. The mechanism by which resveratrol activates these channels was not, however, explored; moreover these experiments were not designed to specifically assess whether BK_{Ca} and/or mitochondrial K_{ATP} channels contribute to resveratrol postconditioning. Resveratrol enhances the bioactivity of nitric oxide (7, 25, 27, 19, 42). Resveratrol activation of cardiomyocyte K_{ATP} or BK_{Ca} channels in the present study may have been a direct action, as occurs in endothelial cells (38). Alternatively, the channels might have been activated by resveratrol-induced increases in nitric oxide (15) or decreases in ROS production (1, 16). Furthermore, mitochondrial K_{ATP} channel activation in the heart is downstream of the cell survival kinase Akt (18, 36).

Resveratrol protection of the intact heart

The ability of resveratrol to protect the intact heart from I–R injury has some precedent (3, 8, 9, 19–21, 30). Of these previous studies, however, the majority only explored cardioprotection when resveratrol was used as a preconditioning stimulus (3, 8, 9, 19–21). An intravenous resveratrol bolus administered 15 min prior to occlusion of the left main coronary artery for 30 min attenuated myocardial injury (on LDH analysis) on reperfusion in anesthetized rats *in vivo* (19). Similarly, a 15 min resveratrol infusion prior to 30 min ischemia reduces infarct size and improves recovery of left ventricular function in the isolated working rat heart *in vitro* (8, 9). Neither of these studies continued to provide the myocardium with resveratrol for the duration of the ischemic insult or following recovery. Furthermore, the impact of resveratrol on I–R damage to the cardiomyocytes has never been sought. The landmark study of Ray *et al*. (30) demonstrated improved recovery of myocardial function and decreased infarct size with resveratrol present during and following the ischemic insult. This clinically relevant study did not, however, investigate the mechanism of those effects, whether they were evident at the level of cardiomyocytes, nor were vehicle controls included. Previously, cardioprotection observed with resveratrol preconditioning (distinct to the longer and later time period employed here), implicated a role for activation of the serine/threonine cell survival protein kinase Akt (8, 9). Prior to our study, the precise mechanism and site of resveratrol action during and/or following ischemia, however, remained largely unresolved. We now demonstrate the first evidence of protective resveratrol actions during an I–R insult (as opposed to preconditioning) compared to vehicle-treated controls. Cardioprotection is evident on both myocardial injury (LDH) and recovery of myocardial contractile function (LVSP, LVEDP, LVDP, and LV + dP/dt_{max}). Our evidence that resveratrol potently improves postischemic diastolic dysfunction is of particular clinical importance. Moreover, protection is also evident in cardiomyocytes. Preserved mitochondrial integrity and an approximately twofold increase in Akt activation (within the optimal physiological range, 26), in addi-

tion to activation of p38MAPK and inactivation of GSK-3β, accompany myocardial protection. Taken together with the dependence of resveratrol cardiomyocyte protection on mitochondrial K_{ATP} channels, upstream of p38MAPK activation in cardioprotection (41), we suggest that resveratrol protection in the whole heart in our own studies was also potassium channel dependent. Downstream signals of Akt include GSK-3β and eNOS (17, 18). In the trigger phase of late delayed ischemic preconditioning, eNOS-derived NO serves as the initiator of a cascade of molecular events that culminates in the delayed activation of iNOS, which then confers protection (22). In the present study, however, resveratrol failed to increase expression of either eNOS or iNOS. The absence of effect on iNOS is perhaps predictable as iNOS is not constitutively expressed in the normal heart (32) and its induction requires at least 6 h (34).

Resveratrol protection of the vasculature

In the present study, resveratrol elicited dose-dependent vascular relaxation and suppression of vascular superoxide levels compared to vehicle-treated control arteries *in vitro*, confirming earlier reports (7, 25, 27, 42). In addition, we now provide the first evidence that resveratrol also improves endothelial function (shifting the acetylcholine concentration–response curve leftward, indicative of improved nitric oxide bioavailability), under both normal and oxidant stress-induced (using the superoxide generator pyrogallol) conditions *in vitro*. Our vascular studies thus clearly demonstrate that resveratrol acutely prevents the adverse effects of superoxide, reacting with superoxide in a very rapid fashion. Taken together with improved nitric oxide bioavailability, these vascular protective actions of resveratrol are clearly advantageous for improving postischemic vasodilator reserve and reducing the no-flow phenomenon, of significance for potential management of acute myocardial infarction (37). Over the longer term, these vascular resveratrol actions result in further augmentation of myocardial blood flow *in vivo* (23). Resveratrol induced enhancement of endothelial function and vascular superoxide suppression is consistent with resveratrol superoxide-scavenging actions, which would likely play a causal role in resveratrol prevention of myocardial injury post I–R in the intact heart.

CONCLUSIONS

This study documents the spectrum of cardioprotective actions of resveratrol across cardiomyocytes, vessels, and intact isolated hearts following I–R. Cardioprotection by resveratrol was evident at the same concentrations in all three preparations, and was specifically attributable to resveratrol and not its solvent vehicle (as has confounded findings in previous studies). Our findings provide compelling evidence that resveratrol specifically protects cardiomyocytes from I–R injury. Moreover, important mechanistic insights were revealed; resveratrol-mediated cardioprotection involves inhibition of cardiomyocyte and vascular superoxide levels, activation of mitochondrial K_{ATP} and BK_{Ca} channels in cardiomyocytes, and preservation of endothelial function and mitochondrial integrity, thus permitting improved postischemic/hypoxic recovery of myocardial contractile function and attenuation of myocardial damage. Akt activation downstream of PI-3 kinase triggers a cascade of events involving serial phosphorylation and activation of eNOS, opening of mitochondrial K_{ATP} channels with subsequent activation of mediators of cardioprotection, including p38MAPK (18). Our evidence that resveratrol cardioprotection, when present for the duration of I–R, encompasses activation of Akt, p38MAPK, mitochondrial K_{ATP} and BK_{Ca} channels, inactivation of GSK-3β, and improved endothelial NO bioavailability (even in the face of no effect on myocardial eNOS expression) is in line with cardioprotective signaling implicated in both preconditioning and postconditioning (18). Thus, resveratrol targets a number of outcomes of myocardial I–R injury (28, 35), including release of reactive oxygen species, loss of recovery of contractile function, and impaired endothelium-dependent vasodilatation.

Clinical significance of resveratrol

Coronary heart disease remains a major cause of morbidity and mortality in developed nations. Numerous interventions have been reported to protect the ischemic myocardium in experimental animals. Only strategies that permit more rapid reperfusion, including thrombolysis and percutaneous coronary interventions, have, however, translated into clinical practice (2, 4, 13, 24). Moderate red wine consumption, however, has cardiovascular benefits and is a key contributing factor to the relatively low incidence of coronary heart disease in France, known as the "French paradox" (11, 27, 29). In recent years, resveratrol, an antioxidant abundant in red wine, has been increasingly implicated in this phenomenon, and certainly has cardioprotective actions when used as a preconditioning stimulus (3, 8, 9, 19–21). This early time point of cardioprotection strongly favors the prophylactic potential of resveratrol in coronary heart disease, for example, as a dietary additive. Given that myocardial ischemic events in humans cannot be predicted, our observations that resveratrol-induced cardioprotection can be evoked even when administered only on recovery from an ischemic insult (analogous to postconditioning, 18), indicates promising clinical relevance. Resveratrol-based therapy may thus provide a valuable clinical strategy to limit acute myocardial reperfusion injury in patients with coronary artery disease presenting for angioplasty or coronary artery bypass graft surgery.

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ABBREVIATIONS

DMSO, dimethylsulphoxide; eNOS, constitutive nitric oxide synthase; GSK-3β, glycogen synthase kinase-3β; 5-

HD, 5-hydroxydecanoate; iNOS, inducible nitric oxide synthase; I–R, ischemia-reperfusion; K_{ATP} channels, ATP-sensitive potassium; K_{Ca} channels, calcium-sensitive potassium channels; LDH, lactate dehydrogenase; LV, left ventricular; LVDP, LV developed pressure; LV + dP/dt_{max}, peak rate of rise of LV pressure; LV − dP/dt_{min}, peak rate of decline of LV pressure; LVEDP, LV end-diastolic pressure; LVSP, LV systolic pressure; p38MAPK, p38 mitogen-activated protein kinase; ROS, reactive oxygen species; SNK, Student–Newman–Keuls; SOD, superoxide dismutase; TEA, tetraethylammonium bromide.

REFERENCES

1. Barlow RS and White RE. Hydrogen peroxide relaxes porcine coronary arteries by stimulating BK_{Ca} channel activity. *Am J Physiol* 275: H1283–H1289, 1998.
2. Bolli R, Becker L, Gross G, Mentzer R, Balshaw D, and Lathrop DA. Myocardial protection at a crossroads. The need for translation into clinical practice. *Circulation* 95: 125–134, 2004.
3. Bradamante S, Barenghi L, Piccinini F, Bertelli AA, De Jonge R, Beemster P, and De Jong JW. Resveratrol provides late-phase cardioprotection by means of a nitric oxide- and adenosine-mediated mechanism. *Eur J Pharmacol* 465: 115–123, 2003.
4. Cannon RO. Mechanisms, management and future directions for reperfusion injury after acute myocardial infarction. *Nature Clin Practice Cardiovasc Med* 2: 88–94, 2005.
5. Cao Z and Li Y. Potent induction of cellular antioxidants and phase 2 enzymes by resveratrol in cardiomyocytes: protection against oxidative and electrophilic injury. *Eur J Pharmacol* 489: 39–48, 2004.
6. Chan ECH, GR Drummond, and OL Woodman. 3′,4′-Dihydroxyflavonol enhances nitric oxide bioavailability and improves vascular function after ischaemia and reperfusion injury in the rat. *J Cardiovasc Pharmacol* 42: 727–735, 2003.
7. Chen CK and Pace–Asciak CR. Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta. *Gen Pharmacol* 27:363–366, 1996.
8. Das S, Tosaki A, Bagchi D, Maulik N, and Das DK. Resveratrol-mediated activation of cAMP response element-binding protein through adenosine A_3 receptor by Akt-dependent and -independent pathways. *J Pharmacol Exp Ther* 314: 762–769, 2005.
9. Das S, Tosaki A, Bagchi D, Maulik N, and Das DK. Potentiation of a survival signal in the ischemic heart by resveratrol through p38 mitogen-activated protein kinase/mitogen- and stress-activated protein kinase 1/cAMP response element-binding protein signaling. *J Pharmacol Exp Ther* 317: 980–988, 2006.
10. Delmas D, Jannin B, and Latruffe N. Resveratrol: preventing properties against vascular alterations and ageing. *Mol Nutr Food Res* 49: 377–395, 2005.
11. De Lorgeril M, Salen P, Paillard F, Laporte L, Boucher F, and De Leiris J. Mediterranean diet and the French paradox: Two distinct biogeographic concepts for one consolidated scientific theory on the role of nutrition in coronary heart disease. *Cardiovasc Res* 54: 503–515, 2002.
12. Eigel BN, Gursahani H, and Hadley RW. ROS are required for rapid reactivation of Na^+/Ca^{2+} exchanger in hypoxic reoxygenated guinea pig ventricular myocytes. *Am J Physiol* 286: H955–H963, 2004.
13. Faxon DP. Coronary interventions and their impact on post myocardial infarction survival. *Clin Cardiol* 28: I38–I44, 2005.
14. Garlid KD, Dos Santos P, Xie ZJ, Costa ADT, and Paucek P. Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K^+ channel in cardiac function and cardioprotection. *Biochim Biophys Acta* 1606: 1–21, 2003.
15. Garreffa AM, Woodman OL, Cao AH, and Ritchie RH. Sodium nitroprusside protects adult rat cardiac myocytes from cellular injury induced by simulated ischemia: role for a non-cGMP-dependent mechanism of nitric oxide protection. *J Cardiovasc Pharmacol* 47: 1–8, 2006.
16. Gong L, Gao TM, Huang H, and Tong Z. Redox modulation of large conductance calcium-activated potassium channels in CA1 pyramidal neurons from adult rat hippocampus. *Neurosci Lett* 286: 191–194, 2000.
17. Hardt SE and Sadoshima J. Glycogen synthase kinase-3beta: a novel regulator of cardiac hypertrophy and development. *Circ Res* 90: 1055–1063, 2002.
18. Hausenloy DJ, Tsang A, and Yellon DM. The reperfusion injury salvage kinase pathway: a common target for both ischemic preconditioning and postconditioning. *Trends Cardiovasc Med* 15: 69–75, 2005.
19. Hung LM, Chen JK, Huang SS, Lee RS, and Su MJ. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc Res* 47: 549–555, 2000.
20. Hung LM, Su MJ, Chu WK, Chiao CW, Chan WF, and Chen JK. The protective effect of resveratrol on ischaemia-reperfusion injuries of rat hearts is correlated with antioxidant efficacy. *Br J Pharmacol* 135: 1627–1633, 2002.
21. Imamura G, Bertelli AA, Bertelli A, Otani H, Maulik N, and Das DK. Pharmacological preconditioning with resveratrol: an insight with iNOS knockout mice. *Am J Physiol* 282: H1996–H2003, 2002.
22. Jones SP and Bolli R. The ubiquitous role of nitric oxide in cardioprotection. *J Mol Cell Cardiol* 40: 16–23, 2006.
23. Kaga S, Zhan L, Matumoto M, and Maulik N. Resveratrol enhances neovascularization in the infarcted rat myocardium through the induction of thioredoxin-1, heme-oxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 39: 813–822, 2005.
24. Kloner RA and Rezkalla SH. Cardiac protection during acute myocardial infarction: Where do we stand in 2004? *J Am Coll Cardiol* 44: 276–286, 2004.
25. Miatello R, Vazquez M, Renna N, Cruzado M, Zumino AP, and Risler N. Chronic administration of resveratrol prevents biochemical cardiovascular changes in fructose-fed rats. *Am J Hypertens* 18: 864–870, 2005.
26. O'Neill BT and Abel ED. Akt1 in the cardiovascular system: friend or foe? *J Clin Invest* 115: 2059–2064, 2005.
27. Orallo F, Alvarez E, Camina M, Leiro JM, Gomez E, and Fernandez P. The possible implication of trans-resveratrol in the cardioprotective effects of long-term moderate wine consumption. *Mol Pharmacol* 61, 294–302, 2002.
28. Qi XL, Nguyen TL, Andries L, Sys SU, and Rouleau JL. Vascular endothelial dysfunction contributes to myocardial depression in ischemia-reperfusion injury in the rat. *Can J Physiol Pharmacol* 76: 35–45, 1998.
29. Rakotovao A, Berthonneche C, Guiraud A, De Lorgeril M, Salen P, De Leiris J, and Boucher F. Ethanol, wine, and experimental cardioprotection in ischemia/reperfusion: role of the prooxidant/antioxidant balance. *Antiox Redox Signal* 6: 431–438, 2004.
30. Ray PS, Maulik G, Cordis GA, Bertelli AA, Bertelli A, and Das DK. The red wine antioxidant resveratrol protects isolated rat hearts from ischemia reperfusion injury. *Free Radic Biol Med* 27: 160–169, 1999.
31. Ritchie RH, Gordon JG, Cao AH, Woodman OL, and Dusting GJ. Annexin-1 peptide $Anx\text{-}1_{2–26}$ protects adult rat cardiac myocytes from cellular injury induced by simulated ischaemia. *Br J Pharmacol* 145: 495–502, 2005.
32. Ritchie RH, Sun XS, Bilszta JL, Gulluyan LM, and Dusting GJ. Cardioprotective actions of an N-terminal fragment of annexin-1 in rat myocardium *in vitro. Eur J Pharmacol* 461: 171–179, 2003.
33. Sawyer DB, Siwik DA, Xiao L, Pimentel DR, Singh K, and Colucci WS. Role of oxidative stress in myocardial hypertrophy and failure. *J Mol Cell Cardiol* 34: 379–388, 2002.
34. Smart N, Mojet MH, Latchman DS, Marber MS, Duchen MR, and Heads RJ. IL-6 induces PI 3-kinase and nitric oxide-dependent

protection and preserves mitochondrial function in cardiomyocytes. *Cardiovasc Res* 69: 164–177, 2006.
35. Wang QD, Pernow J, Sjoquist PO, and Ryden L. Pharmacological possibilities for protection against myocardial reperfusion injury. *Cardiovasc Res* 55: 25–37, 2002.
36. Wang Y, Ahmad N, Kudo M, and Ashraf M. Contribution of Akt and endothelial nitric oxide synthase to diazoxide-induced late preconditioning. *Am J Physiol* 287: H1125–H1131, 2004.
37. Woodman OL and Chan ECH. Vascular and anti-oxidant actions of flavonols and flavones. *Clin Exp Pharmacol Physiol* 31: 786–790, 2004.
38. Wu SN. Large-conductance Ca^{2+}-activated K^+ channels: physiological role and pharmacology. *Curr Med Chem* 10: 649–661, 2003.
39. Xu W, Liu Y, Wang S, Mcdonald T, Van Eyk JE, Sidor A, and O'Rourke B. Cytoprotective role of Ca^{2+}-activated K^+ channels in the cardiac inner mitochondrial membrane. *Science* 298: 1029–1033, 2002.
40. Younes A, Pepe S, Barron BA, Spurgeon HA, Lakatta EG, and Caffrey JL. Cardiac synthesis, processing, and coronary release of enkephalin-related peptides. *Am J Physiol* 279: H1989–H1998, 2000.
41. Yue Y, Qin Q, Cohen MV, Downey JM, and Critz SD. The relative order of mK(ATP) channels, free radicals and p38 MAPK in preconditioning's protective pathway in rat heart. *Cardiovasc Res* 55: 681–689, 2002.
42. Zou JG, Wang ZR, Huang YZ, Cao KJ, and Wu JM. Effect of red wine and wine polyphenol resveratrol on endothelial function in hypercholesterolemic rabbits. *Int J Mol Med* 11: 317–320, 2003.

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2. Jay H. Chung, Vincent Manganiello, Jason R.B. Dyck. 2012. Resveratrol as a calorie restriction mimetic: therapeutic implications. *Trends in Cell Biology* **22**:10, 546-554. [CrossRef]
3. Belma Turan, Erkan Tuncay, Guy Vassort. 2012. Resveratrol and diabetic cardiac function: focus on recent in vitro and in vivo studies. *Journal of Bioenergetics and Biomembranes* . [CrossRef]
4. Shijun Wang, Yiming Qian, Dandan Gong, Yingyu Zhang, Yu Fan. 2011. Resveratrol attenuates acute hypoxic injury in cardiomyocytes: Correlation with inhibition of iNOS–NO signaling pathway. *European Journal of Pharmaceutical Sciences* . [CrossRef]
5. Sergiy M. Nadtochiy, Emily K. Redman. 2011. Mediterranean diet and cardioprotection: The role of nitrite, polyunsaturated fatty acids, and polyphenols. *Nutrition* **27**:7-8, 733-744. [CrossRef]
6. Cheng Xue Qin, Spencer J. Williams, Owen L. Woodman. 2011. Antioxidant activity contributes to flavonol cardioprotection during reperfusion of rat hearts. *Free Radical Biology and Medicine* . [CrossRef]
7. Vernon W. Dolinsky, Jason R.B. Dyck. 2011. Calorie restriction and resveratrol in cardiovascular health and disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* . [CrossRef]
8. Carlotta Giorgi, Chiara Agnoletto, Angela Bononi, Massimo Bonora, Elena De Marchi, Saverio Marchi, Sonia Missiroli, Simone Patergnani, Federica Poletti, Alessandro Rimessi, Jan M. Suski, Mariusz R. Wieckowski, Paolo Pinton. 2011. Mitochondrial calcium homeostasis as potential target for mitochondrial medicine. *Mitochondrion* . [CrossRef]
9. Suwan Yap, Chengxue Qin, Owen L. Woodman. 2010. Effects of resveratrol and flavonols on cardiovascular function: Physiological mechanisms. *BioFactors* **36**:5, 350-359. [CrossRef]
10. R.H.X. Wong, P.R.C. Howe, J.D. Buckley, A.M. Coates, I. Kunz, N.M. Berry. 2010. Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. *Nutrition, Metabolism and Cardiovascular Diseases* . [CrossRef]
11. Gerhard Spiteller . 2010. Is Lipid Peroxidation of Polyunsaturated Acids the Only Source of Free Radicals That Induce Aging and Age-Related Diseases?. *Rejuvenation Research* **13**:1, 91-103. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
12. Chengxue Qin, Suwan Yap, Owen L Woodman. 2009. Antioxidants in the prevention of myocardial ischemia/reperfusion injury. *Expert Review of Clinical Pharmacology* **2**:6, 673-695. [CrossRef]
13. Wen-juan Li, Shao-ping Nie, Yan Yan, Shang-bin Zhu, Ming-yong Xie. 2009. The protective effect of Ganoderma atrum polysaccharide against anoxia/reoxygenation injury in neonatal rat cardiomyocytes. *Life Sciences* **85**:17-18, 634-641. [CrossRef]
14. Zuo-Hui Shao, Kimberly R. Wojcik, Anar Dossumbekova, Chinwang Hsu, Sangeeta R. Mehendale, Chang-Qing Li, Yimin Qin, Willard W. Sharp, Wei-Tien Chang, Kimm J. Hamann, Chun-Su Yuan, Terry L. Vanden Hoek. 2009. Grape seed proanthocyanidins protect cardiomyocytes from ischemia and reperfusion injury via Akt-NOS signaling. *Journal of Cellular Biochemistry* **107**:4, 697-705. [CrossRef]
15. Elizabeth D. Brookins Danz, Jeremy Skramsted, Nicholas Henry, James A. Bennett, Rebecca S. Keller. 2009. Resveratrol prevents doxorubicin cardiotoxicity through mitochondrial stabilization and the Sirt1 pathway. *Free Radical Biology and Medicine* **46**:12, 1589-1597. [CrossRef]
16. Michael N. Sack. 2009. Type 2 diabetes, mitochondrial biology and the heart. *Journal of Molecular and Cellular Cardiology* **46**:6, 842-849. [CrossRef]
17. Anita Palfi, Eva Bartha, Laszlo Copf, Laszlo Mark, Ferenc Gallyas, Balazs Veres, Endre Kalman, Laszlo Pajor, Kalman Toth, Robert Ohmacht, Balazs Sumegi. 2009. Alcohol-free red wine inhibits isoproterenol-induced cardiac remodeling in rats by the regulation of Akt1 and protein kinase C #/# II. *The Journal of Nutritional Biochemistry* **20**:6, 418-425. [CrossRef]
18. R RITCHIE. 2009. Evidence for a Causal Role of Oxidative Stress in the Myocardial Complications of Insulin Resistance. *Heart, Lung and Circulation* **18**:1, 11-18. [CrossRef]
19. Prabir K. Chakraborty, Soumyajit Banerjee Mustafi, Sudipto Ganguly, Mitali Chatterjee, Sanghamitra Raha. 2008. Resveratrol induces apoptosis in K562 (chronic myelogenous leukemia) cells by targeting a key survival protein, heat shock protein 70. *Cancer Science* **99**:6, 1109-1116. [CrossRef]

20. Alicja Mortensen, Ilona K. Sorensen, Colin Wilde, Stefania Dragoni, Dana Mullerová, Olivier Toussaint, Zden#k Zloch, Giampietro Sgaragli, Jaroslava Ovesná. 2008. Biological models for phytochemical research: from cell to human organism. *British Journal of Nutrition* **99**:E-S1. . [CrossRef]
21. Cheng Li , Parastu Hossieny , Ben J. Wu , Abdelqader Qawasmeh , Konstanze Beck , Roland Stocker . 2007. Pharmacologic Induction of Heme Oxygenase-1. *Antioxidants & Redox Signaling* **9**:12, 2227-2240. [Abstract] [Full Text PDF] [Full Text PDF with Links]
22. Yim Tong Szeto. 2007. Single-cell gel electrophoresis: a tool for investigation of DNA protection or damage mediated by dietary antioxidants. *Journal of the Science of Food and Agriculture* **87**:13, 2359-2381. [CrossRef]