Reactive Oxygen Species and Mitochondrial Adenosine Triphosphate–Regulated Potassium Channels Mediate Helium-Induced Preconditioning Against Myocardial Infarction In Vivo

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Objectives: **Helium produces preconditioning by activating prosurvival kinases, but the roles of reactive oxygen species (ROS) or mitochondrial adenosine triphosphate–regulated potassium (K_{ATP}) channels in this process are unknown. The authors tested the hypothesis that ROS and mitochondrial K_{ATP} channels mediate helium-induced preconditioning in vivo.**

Design: **A randomized, prospective study.**

Setting: **A university research laboratory.**

Participants: **Male New Zealand white rabbits.**

Interventions: **Rabbits (n = 64) were instrumented for the measurement of systemic hemodynamics and subjected to a 30-minute left anterior descending coronary artery (LAD) occlusion and 3 hours of reperfusion. In separate experimental groups, rabbits (n = 7 or 8 per group) were randomly assigned to receive 0.9% saline (control) or 3 cycles of 70% helium–30% oxygen administered for 5 minutes interspersed with 5 minutes of an air-oxygen mixture before LAD occlusion with or without the ROS scavengers *N*-acetylcysteine (NAC; 150 mg/kg) or *N*-2 mercaptoproprionyl glycine (2-MPG; 75 mg/kg), or the mitochondrial K_{ATP} antagonist 5-hydroxydecanoate (5-HD; 5 mg/kg). Statistical analysis of data was performed with analysis of variance for repeated measures followed by Bonferroni's modification of a Student *t* test.**

Measurements and Main Results: **The myocardial infarct size was determined by using triphenyltetrazolium chloride staining and presented as a percentage of the left ventricular area at risk. Helium significantly ($p < 0.05$) reduced infarct size (23 ± 4% of the area at risk; mean ± standard deviation) compared with control (46 ± 3%). NAC, 2-MPG, and 5-HD did not affect irreversible ischemic injury when administered alone (49 ± 5%, 45 ± 6%, and 45 ± 3%), but these drugs blocked reductions in infarct size produced by helium (45 ± 4%, 45 ± 2%, and 44 ± 3%).**

Conclusions: **The results suggest that ROS and mitochondrial K_{ATP} channels mediate helium-induced preconditioning in vivo.**

KEY WORDS: myocardial ischemia, preconditioning, helium, reactive oxygen species, mitochondrial adenosine triphosphate–regulated potassium channels

REPERFUSION AFTER coronary artery occlusion produces large quantities of reactive oxygen species (ROS) that contribute substantially to myocardial injury.[1,2] In contrast, small amounts of ROS released from mitochondria during a brief episode of ischemia before prolonged coronary occlusion and reperfusion cause preconditioning.[3,4] Pretreatment with free radical scavengers abolished cardioprotection produced by ischemic preconditioning.[5] Volatile anesthetics also directly produce small quantities of ROS[6,7] (most likely from mitochondrial electron transport chain complex III[8]) through activation of mitochondrial adenosine triphosphate–regulated potassium (K_{ATP}) channels.[9,10] As observed during ischemic preconditioning, ROS generated by this form of pharmacologic preconditioning mediated reductions in myocardial infarct size produced by the volatile agent.[6,7,10] Brief, intermittent administration of helium before prolonged coronary artery occlusion and reperfusion was recently shown to protect myocardium against infarction by activating prosurvival signaling kinases (eg, phosphotidylinositol-3-kinase, extracellular signal-regulated kinases, and endothelial nitric oxide synthase), attenuating the detrimental actions of glycogen synthase kinase, and inhibiting mitochondrial transition in vivo.[11-13] Whether oxygen-derived free radical intermediates play a role in helium-induced preconditioning is unknown. Brief exposure to the anesthetic noble gas xenon produced cardioprotection by activating mitochondrial K_{ATP} channels,[14] but the role of mitochondrial K_{ATP} channels in cardioprotection by nonanesthetic noble gases remains undefined. Thus, the current investigation also tested the hypothesis that the activation of mitochondrial K_{ATP} channels mediates helium-induced preconditioning in rabbits.

METHODS

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin. Furthermore, all conformed to the *Guiding Principles in the Care and Use of Animals of the American Physiologic Society* and were in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Male New Zealand white rabbits weighing between 2.5 and 3.0 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg) as previously described.[11] Additional doses of pentobarbital were titrated as required to ensure that pedal and palpebral reflexes were absent throughout the experiment. Briefly, a tracheostomy was performed through a midline incision, and each rabbit was ventilated with positive

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pressure using an air-oxygen mixture (fractional inspired oxygen concentration = 0.30). Arterial blood gas tensions and acid-base status were maintained within a normal physiologic range by adjusting the respiratory rate or tidal volume throughout the experiment. A pulse oximeter was placed on the right hind paw of each rabbit for the measurement of continuous arterial oxygen saturation. Heparin-filled catheters were positioned in the right carotid artery and the left jugular vein for measurement of arterial blood pressure and fluid or drug administration, respectively. Maintenance fluids (0.9% saline; 15 mL/kg/min) were continued for the duration of each experiment. A thoracotomy was performed at the left fourth intercostal space, and the heart was suspended in a pericardial cradle. A prominent branch of the left anterior descending coronary artery (LAD) was identified, and a silk ligature was placed around this vessel approximately halfway between the base and the apex for the production of coronary artery occlusion and reperfusion. Intravenous heparin (500 U) was administered immediately before LAD occlusion. Coronary artery occlusion was verified by the presence of epicardial cyanosis and regional dyskinesia in the ischemic zone, and reperfusion was confirmed by observing an epicardial hyperemic response. Hemodynamics were continuously recorded on a polygraph throughout each experiment.

The experimental design is shown in Figure 1. Baseline hemodynamics, arterial blood gas tensions, and arterial oxygen saturation were recorded 30 minutes after instrumentation was completed. All rabbits underwent a 30-minute LAD occlusion followed by 3 hours of reperfusion. In 8 separate groups, rabbits (n = 7 to 8 per group) were randomly assigned (Latin square design) to receive 0.9% saline (control) or 3 cycles of 70% helium–30% oxygen administered for 5 minutes interspersed with 5 minutes of 70% nitrogen–30% oxygen before coronary artery occlusion in the presence or absence of the ROS scavengers *N*-acetylcysteine (NAC, 150 mg/kg) or *N*-2 mercaptopropionyl glycine (2-MPG, 75 mg/kg) or the selective mitochondrial K_{ATP} channel antagonist 5-hydroxydeconate (5-HD, 5 mg/kg). NAC and 2-MPG were dissolved in 0.9% saline and administered as intravenous infusions over 30 and 75 minutes, respectively. 5-HD was dissolved in 0.9% saline and administered intravenously 30 minutes before LAD occlusion. The doses of NAC, 2-MPG, and 5-HD used in the current investigation did not produce hemodynamic effects nor affect infarct size when administered alone in an identical rabbit model.[7,10] The doses of NAC, 2-MPG, and 5-HD also abolished isoflurane-induced production of ROS as detected by dihydroethidium staining independent of prolonged coronary artery occlusion and reperfusion in rabbits.[7,10] Taken together, these previous findings suggest that these particular antagonists are effective and selective in the doses that were used in the current investigation.

Myocardial infarct size was measured as previously described.[15] Briefly, the LAD was reoccluded at the completion of each experiment, and 3 mL of patent blue dye were injected intravenously. The left ventricular area at risk for infarction was separated from the surrounding normal areas (stained blue), and the 2 regions were incubated at

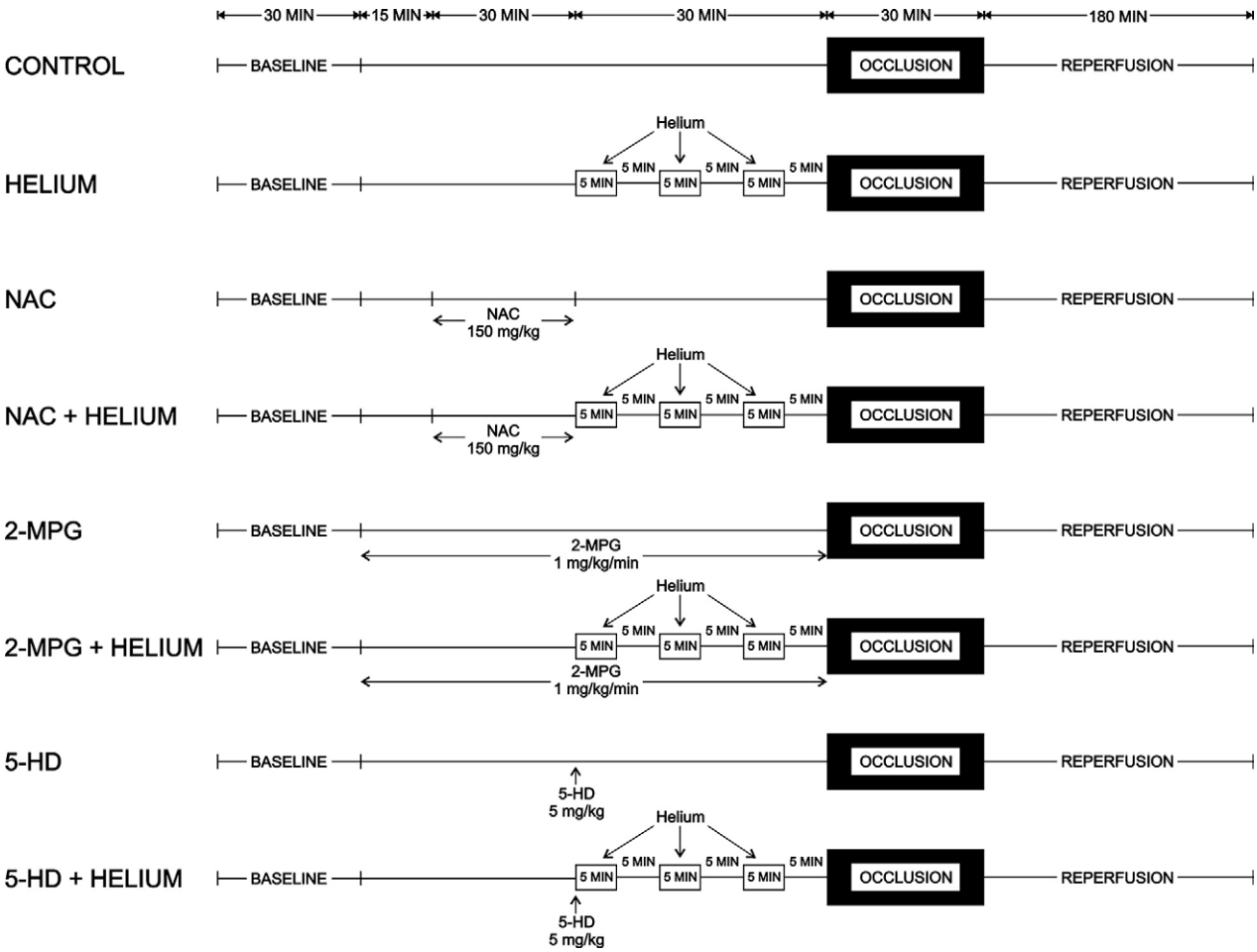

Fig 1. Schematic illustration depicting the experimental protocol used in the current investigation.

Table 1. Hemodynamics

	Baseline	Intervention	Occlusion	Reperfusion (min)		
				60	120	180
HR (beats/min)						
CON	236 ± 23	226 ± 21	230 ± 27	216 ± 16*	211 ± 21*	203 ± 20*
He	255 ± 29	238 ± 28	228 ± 14*	220 ± 29*	209 ± 25*	200 ± 27*
5-HD	243 ± 28	236 ± 22	225 ± 27	221 ± 29	208 ± 26*	203 ± 27*
5-HD + He	229 ± 24	219 ± 22	206 ± 19*	196 ± 16*	189 ± 10*	184 ± 9*
NAC	253 ± 28	253 ± 22	244 ± 31	238 ± 33	235 ± 37	232 ± 39
NAC + He	246 ± 28	234 ± 24	223 ± 18	214 ± 22*	207 ± 23*	196 ± 29*
2-MPG	236 ± 24	254 ± 26	261 ± 35	236 ± 11	229 ± 25	237 ± 34
2-MPG + He	226 ± 23	221 ± 20	206 ± 25	205 ± 23	192 ± 19*	186 ± 18*
MAP (mmHg)						
CON	71 ± 9	65 ± 9	61 ± 10	62 ± 10	63 ± 11	62 ± 11
He	75 ± 8	80 ± 17	69 ± 7	66 ± 10	68 ± 6	67 ± 16
5-HD	69 ± 2	74 ± 10	61 ± 11	60 ± 12	55 ± 10	54 ± 9
5-HD + He	71 ± 7	71 ± 9	61 ± 11	65 ± 10	64 ± 10	66 ± 7
NAC	85 ± 9	86 ± 6†	76 ± 11	71 ± 11*	72 ± 12*	73 ± 13*
NAC + He	75 ± 8	74 ± 6	60 ± 13	61 ± 8*	62 ± 10*	61 ± 6*
2-MPG	89 ± 8†	84 ± 6†	68 ± 16*	68 ± 15*	73 ± 21	75 ± 19
2-MPG + He	72 ± 4	71 ± 5	55 ± 12*	63 ± 10	63 ± 10	65 ± 9
RPP (min/mmHg/10^3)						
CON	18.9 ± 2.4	17.0 ± 2.9	16.1 ± 1.8	15.6 ± 2.6*	15.5 ± 3.2*	14.7 ± 3.4*
He	21.6 ± 3.7	21.6 ± 6.1	17.9 ± 2.3	16.8 ± 3.3*	16.4 ± 2.4*	15.3 ± 4.1*
5-HD	19.4 ± 2.3	19.6 ± 2.7	15.6 ± 1.6	15.5 ± 3.2*	13.3 ± 3.1*	13.2 ± 2.7*
5-HD + He	18.5 ± 2.7	17.7 ± 3.1	14.7 ± 3.2*	14.8 ± 3.0*	14.1 ± 2.3*	14.1 ± 1.8*
NAC	24.5 ± 4.5	24.4 ± 3.0†	21.2 ± 4.1	19.4 ± 4.9*	19.7 ± 5.6*	19.8 ± 6.1
NAC + He	21.0 ± 3.5	19.7 ± 3.2	15.3 ± 3.3*	15.1 ± 2.0*	14.9 ± 2.3*	13.8 ± 2.0*
2-MPG	23.6 ± 2.9	24.5 ± 3.5†	20.2 ± 4.2	18.3 ± 3.5*	19.1 ± 5.2*	20.1 ± 4.9
2-MPG + He	18.5 ± 2.0	17.9 ± 2.5	13.6 ± 4.0*	15.0 ± 2.8*	14.0 ± 2.5*	14.0 ± 2.5*

NOTE. Data are mean ± standard deviation.

Abbreviations: HR, heart rate; MAP, mean arterial pressure; RPP, rate-pressure product; CON, control; He, helium.

*Significantly ($p < 0.05$) different from baseline.

†Significantly ($p < 0.05$) different from the corresponding control.

37°C for 20 minutes in 1% 2,3,5 triphenyltetrazolium chloride in 0.1 mol/L of phosphate buffer adjusted to pH 7.4. Infarcted and noninfarcted myocardium within the area at risk were carefully separated and weighed after storage overnight in 10% formaldehyde. Myocardial infarct size was expressed as a percentage of the area at risk. Rabbits that developed intractable ventricular fibrillation and those with an area at risk less than 15% of the total left ventricular mass were excluded from subsequent analysis.

Statistical analysis of data within and between groups was performed with multiple analysis of variance for repeated measures followed by Bonferroni's modification of a Student t test.[16] Changes were considered statistically significant when $p < 0.05$. All data are expressed as mean ± standard deviation.

RESULTS

Sixty-four rabbits were instrumented to obtain 59 successful infarct size experiments. Two rabbits were excluded because the left ventricular area at risk was less than 15% of the total left ventricular mass. Three rabbits were excluded because intractable ventricular fibrillation occurred during coronary artery occlusion. Arterial blood gas tensions were maintained within the physiologic range during the administration of helium in all groups (data not shown). Arterial oxygen saturation remained at 100% during and after the administration of helium with or without other drug interventions (data not shown). Baseline systemic hemodynamics were similar between groups (Table 1), but the mean arterial pressure was greater in rabbits randomized to receive 2-MPG compared with 0.9% saline. Helium did not affect hemodynamics. The mean arterial pressure and rate-pressure product were greater in rabbits receiving NAC or 2-MPG alone before LAD occlusion compared with those treated with 0.9% saline. Brief coronary artery occlusion and reperfusion significantly ($p < 0.05$) reduced rate-pressure product in all experimental groups. There were no differences in hemodynamics between groups during LAD occlusion and reperfusion. Body weight, left ventricular mass, area at risk weight, and the ratio of area at risk to left ventricular mass were similar between groups (Table 2). Left ventricular weight and area at risk weight were less in rabbits randomized to receive NAC compared with 0.9% saline, but the ratio of area at risk to the left ventricular mass was similar between these groups. Brief, intermittent exposure to 70% helium before LAD occlusion reduced myocardial infarct size (23 ± 4% of the left ventricular area at risk) as compared with control rabbits (46 ± 3%, Fig 2). The administration of NAC, 2-MPG, or 5-HD alone did not affect infarct size (49 ± 5%, 45 ± 6%, and 45 ± 3%,

Table 2. Left Ventricular Area at Risk

	N	Body Weight (g)	LV (g)	AAR (g)	AAR/LV (%)
CON	8	2,703 ± 298	3.90 ± 0.46	1.63 ± 0.20	42 ± 5
He	8	2,803 ± 243	3.84 ± 0.21	1.26 ± 0.08	33 ± 2
5-HD	7	2,789 ± 231	3.49 ± 0.39	1.36 ± 0.34	39 ± 6
5-HD + He	7	2,487 ± 72	3.58 ± 0.25	1.34 ± 0.17	37 ± 4
NAC	8	2,709 ± 280	3.08 ± 0.48*	1.02 ± 0.33*	33 ± 6
NAC + He	7	2,594 ± 82	3.74 ± 0.45	1.35 ± 0.37	36 ± 7
2-MPG	7	2,847 ± 170	3.32 ± 0.63	1.18 ± 0.48	35 ± 9
2-MPG + He	7	2,726 ± 112	3.58 ± 0.44	1.47 ± 0.25	41 ± 4

NOTE. Data are mean ± standard deviation.
Abbreviations: LV, left ventricle; AAR, area at risk; CON, control; He, helium.
*Significantly ($p < 0.05$) different from the corresponding CON value.

respectively), but these drugs abolished helium-induced cardioprotection (45 ± 4%, 44 ± 2%, and 44 ± 3%, respectively).

DISCUSSION

The current results confirm the previous findings,[11-13] indicating that 3 cycles of 5 minutes of 70% helium–30% oxygen preconditioning interspersed with 5-minute washout periods of an air-oxygen mixture reduce myocardial necrosis after prolonged coronary artery occlusion and reperfusion. The results show for the first time that pretreatment with NAC or 2-MPG abolishes this helium-induced cardioprotection, suggesting that ROS mediate preconditioning by the nonanesthetic noble gas in vivo. The results further indicate that 5-HD pretreatment blocks reductions in myocardial infarct size produced by brief, intermittent administration of helium, implicating mitochondrial K_{ATP} channels in this process as well. Weber et al[14] showed that mitochondrial K_{ATP} channels mediated cardioprotection produced by the anesthetic inert gas xenon,[14] and the current results extend this observation to another noble gas that is devoid of anesthetic properties, even under extreme hyperbaric conditions (>200 atm).[17] Collectively, the current and previous[14] data with noble gases also lend support to the hypothesis that ROS are a ubiquitous feature in preconditioning phenomena in conjunction with mitochondrial K_{ATP} channels. Pretreatment with low concentrations of ROS mimicked the beneficial effects of ischemic preconditioning,[18] and small quantities of ROS generated by mitochondria during brief periods of ischemia[3,4] or after exposure to the mitochondrial K_{ATP} channel agonist diazoxide[19,20] have been shown to play a central role in ischemic and pharmacologic preconditioning, respectively. Evidence that mitochondrial K_{ATP} activation mediates this ROS-induced cardioprotection also was provided by the observations that diazoxide enhances oxidation of the ROS probe Mitotracker orange[20] (Molecular Probes, Eugene, OR) and increases ROS production as measured using 2′,7′-dichlorofluorescein diacetate in rat ventricular myocytes or isolated hearts.[21] Pretreatment with 5-HD abolished ROS generation produced by the mitochondrial K_{ATP} channel openers nicorandil and cromakalim in isolated rat hearts.[22] Similarly, the ROS scavengers NAC, 2-MPG, and Mn(III)tetrakis (4-benzoic acid) porphyrin chloride inhibited the cardioprotective effects of isoflurane in isolated[6] and intact rabbit hearts.[7,10] Scavengers of ROS also

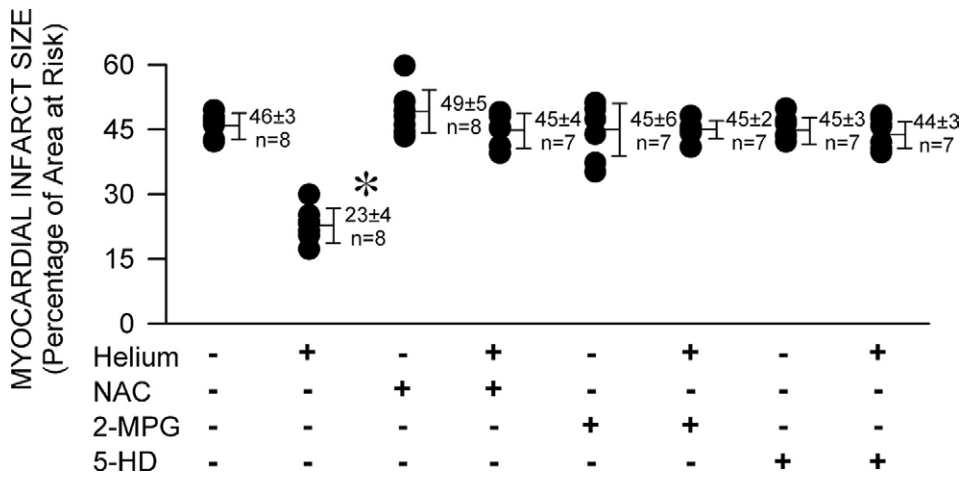

Fig 2. Myocardial infarct size depicted as a percentage of left ventricular area at risk in rabbits receiving 0.9% saline (control, CON) or 3 cycles of 70% helium–30% oxygen administered for 5 minutes interspersed with 5 minutes of an air-oxygen mixture ($F_IO_2 = 0.30$) in the presence or absence of pretreatment with the ROS scavengers NAC (150 mg/kg) or 2-MPG (75 mg/kg) or the selective mitochondrial K_{ATP} channel antagonist 5-HD (5 mg/kg) before prolonged coronary artery occlusion and reperfusion. Each point represents a single experiment. All data are mean ± standard deviation. *Significantly ($p < 0.05$) different from CON.

abolished the salutary actions of sevoflurane against ischemic damage in isolated guinea pig hearts.[23,24] Preconditioning by isoflurane directly increased the production of superoxide anion (O_2^-) as determined using dihydroethidium staining through a mitochondrial K_{ATP} channel-mediated mechanism.[8,10] Thus, it has become abundantly clear that ROS and mitochondrial K_{ATP} channels play complementary roles during ischemic, pharmacologic, and anesthetic preconditioning, and the current results with helium suggest that ROS and mitochondrial K_{ATP} channels are also essential in preconditioning by the nonanesthetic noble gas.

The current results must be interpreted within the constraints of several potential limitations. Based on previous experiments conducted with volatile anesthetics,[7,8,10] it appears highly likely that O_2^- generated from the electron transport chain though mitochondrial K_{ATP} channel opening may also be responsible for the observed results with helium. The sulfhydryl-containing glutathione precursor NAC produces antioxidant effects by enhancing glutathione synthesis, acting as a substrate for glutathione peroxidase, and facilitating metabolism of hydrogen peroxide (H_2O_2) by univalent reduction of O_2^- via preservation of intracellular-reduced glutathione concentration.[25] Large amounts of superoxide dismutase are contained within mitochondria, and this enzyme is primarily responsible for the chemical conversion of O_2^- to H_2O_2 and water. The subsequent reduction of H_2O_2 is catalyzed by glutathione peroxidase to which reduced glutathione serves as an electron donor during the reaction. Thus, the current observation that NAC abolishes helium-induced preconditioning indirectly infers that O_2^- or one of its immediate derivatives is responsible for this cardioprotective effect; 2-MPG also donates sulfhydryl groups to glutathione peroxidase and may be more mitochondria-specific than NAC.[26-28] These observations also indirectly suggest that O_2^- derived from mitochondria or another oxygen-derived free radical intermediate produced by O_2^- metabolism is involved in cardioprotection by helium. Nevertheless, such conclusions must be qualified because the authors did not specifically determine the identity or define the source of the ROS involved in helium-induced preconditioning in the current investigation. It is also unclear based on the current results whether mitochondrial K_{ATP} opening acts as a trigger or end-effector for preconditioning by helium through ROS generation. The authors' laboratory is currently examining this hypothesis.

In addition to previously described limitations, the current results must be interpreted within the constraints of several other potential shortcomings. The duration of administration of NAC, 2-MPG, and 5-HD were heterogenous, and these pharmacokinetic factors may have influenced the results. Plasma concentrations of NAC, 2-MPG, and 5-HD also were not determined nor were dose-response relationships to these drugs performed. Myocardial infarct size is determined primarily by the size of the area at risk and the extent of coronary collateral perfusion. The area at risk expressed as a percentage of total left ventricular mass was similar between groups in the current investigation, and coronary collateral blood flow has been shown to be minimal in rabbits.[29] Thus, differences in collateral perfusion between groups probably did not account for the observed results, but coronary collateral blood flow was not specifically quantified. The reductions in myocardial necrosis produced by helium in the absence or presence of other drug interventions occurred independent of changes in major determinants of myocardial oxygen consumption. Nevertheless, coronary venous oxygen tension was not directly measured nor was myocardial oxygen consumption calculated. Notably, no significant differences in hemodynamics were observed between groups before and during coronary artery occlusion that may account for differences in infarct size observed between groups. Finally, the current results implicating a role for ROS and mitochondrial K_{ATP} channels in helium-induced cardioprotection were obtained in barbiturate-anesthetized, acutely instrumented rabbits. Whether similar results occur in other animal species or humans is unknown. Helium preconditioning has yet to be established in humans, but the administration of this noble gas before a defined period of myocardial ischemia may be beneficial in a clinical setting in which an anesthetic is not required (eg, inflation of an angioplasty balloon during cardiac catheterization). However, additional investigation will be required to test this intriguing hypothesis.

In summary, the current results confirm that brief, intermittent administration of helium before prolonged coronary artery occlusion and reperfusion protects myocardium against infarction. The findings further suggest that ROS and mitochondrial K_{ATP} channels mediate this helium-induced preconditioning in vivo.

REFERENCES

1. Zweier JL, Flaherty JT, Weisfeldt ML: Direct measurement of free radical generation following reperfusion of ischemic myocardium. Proc Natl Acad Sci U S A 84:1404-1407, 1987

2. Ambrosio G, Zweier JL, Duilio C, et al: Evidence that mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact rabbit hearts subjected to ischemia and reflow. J Biol Chem 268:18532-18541, 1993

3. Baines CP, Goto M, Downey JM: Oxygen radicals released during ischemic preconditioning contribute to cardioprotection in the rabbit myocardium. J Mol Cell Cardiol 29:207-216, 1997

4. Vanden Hoek TL, Becker LB, Shao Z, et al: Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. J Biol Chem 273:18092-18098, 1998

5. Tanaka M, Fujiwara H, Yamasaki K, et al: Superoxide dismutase and N-2mercaptopropionyl glycine attenuate infarct size limitation effect of ischaemic preconditioning in the rabbit. Cardiovasc Res 28:980-986, 1994

6. Mullenheim J, Ebel D, Frassdorf J, et al: Isoflurane preconditions myocardium against infarction via release of free radicals. Anesthesiology 96:934-940, 2002

7. Tanaka K, Weihrauch D, Kehl F, et al: Mechanism of preconditioning by isoflurane in rabbits: A direct role for reactive oxygen species. Anesthesiology 97:1485-1490, 2002

8. Ludwig LM, Tanaka K, Eells JT, et al: Isoflurane-induced preconditioning is mediated by reactive oxygen species generated by mitochondrial electron transport chain complex III. Anesth Analg 99: 1308-1315, 2004

9. Kohro S, Hogan QH, Nakae Y, et al: Anesthetic effects on mitochondrial ATP-sensitive K channel. Anesthesiology 95:1435-1440, 2001

10. Tanaka K, Weihrauch D, Ludwig LM, et al: Mitochondrial adenosine triphosphate-regulated potassium channel opening acts as a trigger for isoflurane-induced preconditioning by generating reactive oxygen species. Anesthesiology 98:935-943, 2003

11. Pagel PS, Krolikowski JG, Venkatapuram S, et al: Noble gases without anesthetic properties protect myocardium against infarction by activating prosurvival signaling kinases and inhibiting mitochondrial permeability transition in vivo. Anesth Analg 105:562-569, 2007

12. Pagel PS, Krolikowski JG, Weihrauch D, et al: Inhibition of glycogen synthase kinase or the apoptotic protein p53 lowers the threshold of helium cardioprotection in vivo: Role of mitochondrial permeability transition. Anesth Analg (in press)

13. Pagel PS, Krolikowski JG, Pratt PF Jr, et al: Mechanism of helium-induced preconditioning: A direct role for nitric oxide in rabbits. Anesth Analg (in press)

14. Weber NC, Toma O, Damla H, et al: Upstream signaling of PKC-e in xenon-induced pharmacological preconditioning. Implication of mitochondrial K_{ATP} channels and PDK-1. Eur J Pharmacol 539:1-9, 2006

15. Warltier DC, Zyvoloski MG, Gross GJ, et al: Determination of experimental myocardial infarct size. J Pharmacol Methods 6:199-210, 1981

16. Wallenstein S, Zucker CL, Fleiss JL: Some statistical methods useful in circulation research. Circ Res 47:1-9, 1980

17. Koblin DD, Fang Z, Eger EI II, et al: Minimum alveolar concentrations of noble gases, nitrogen, and sulfur hexafluoride in rats: Helium and neon as nonimmobilizers (nonanesthetics). Anesth Analg 87:419-424, 1998

18. Tritto I, D'Andrea D, Eramo N, et al: Oxygen radicals can induce preconditioning in rabbit hearts. Circ Res 80:743-748, 1997

19. Pain T, Yang XM, Critz SD, et al: Opening of mitochondrial K_{ATP} channels triggers the preconditioned state by generating free radicals. Circ Res 87:460-466, 2000

20. Carroll R, Grant VA, Yellon DM: Mitochondrial K(ATP) channel opening protects a human atrial-derived cell line by a mechanism involving free radical generation. Cardiovasc Res 51:691-700, 2001

21. Forbes RA, Steenbergen C, Murphy E: Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. Circ Res 88:802-809, 2001

22. Obata T, Yamanaka Y: Block of cardiac ATP-sensitive K(+) channels reduces hydroxyl radicals in the rat myocardium. Arch Biochem Biophys 378:195-200, 2000

23. Novalija E, Varadarajan SG, Camara AK, et al: Anesthetic preconditioning: Triggering role of reactive oxygen and nitrogen species in isolated hearts. Am J Physiol Heart Circ Physiol 283:H44-H52, 2002

24. Kevin LG, Novalija E, Riess ML, et al: Sevoflurane exposure generates superoxide but leads to decreased superoxide during ischemia and reperfusion in isolated hearts. Anesth Analg 96:949-955, 2003

25. Aruoma OI, Halliwell B, Hoey BM, et al: The antioxidant action of N-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med 6:593-597, 1989

26. Beyersdorf F, Fuchs J, Eberhardt B, et al: Myocardial protection by 2mercaptopropionylglycine during global ischemia in dogs. Arzneimittelforschung 39:46-49, 1989

27. Beyersdorf F, Zimmer G, Fuchs J, et al: Improvement of myocardial function after global hypoxia by protection of the inner mitochondrial membrane. Arzneimittelforschung 37:142-149, 1987

28. Fuchs J, Mainka L, Zimmer G: 2-mercaptopropionylglycine and related compounds in treatment of mitochondrial dysfunction and postischemic myocardial damage. Arzneimittelforschung 35:1394-1402, 1985

29. Maxwell MP, Hearse DJ, Yellon DM: Species variation in the coronary collateral circulation during regional myocardial ischaemia: A critical determinant of the rate of evolution and extent of myocardial infarction. Cardiovasc Res 21:737-746, 1987