Noble Gases Without Anesthetic Properties Protect Myocardium Against Infarction by Activating Prosurvival Signaling Kinases and Inhibiting Mitochondrial Permeability Transition *In Vivo*

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BACKGROUND: The anesthetic noble gas, xenon, produces cardioprotection. We hypothesized that other noble gases without anesthetic properties [helium (He), neon (Ne), argon (Ar)] also produce cardioprotection, and further hypothesized that this beneficial effect is mediated by activation of prosurvival signaling kinases [including phosphatidylinositol-3-kinase, extracellular signal-regulated kinase, and 70-kDa ribosomal protein s6 kinase] and inhibition of mitochondrial permeability transition pore (mPTP) opening *in vivo*.

METHODS: Rabbits ($n = 98$) instrumented for hemodynamic measurement and subjected to a 30-min left anterior descending coronary artery (LAD) occlusion and 3 h reperfusion received 0.9% saline (control), three cycles of 70% He-, Ne-, or Ar-30% O_2 administered for 5 min interspersed with 5 min of 70% N_2–30% O_2 before LAD occlusion, or three cycles of brief (5 min) ischemia interspersed with 5 min reperfusion before prolonged LAD occlusion and reperfusion (ischemic preconditioning). Additional groups of rabbits received selective inhibitors of phosphatidylinositol-3-kinase (wortmannin; 0.6 mg/kg), extracellular signal-regulated kinase (PD 098059; 2 mg/kg), or 70-kDa ribosomal protein s6 kinase (rapamycin; 0.25 mg/kg) or mPTP opener atractyloside (5 mg/kg) in the absence or presence of He pretreatment.

RESULTS: He, Ne, Ar, and ischemic preconditioning significantly ($P < 0.05$) reduced myocardial infarct size [23% ± 4%, 20% ± 3%, 22% ± 2%, 17% ± 3% of the left ventricular area at risk (mean ± SD); triphenyltetrazolium chloride staining] versus control (45% ± 5%). Wortmannin, PD 098059, rapamycin, and atractyloside alone did not affect infarct size, but these drugs abolished He-induced cardioprotection.

CONCLUSIONS: The results indicate that noble gases without anesthetic properties produce cardioprotection by activating prosurvival signaling kinases and inhibiting mPTP opening in rabbits.

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There is evidence suggesting that brief exposure to the anesthetic noble gas xenon before prolonged coronary artery occlusion and reperfusion protects myocardium against infarction (1,2). The mechanisms by which xenon produces these beneficial effects are incompletely characterized (2–5), but share many similarities with those described for ischemic and volatile anesthetic-induced preconditioning (6). Xenon and volatile anesthetics appear to exert similar actions on cardiac and neural signal transduction, and as a result, the reductions in infarct size observed with administration of xenon were initially assumed to be related, at least in part, to its anesthetic action (7). Because xenon is chemically inert, we tested the hypothesis that other noble gases that do not produce anesthesia [e.g., helium, neon, argon (8,9)] may also cause cardioprotection. We further hypothesized that this noble gas-induced cardioprotection is mediated

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by activation of the "reperfusion injury salvage kinase" (RISK) pathway (10). This prosurvival signaling cascade, which includes the key regulatory proteins phosphatidylinositol-3-kinase (PI3K)-Akt, extracellular signal-regulated kinases [Erk1/2; also known as p44/p42 mitogen activated protein kinase (MAPK)], and 70-kDa ribosomal protein s6 kinase (p70s6K), has been implicated in protection against myocardial necrosis and apoptosis (programmed cell death) during ischemia-reperfusion injury produced by ischemic, opioid, and anesthetic-induced pre- and postconditioning (7,10–14).

Many of the signaling molecules of the RISK pathway prevent cellular damage by converging on, and inhibiting opening of, the mitochondrial permeability transition pore (mPTP) (15–18). The mPTP is contained within the inner mitochondrial membrane. It remains closed under normal physiologic conditions and during ischemia, but selectively opens upon reperfusion in response to intracellular calcium overload and large quantities of reactive oxygen species (19,20). Opening of mPTP eliminates the mitochondrial membrane potential ($\delta\psi_m$), inhibits oxidative phosphorylation, and stimulates the release or formation of proapoptotic proteins that accelerate cell death (21). PI3K, Erk1/2, and p70s6K inhibit mPTP opening by their actions on several downstream signaling molecules that directly modulate the transition state of the pore (e.g., endothelial nitric oxide synthase, p53, glycogen synthase kinase 3β) or indirectly influence pore permeability by affecting the relative balance of pro- and antiapoptotic B-cell lymphoma protein derivatives (e.g., Bcl-2, Bax, Bad) (10,22–24). A central role for mPTP has been implicated in pre- and postconditioning by volatile anesthetics (25,26), but whether cardioprotection produced by xenon or other nonanesthetic noble gases is mediated by mitochondrial permeability transition has yet to be defined. Thus, we also tested the hypothesis that mPTP mediates cardioprotection by helium *in vivo*.

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*.

Experimental Preparation

Male New Zealand white rabbits weighing between 2.5 and 3.0 kg were anesthetized with IV sodium pentobarbital (30 mg/kg) as previously described (27). Additional doses of pentobarbital were titrated as required to assure 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 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. Body temperature was maintained with a heating blanket. A pulse oximeter (Model NPB-40, Nellcor Puritan Bennett, Pleasanton, CA) was placed on the right hind paw of each rabbit for measurement of continuous arterial oxygen saturation. Heparin-filled catheters were inserted into the right carotid artery and the left jugular vein for measurement of arterial blood pressure and fluid or drug administration, respectively. Maintenance fluids consisted of 0.9% saline ($15\ mL \cdot kg^{-1} \cdot min^{-1}$) and were continued for the duration of the 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. IV 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 was continuously recorded on a polygraph throughout each experiment.

Experimental Protocol

The experimental design is illustrated in Figure 1. Baseline hemodynamics and arterial blood gas tensions were recorded 30 min after instrumentation was completed. All rabbits underwent a 30-min LAD occlusion, followed by 3 h of reperfusion. In separate experimental groups, rabbits (n = 7–8 per group) were randomly assigned to receive 0.9% saline (control), three cycles of 70% helium–30% oxygen administered for 5 min interspersed with 5 min of 70% nitrogen–30% oxygen before coronary artery occlusion, or the PI3K antagonist wortmannin (0.6 mg/kg), the MEK-1 inhibitor PD 098059 [2 mg/kg; Erk1/2 is activated by phosphorylation via the upstream kinase MEK-1 (28)], the p70s6K inhibitor rapamycin (0.25 mg/kg), or the mPTP opener atractyloside (5 mg/kg) in the absence or presence of helium pretreatment. Wortmannin, PD 098059, and rapamycin were dissolved in dimethylsulfoxide and administered over 3 min as an IV infusion 30 min before LAD occlusion. We have previously demonstrated that dimethylsulfoxide does not affect myocardial infarct size in an identical rabbit model (12). Atractyloside was dissolved in 2 mL of distilled water and administered over 2 min as an IV infusion 30 min before LAD occlusion. Two additional groups of rabbits were

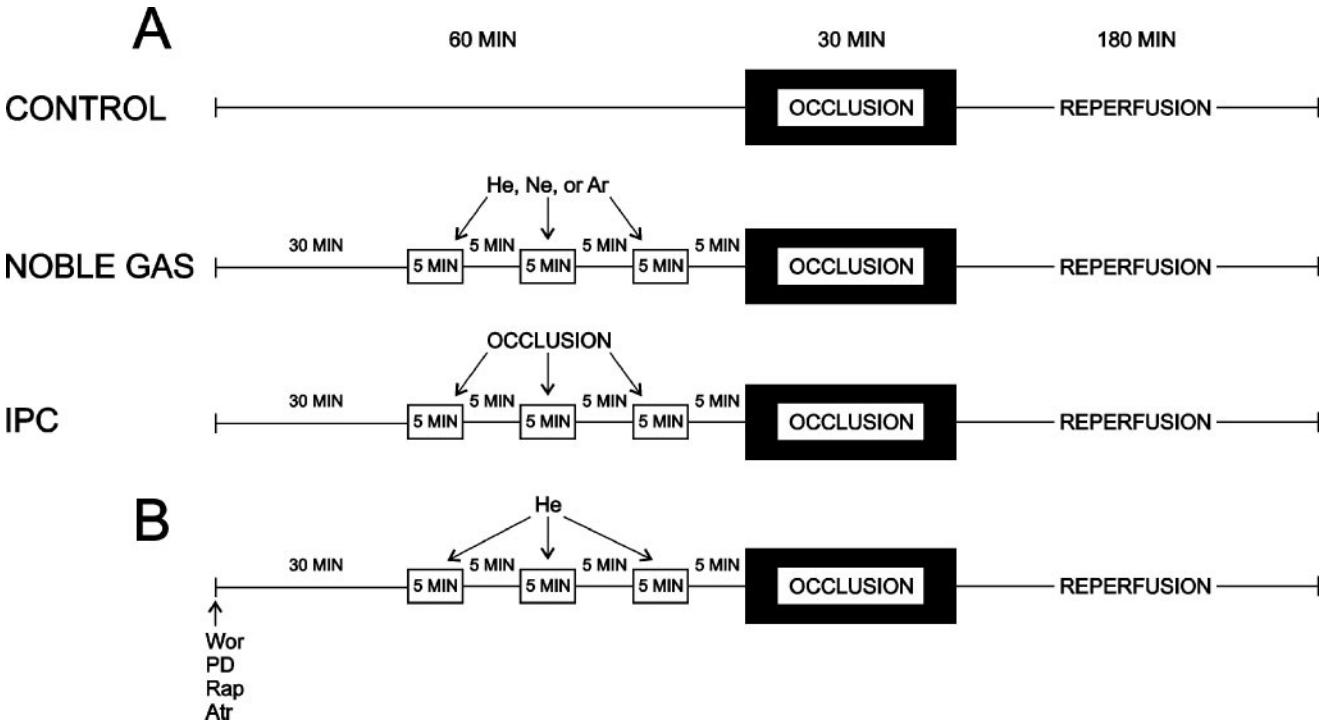

Figure 1. Schematic illustration of the experimental protocol used in the investigation. He = helium; Ne = neon; Ar = Argon; IPC = ischemic preconditioning; Wor = wortmannin; PD = PD 098059; Rap = rapamycin; Atr = atractyloside.

randomly assigned to receive three cycles of 70% neon- or argon-30% oxygen administered for 5 min interspersed with 5 min of the air-oxygen mixture before LAD occlusion. A final group of rabbits was preconditioned with three cycles of brief (5 min) ischemia produced by LAD occlusion interspersed with 5 min reperfusion before prolonged LAD occlusion as a positive control.

Measurement of Myocardial Infarct Size

Myocardial infarct size was measured as previously described (29). Briefly, the LAD was reoccluded at the completion of each experiment, and 3 mL of patent blue dye was injected IV. The left ventricular area at risk for infarction was separated from surrounding normal areas (stained blue), and the two regions were incubated at 37°C for 20 min in 1% 2,3,5-triphenyltetrazolium chloride in 0.1 M 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 <15% of total left ventricular mass were excluded from subsequent analysis.

Statistical Analysis

Statistical analysis of data within and between groups was performed with analysis of variance for repeated measures, followed by Bonferroni's modification of Student's *t*-test. Changes were considered statistically significant when $P < 0.05$. All data are expressed as mean ± SD.

RESULTS

One-hundred-three rabbits were instrumented to obtain 98 successful infarct size experiments. One rabbit (control) was excluded because the left ventricular area at risk was <15% of the total left ventricular mass. Four rabbits were excluded because intractable ventricular fibrillation occurred during coronary artery occlusion (1 wortmannin alone; 1 PD 098059 alone; 1 helium plus rapamycin; 1 atractyloside alone). Arterial blood gas tensions were maintained within the physiologic range during administration of helium, neon, and argon in all groups (data not shown). Arterial oxygen saturation remained at 100% during and after administration of helium, neon, and argon with or without other drug interventions (data not shown). Baseline systemic hemodynamics were similar between groups (Table 1). Helium, neon, and argon did not affect hemodynamics. In contrast, brief coronary artery occlusion significantly ($P < 0.05$) reduced mean arterial blood pressure and rate-pressure product. Declines in heart rate, mean arterial blood pressure, and rate-pressure product occurred during reperfusion in most experimental groups. Heart rate was decreased in rabbits pretreated with rapamycin alone when compared with control 1 h after reperfusion. Mean arterial blood pressure was greater during LAD occlusion in wortmannin-pretreated rabbits in the absence of helium when compared with control. Mean arterial blood pressure was reduced in rabbits receiving PD 098059 alone versus control 2 h after reperfusion. Rate-pressure product was decreased in rabbits pretreated with PD 098059 or rapamycin alone

Table 1. Hemodynamics

				Reperfusion		
	Baseline	Intervention	Occlusion	1 h	2 h	3 h
HR (min^{-1})						
CON	267 ± 36	265 ± 34	249 ± 31	240 ± 22	231 ± 25*	218 ± 25*
He	245 ± 31	234 ± 29	219 ± 15	207 ± 21*	199 ± 19*	191 ± 23*
Ne	240 ± 32	236 ± 32	223 ± 21	222 ± 21	215 ± 24*	204 ± 27*
Ar	259 ± 26	246 ± 15	234 ± 25	224 ± 16*	218 ± 15*	209 ± 12*
IPC	254 ± 26	231 ± 18	237 ± 21	217 ± 21*	206 ± 27*	199 ± 24*
WOR (0.6 mg/kg)	276 ± 28	253 ± 39	262 ± 36	234 ± 25	219 ± 16*	209 ± 23*
WOR (0.6 mg/kg) + He	253 ± 31	231 ± 23	239 ± 32	219 ± 26*	213 ± 27*	203 ± 25*
PD (2 mg/kg)	242 ± 32	233 ± 32	216 ± 26	202 ± 18*	194 ± 14*	191 ± 18*
PD (2 mg/kg) + He	238 ± 18	228 ± 18	231 ± 17	211 ± 12*	209 ± 14*	203 ± 8*
RAP (0.25 mg/kg)	242 ± 21	238 ± 25	209 ± 24	197 ± 30*†	203 ± 19*	195 ± 16*
RAP (0.25 mg/kg) + He	233 ± 15	227 ± 14	216 ± 21	205 ± 18	200 ± 18*	193 ± 16*
ATR (5 mg/kg)	247 ± 35	233 ± 21	220 ± 22	210 ± 30	198 ± 23*	193 ± 21*
ATR (5 mg/kg) + He	251 ± 21	254 ± 17	227 ± 20*	225 ± 18*	213 ± 18*	211 ± 17*
MAP (mm Hg)						
CON	80 ± 6	75 ± 8	64 ± 4*	65 ± 7*	69 ± 9	65 ± 8*
He	74 ± 10	83 ± 14	63 ± 14*	63 ± 11*	64 ± 9*	65 ± 18*
Ne	80 ± 8	81 ± 8	66 ± 6*	66 ± 7*	68 ± 9*	64 ± 9*
Ar	79 ± 5	77 ± 7	62 ± 7*	64 ± 3*	63 ± 5*	62 ± 5*
IPC	81 ± 14	67 ± 9*	68 ± 10*	65 ± 15	68 ± 12	71 ± 15
WOR (0.6 mg/kg)	85 ± 15	94 ± 14	87 ± 14†	66 ± 8*	60 ± 14*	53 ± 12*
WOR (0.6 mg/kg) + He	74 ± 11	88 ± 10	71 ± 12	65 ± 14	63 ± 15	61 ± 12
PD (2 mg/kg)	70 ± 16	62 ± 14	54 ± 17	53 ± 18	45 ± 14*†	48 ± 16*
PD (2 mg/kg) + He	76 ± 13	80 ± 10	63 ± 6*	61 ± 7	59 ± 9*	60 ± 7*
RAP (0.25 mg/kg)	72 ± 12	74 ± 8	52 ± 12*	51 ± 16*	55 ± 10	60 ± 10
RAP (0.25 mg/kg) + He	77 ± 16	85 ± 14	65 ± 14*	62 ± 12*	61 ± 10*	66 ± 12*
ATR (5 mg/kg)	73 ± 14	73 ± 11	63 ± 11	55 ± 10*	51 ± 11*	61 ± 9*
ATR (5 mg/kg) + He	76 ± 5	77 ± 18	58 ± 7*	57 ± 7*	57 ± 9*	59 ± 9*
RPP ($min^{-1} \cdot$ mm Hg 10^{-3})						
CON	24.5 ± 4.4	23.5 ± 4.0	19.2 ± 3.0*	19.3 ± 3.0*	18.7 ± 2.3*	16.3 ± 1.6*
He	20.7 ± 4.4	22.2 ± 5.9	16.1 ± 3.5*	15.2 ± 3.1*	14.9 ± 1.9*	14.3 ± 4.5*
Ne	21.7 ± 4.8	21.6 ± 3.7	16.9 ± 2.3*	16.9 ± 2.2*	16.6 ± 1.7*	15.0 ± 2.5*
Ar	22.9 ± 2.8	21.3 ± 0.7	16.6 ± 2.2*	16.7 ± 1.6*	15.8 ± 1.1*	15.0 ± 1.3*
IPC	23.0 ± 4.1	17.7 ± 3.3*	18.2 ± 3.5*	16.6 ± 4.6*	16.2 ± 4.1*	16.2 ± 4.6*
WOR (0.6 mg/kg)	26.4 ± 3.8	26.3 ± 5.0	25.5 ± 5.0	18.1 ± 2.5*	15.2 ± 3.1*	13.3 ± 3.4*
WOR (0.6 mg/kg) + He	21.3 ± 4.4	22.6 ± 4.1	19.5 ± 4.8	16.6 ± 4.6	15.6 ± 4.1	14.3 ± 2.6
PD (2 mg/kg)	20.0 ± 5.5	17.5 ± 5.0	14.2 ± 4.9*	12.9 ± 4.5*†	10.8 ± 2.6*†	11.2 ± 2.8*
PD (2 mg/kg) + He	20.4 ± 3.6	20.4 ± 3.4	16.6 ± 2.1*	15.0 ± 1.8*	14.3 ± 2.7*	14.2 ± 1.3*
RAP (0.25 mg/kg)	19.7 ± 3.7	20.4 ± 3.6	13.3 ± 3.7*	12.3 ± 4.7*†	13.4 ± 2.5*†	13.5 ± 2.4*
RAP (0.25 mg/kg) + He	20.3 ± 3.8	20.6 ± 3.4	16.0 ± 4.3*	14.8 ± 3.7*	14.1 ± 3.1*	14.6 ± 3.5*
ATR (5 mg/kg)	20.8 ± 6.0	19.3 ± 3.8	16.0 ± 3.4*	13.7 ± 2.8*	12.3 ± 2.4*	13.6 ± 2.4*
ATR (5 mg/kg) + He	21.5 ± 2.7	22.1 ± 3.0	15.7 ± 2.6*	15.3 ± 1.5*	14.5 ± 1.5*	14.5 ± 1.3*

Data are mean ± SD.

HR = heart rate; MAP = mean arterial blood pressure; RPP = rate pressure product; CON = control; He = helium; Ne = neon; Ar = Argon; IPC = ischemic preconditioning; WOR = wortmannin; PD = PD 098059; RAP = rapamycin; ATR = atractyloside.

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

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

when compared with control 1 and 2 h after reperfusion. No other significant differences in hemodynamics were observed among experimental groups.

Body weight, left ventricular mass, area at risk weight, and the ratio of area at risk to left ventricular mass were similar among groups (Table 2). Brief, intermittent exposure to 70% helium, neon, or argon before LAD occlusion reduced myocardial infarct size (23% ± 4%, 20% ± 3%, and 22% ± 2%, respectively, of the left ventricular area at risk) when compared with control (45% ± 5%; Fig. 2). Ischemic preconditioning also produced a protective effect (17% ± 3%). Administration of wortmannin, PD 098059, rapamycin, or atractyloside alone did not affect infarct size (38% ± 6%, 45% ± 2%, 47% ± 2%, and 46% ± 2%, respectively), but these drugs abolished helium-induced cardioprotection (43% ± 9%, 46% ± 3%, 46% ± 3%, and 46% ± 5%, respectively).

DISCUSSION

The results of this investigation demonstrate for the first time that brief, repetitive exposure to helium, neon, or argon protects myocardium against irreversible ischemia injury. Helium and neon have been shown to produce convulsions, but do not cause

Table 2. Left Ventricular Area at Risk

	N	Body weight (g)	LV (g)	AAR (g)	AAR/LV (%)
CON	7	2628 ± 238	3.21 ± 0.29	1.11 ± 0.28	34 ± 8
He	8	2574 ± 141	3.82 ± 0.26	1.28 ± 0.08	34 ± 2
Ne	8	2614 ± 117	3.47 ± 0.27	1.29 ± 0.09	37 ± 2
Ar	8	2348 ± 146	3.42 ± 0.41	1.16 ± 0.15	34 ± 4
IPC	8	2637 ± 189	3.19 ± 0.62	1.02 ± 0.29	32 ± 8
WOR (0.6 mg/kg)	7	2478 ± 136	3.59 ± 0.44	1.28 ± 0.30	36 ± 10
WOR (0.6 mg/kg) + He	8	2513 ± 181	3.96 ± 0.29*	1.41 ± 0.25	36 ± 5
PD (2 mg/kg)	7	2700 ± 277	3.69 ± 0.33	1.44 ± 0.22	39 ± 4
PD (2 mg/kg) + He	8	2508 ± 86	3.87 ± 0.37	1.47 ± 0.25	38 ± 6
RAP (0.25 mg/kg)	7	2786 ± 227	3.80 ± 0.43	1.38 ± 0.34	37 ± 3
RAP (0.25 mg/kg) + He	7	2390 ± 128	3.26 ± 0.23	1.26 ± 0.14	36 ± 7
ATR (5 mg/kg)	7	2643 ± 151	3.81 ± 0.25	1.50 ± 0.30	40 ± 8
ATR (5 mg/kg) + He	8	2393 ± 160	3.65 ± 0.33	1.44 ± 0.29	39 ± 6

Data are mean ± SD.

LV = left ventricle; AAR = area at risk; CON = control; He = helium; Ne = neon; Ar = Argon; IPC = ischemic preconditioning; WOR = wortmannin; PD = PD 098059; RAP = rapamycin; ATR = atractyloside.

* Significantly ($P < 0.05$) different from corresponding CON value.

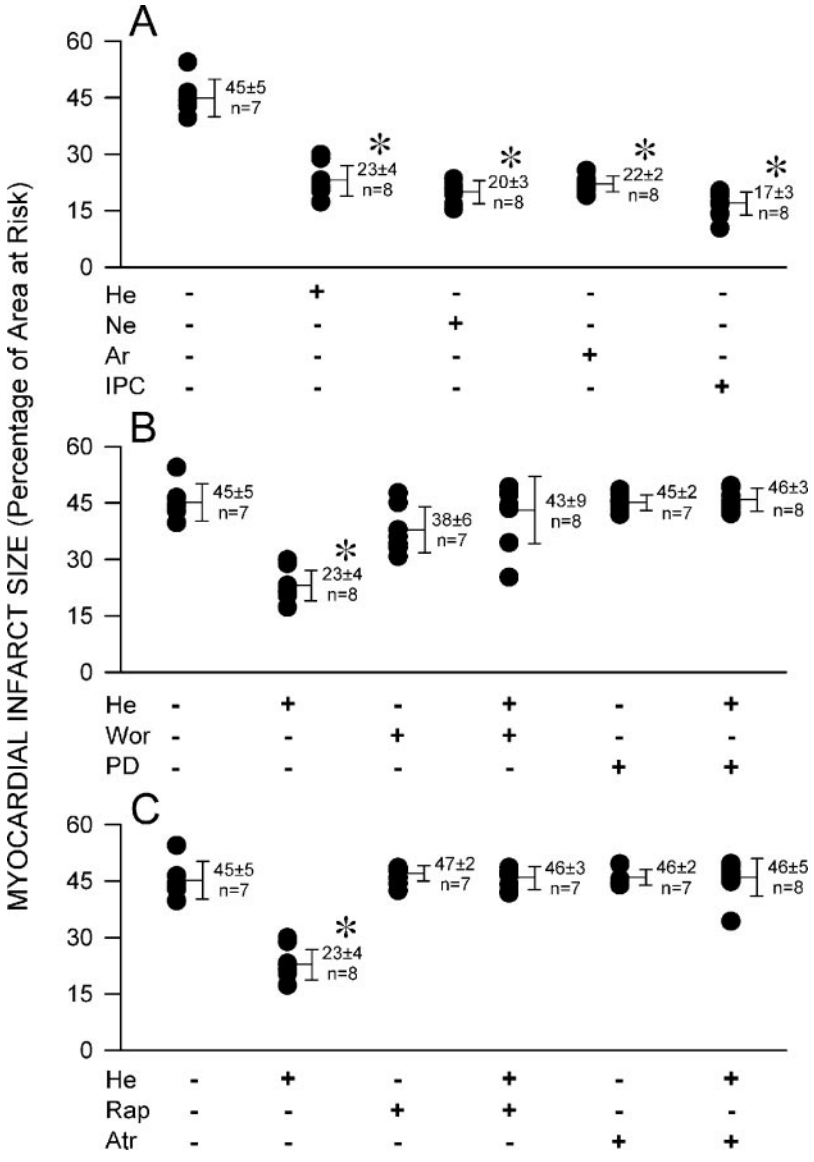

Figure 2. Myocardial infarct size expressed as a percentage of the left ventricular area at risk for infarction in rabbits in the absence (−) or presence (+) of three 5-min preconditioning cycles of 70% helium (He), neon (Ne), and argon (Ar) or three 5-min periods of ischemia (IPC; ischemic preconditioning) before prolonged coronary artery occlusion and reperfusion (panel A, top). Infarct sizes in the absence (−) or presence (+) of 70% helium preconditioning in rabbits receiving the selective PI3K antagonist wortmannin (Wor) or the selective MEK-1 antagonist PD 098059 (PD) are depicted in panel B (middle). Infarct sizes in the absence (−) or presence (+) of 70% helium preconditioning in rabbits receiving the selective p70s6K antagonist rapamycin (Rap) or the mitochondrial permeability transition pore (mPTP) opener atractyloside (Atr) are depicted in panel C (bottom). Each point represents a single experiment. All data are mean ± SD. *Significantly ($P < 0.05$) different from rabbits receiving 0.9% saline alone in the absence of noble gases or other drug interventions.

anesthesia in rats during extreme hyperbaric conditions (84.6 ± 22.2 atm and 91.3 ± 7.0 atm, respectively) (8). In contrast to helium and neon, argon caused an anesthetic effect at 27.0 ± 2.6 atm, but the concentration of argon (70%) used in the current investigation is equivalent to a minimum alveolar concentration value of approximately 0.026 (8). Thus, the current data indicate that helium, neon, and argon produce cardioprotection independent of an anesthetic effect. The magnitude of cardioprotection produced by the nonanesthetic noble gases was similar to that previously reported for 1.0 minimum alveolar anesthetic concentration isoflurane in pre- and postconditioning experiments in an identical rabbit model of prolonged coronary artery occlusion and reperfusion (12,26,27). Decreases in infarct size caused by three 5-min cycles of 70% helium, neon, and argon were also modestly less than those observed with ischemic preconditioning resulting from three 5-min cycles of ischemia and reperfusion, although dose-response relationships to helium, neon, and argon were not performed. These data with nonanesthetic noble gases are consistent with previous findings demonstrating that volatile anesthetic-induced preconditioning does not provide a similar degree of cardioprotection when compared with ischemic preconditioning (6).

Pretreatment with wortmannin, PD 098059, and rapamycin abolished helium-induced reductions in myocardial necrosis, implicating roles for PI3K, Erk1/2, and p70s6K, respectively, in cardioprotection by the gas. These results with nonanesthetic noble gases support previous findings demonstrating the cardioprotective effects of xenon (7). Pretreatment with 70% xenon before ischemia reduced infarct size in rats (from 51% to 28% of the left ventricular area at risk) by activating the ϵ isoform of protein kinase C and its downstream targets p38 MAPK (2), MAPK-activated protein kinase-2 (5), and heat shock protein 27 (5). More recently, roles for mitochondrial adenosine triphosphate-regulated potassium (K_{ATP}) channels, phosphotidylinositol-dependent kinase-1 (a protein that is immediately upstream to PI3K in the signaling pathway), and Erk1/2 were also implicated in xenon preconditioning (3,30), suggesting that prosurvival signaling may play an important role in xenon-induced cardioprotection. The current results confirm and extend these findings by demonstrating that helium-induced reductions in infarct size are also mediated by several of these prosurvival signaling kinases. Xenon reduced infarct size when the gas was administered solely during early reperfusion in rabbits, suggesting that the anesthetic gas was capable of producing postconditioning as well (1). Whether other nonanesthetic gases are capable of mimicking this xenon-induced postconditioning phenomenon is unknown. However, based on the results implicating RISK cascade proteins in preconditioning by helium, such a contention appears highly plausible, and is being investigated by our laboratory.

The current results also demonstrate for the first time that preservation of myocardial integrity by helium is abolished by atractyloside, a selective opener of mPTP, indicating that helium-induced cardioprotection is mediated by inhibition of mPTP opening *in vivo*. An important interaction between mPTP and mitochondrial K_{ATP} channels through adenine nucleotide translocase (31,32) has been suggested in pharmacologic preconditioning [e.g., diazoxide (15), desflurane (25)] and isoflurane-induced postconditioning (26). The mitochondrial K_{ATP} channel-dependence of xenon preconditioning was recently reported (3), and the reductions in myocardial necrosis produced by brief, intermittent administration of helium that occur *via* inhibition of mPTP opening in the current investigation are consistent with these previous findings, based on the suspected interaction between the mitochondrial K_{ATP} channel and mPTP. The mechanisms by which helium preconditioning inhibits mPTP opening during early reperfusion remain to be fully elucidated. However, PI3K and Erk1/2 signaling pathways activated by helium regulate mitochondrial permeability transition by inhibiting caspase formation and glycogen synthase kinase-3β activity, favorably affecting pro- versus antiapoptotic protein balance, and producing nitric oxide through activation of endothelial nitric oxide synthase within the cardiac myocyte (10,23,33).

The precise mechanisms by which chemically inert noble gases activate endogenous signal transduction pathways to exert myocardial protection are unknown. It has been proposed that intraatomic dipole formation within the outer electron shells ($4d^{10}5s^{2}5p^{6}$) of the relatively large xenon atom may account for its interaction with biologically active molecules (34). Such a hypothesis appears to be highly unlikely when helium is considered, because this atom contains only two electrons that are tightly bound within a stable $1s^{2}$ orbital configuration (35). It is equally unclear how noble gases are capable of selectively activating some signaling kinases while leaving others unaffected (30). Nevertheless, the current and previous data with helium and xenon (7), respectively, provide compelling evidence that brief, intermittent administration of noble gases are capable of exerting important beneficial effects against ischemia-reperfusion injury by activating several signaling kinases known to mediate other forms of pharmacologic and ischemic pre- and postconditioning (10).

The current results must be interpreted within the constraints of several potential limitations. Three cycles of brief administration of 70% helium-, neon-, or argon-30% O_2 interspersed with 70% nitrogen–30% oxygen before prolonged coronary artery occlusion and reperfusion were used as preconditioning stimuli in the current investigation. Dose- or time-response relationships to noble gases were not performed, nor were studies conducted to determine whether more prolonged time periods between noble gas discontinuation and the onset of ischemia also provide cardioprotection (i.e., duration of "memory" period). These investigations are currently

being conducted in our laboratory. A 30-min coronary artery occlusion was used so as to produce myocardial infarction. Whether brief exposure to noble gases also produces cardioprotection after more prolonged periods of coronary artery occlusion is unknown. Activation of PI3K, Erk1/2, and p70s6K produced by helium mediated reductions in myocardial necrosis in the current investigation. These signaling pathways and their proposed putative mPTP end-effector have also been strongly implicated in decreases in apoptotic cell death during reperfusion. We did not examine the role of apoptosis in helium-induced cardioprotection, and further investigation will be required to ascertain whether reductions in apoptosis also mediate salvage of myocardium by noble gases.

Wortmannin, PD 098059, and rapamycin have been shown to be selective inhibitors of PI3K, Erk1/2, and p70s6K, respectively, at the doses used in the current investigation. Nevertheless, dose-response relationships to these selective inhibitors were not performed, and the possibility that these drugs may have inhibited other protein kinases involved in myocardial protection cannot be completely excluded from the analysis. The results also require qualification because the actions of helium with or without atractyloside pretreatment on mPTP channel activity in isolated mitochondrial were not examined. Nevertheless, the current pharmacologic data strongly suggest an important role for mPTP inhibition in helium-induced preconditioning. Myocardial infarct size is determined primarily by the size of the left ventricular area at risk and the extent of coronary collateral perfusion. The ratio of the area at risk to left ventricular mass was similar among groups, and rabbits have minimal coronary collateral blood flow (36). Thus, it is unlikely that differences in collateral perfusion among groups are responsible for the observed results, but coronary collateral blood flow was not specifically quantified. Helium, neon, and argon did not cause systemic hemodynamic effects nor did administration of helium affect heart rate, mean arterial blood pressure, or rate-pressure product in wortmannin-, PD 098059-, or rapamycin-pretreated rabbits. Thus, the beneficial actions of the nonanesthetic noble gases occurred independent of hemodynamic effects. Nevertheless, the results should be qualified because coronary venous oxygen tension was not measured, nor was myocardial oxygen consumption calculated. The results also require qualification because we did not specifically examine the biochemical actions of helium on PI3K-Akt, Erk1/2, and p70s6K phosphorylation, nor did we measure the activity of these kinases in rabbit myocardium before or after ischemia and reperfusion. Western blotting was also not performed to confirm the selective blockade of PI3K-Akt, Erk1/2, and p70s6K using wortmannin, PD 098059, and rapamycin, respectively, and this also represents a limitation. Finally, the current results implicating a role for PI3K, Erk1/2, p70s6K, and mPTP in helium-induced cardioprotection were obtained in barbiturate-anesthetized rabbits. Whether similar results occur in other animal species or humans is unknown.

In summary, the current results demonstrate that brief, intermittent administration of helium, neon, or argon before prolonged coronary artery occlusion and reperfusion protects myocardium against infarction in barbiturate-anesthetized, acutely instrumented rabbits. The findings indicate that selective inhibition of PI3K, Erk1/2, and p70s6K blocks cardioprotection by helium. The results further demonstrate that mPTP opening abolishes helium-induced preconditioning against infarction *in vivo*. The ability to briefly administer a noble gas without anesthetic properties as a therapeutic agent before a predictable episode of ischemia (e.g., percutaneous coronary intervention, cardiopulmonary bypass) may have important clinical ramifications, but further research will be required to test this intriguing hypothesis.

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