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Isoflurane Protects against Myocardial Infarction during Early Reperfusion by Activation of Phosphatidylinositol-3-Kinase Signal Transduction: Evidence for Anesthetic-induced Postconditioning in Rabbits

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Background: **Brief episodes of ischemia during early reperfusion after coronary occlusion reduce the extent of myocardial infarction. Phosphatidylinositol-3-kinase (PI3K) signaling has been implicated in this "postconditioning" phenomenon. The authors tested the hypothesis that isoflurane produces cardioprotection during early reperfusion after myocardial ischemia by a PI3K-dependent mechanism.**

Methods: **Pentobarbital-anesthetized rabbits (n = 80) subjected to a 30-min coronary occlusion followed by 3 h reperfusion were assigned to receive saline (control), three cycles of postconditioning ischemia (10 or 20 s each), isoflurane (0.5 or 1.0 minimum alveolar concentration), or the PI3K inhibitor wortmannin (0.6 mg/kg, intravenously) or its vehicle dimethyl sulfoxide. Additional groups of rabbits were exposed to combined postconditioning ischemia (10 s) and 0.5 minimum alveolar concentration isoflurane in the presence and absence of wortmannin. Phosphorylation of Akt, a downstream target of PI3K, was assessed by Western blotting.**

Results: **Postconditioning ischemia for 20 s, but not 10 s, reduced infarct size ($P < 0.05$) (triphenyltetrazolium staining; 20 ± 3% and 34 ± 3% of the left ventricular area at risk, respectively) as compared with control (41 ± 2%). Exposure to 1.0, but not 0.5, minimum alveolar concentration isoflurane decreased infarct size (21 ± 2% and 43 ± 3%, respectively). Wortmannin abolished the protective effects of postconditioning (20 s) and 1.0 minimum alveolar concentration isoflurane. Combined postconditioning (10 s) and 0.5 minimum alveolar concentration isoflurane markedly reduced infarct size (17 ± 5%). This action was also abolished by wortmannin (44 ± 2%). Isoflurane (1.0 minimum alveolar concentration) increased Akt phosphorylation after ischemia (32 ± 6%), and this action was blocked by wortmannin.**

Conclusions: **Isoflurane acts during early reperfusion after prolonged ischemia to salvage myocardium from infarction and reduces the threshold of ischemic postconditioning by activating PI3K.**

THE first minutes of reperfusion are critical for salvaging ischemic myocardium, but reperfusion also paradoxically worsens ischemic damage.[1,2] Modulation of conditions during reperfusion has been previously shown to favorably influence this postischemic damage.[3–6] Nevertheless, the vast majority of research examining determinants of irreversible ischemia and reperfusion injury has instead focused on interventions conducted before the ischemic insult has occurred. Vinten-Johansen *et al.*[7,8] recently reported that brief episodes of ischemia occurring in early reperfusion after prolonged coronary artery occlusion reduced myocardial infarct size, decreased endothelial dysfunction, attenuated neutrophil accumulation, and partially inhibited generation of cytotoxic reactive oxygen species. The reduction in infarct size observed with this "postconditioning" phenomenon was similar in magnitude to that produced by ischemic preconditioning. The mechanisms responsible for postconditioning are largely unknown, but preliminary data[9,10] suggest that activation of extracellular signal-regulated kinases, phosphatidylinositol-3-kinase (PI3K), and production of nitric oxide may play important roles in this process.

Volatile anesthetics have been shown to produce pharmacological preconditioning in a variety of experimental animal models and in humans.[11] The extent of protection against infarction produced by these agents is similar to that observed during classic ischemic preconditioning. Whether volatile anesthetics are also capable of producing postconditioning during early reperfusion through a PI3K-mediated mechanism is unknown. Volatile anesthetics have been shown by Schlack *et al.* to exert protective effects against ischemic injury when administered solely during reperfusion *in vitro*[12,13] and *in vivo*.[14,15] These previous data evoke the intriguing hypothesis that volatile agents may be capable of mimicking ischemic postconditioning. In this investigation, we tested the hypotheses that brief administration of isoflurane during early reperfusion after prolonged coronary occlusion salvages myocardium from irreversible injury and decreases the time threshold required for ischemic postconditioning. We further tested the hy-

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pothesis that these beneficial actions are mediated by activation of the PI3K signaling pathway.

Materials and 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[16] of the American Physiologic Society and were in accordance with the Guide for the Care and Use of Laboratory Animals.[17]

General Preparation

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.[18] 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 = 0.33). Arterial blood gas tensions and acid-base status were maintained within a normal physiologic range (pH between 7.35 and 7.45, $Paco_2$ between 25 and 40 mmHg, and Pao_2 between 90 and 150 mmHg) by adjusting the respiratory rate or tidal volume. Body temperature was maintained with a heating blanket. Heparin-filled catheters were inserted into the right carotid artery and the left jugular vein for measurement of mean arterial blood pressure and fluid or drug administration, respectively. Maintenance fluids (0.9% saline) were administered at 15 $ml \cdot kg^{-1} \cdot h^{-1}$ 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.

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 seven separate experimental groups, rabbits were randomly assigned to receive 0.9% saline (control), three cycles of postconditioning ischemia (10 or 20 s) during early reperfusion after prolonged (30 min) coronary occlusion, 0.5 or 1.0 minimum alveolar concentration (MAC) isoflurane (1.0 MAC = 2.05% in the rabbit) administered for 3 min before and 2 min after reperfusion, or the selective PI3K inhibitor wortmannin (0.6 mg/kg, intravenously) or its drug vehicle (dimethyl sulfoxide 0.08 ml/kg) before prolonged LAD occlusion. Postconditioning ischemia consisted of three cycles of 10 s or 20 s coronary occlusions separated by 10 s or 20 s, respectively beginning at 10 s or 20 s after initiation of reperfusion, respectively. End-tidal concentrations of volatile anesthetics were measured at the tip of the tracheostomy tube with an infrared gas analyzer that was calibrated with known standards before and during experimentation. Two additional groups of rabbits were pretreated with wortmannin and received three cycles of postconditioning ischemia (20 s) or brief exposure to 1.0 MAC isoflurane. Two final groups of rabbits were exposed to the combination of three cycles of postconditioning ischemia (10 s) and 0.5 MAC isoflurane in the presence and absence of pretreatment with wortmannin (0.6 mg/kg, intravenous).

Determination of Myocardial Infarct Size

Myocardial infarct size was measured as previously described.[19] Briefly, the LAD was reoccluded at the completion of each experiment and 3 ml of patent blue dye was injected intravenously. The left ventricular (LV) area at risk (AAR) 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 AAR were carefully separated and weighed after storage overnight in 10% formaldehyde. Myocardial infarct size was expressed as a percentage of the AAR. Rabbits that developed intractable ventricular fibrillation and those with an AAR less than 15% of total LV mass were excluded from subsequent analysis.

Western Immunoblotting

In seven separate experimental groups (n = 5 rabbits per group), rabbits were randomly assigned to receive 0.9% saline (control), dimethyl sulfoxide, wortmannin (0.6 mg/kg, intravenous), 1.0 MAC isoflurane administered for 3 min before and 2 min after reperfusion in the presence and absence of wortmannin pretreatment, or the combination of postconditioning ischemia (10 s) and 0.5 MAC isoflurane in the presence and absence of wortmannin pretreatment. All animals underwent a 30-min LAD occlusion. Left ventricular tissue samples were collected 5 min after reperfusion, immediately frozen in liquid nitrogen, and stored at −70°C. Tissue was homogenized with a Polytron (IKA® Works Inc., Wilmington, NC) homogenizer in ice-cold lysis buffer containing 20 mM Tris HCl (pH 7.4), 150 mM NaCl, 1 mM Na_2EDTA,

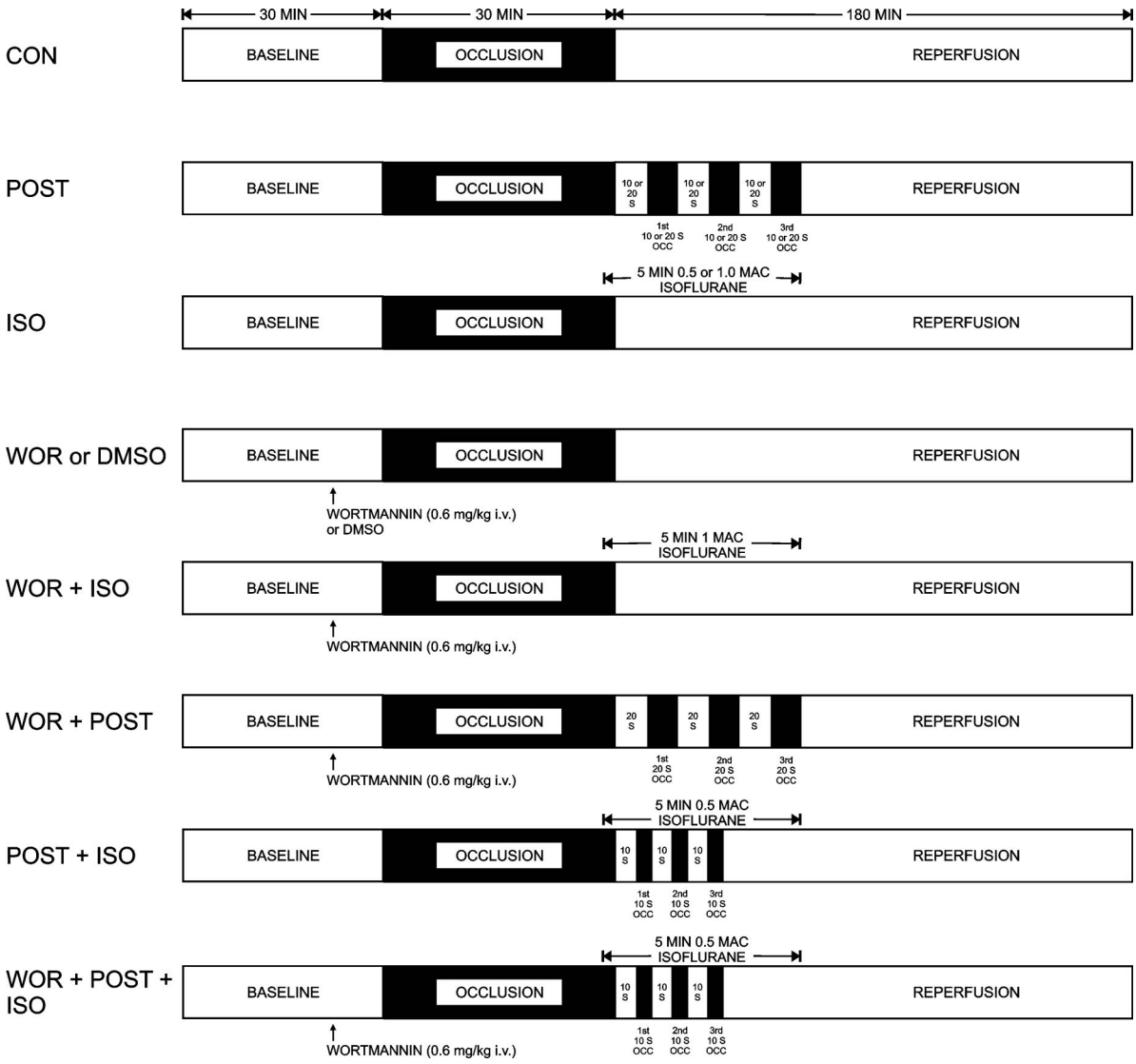

Fig. 1. Schematic diagram depicting the experimental protocol. CON = control; ISO = isoflurane; OCC = occlusion; POST = postconditioning ischemia; WOR = wortmannin.

1 mM EGTA, 1% Nonidet P40, 2.5 mM sodium pyrophosphate, 1 mM Na_3VO_4, and complete proteinase inhibitor cocktail (one tablet per 10 ml; Roche Diagnostics Corporation, Indianapolis, IN). The homogenate was centrifuged at 10,000*g* for 15 min at 4°C to remove cellular debris and isolate total protein. Protein concentration was determined by the Lowry method (BIO-RAD, Hercules, CA). Equivalent amounts (50 μg) of protein samples were mixed with loading buffer and heated at 95°C for 10 min. Samples were separated on a 4–15% polyacrylamide gel (BIO-RAD) and then electrophoretically transferred to a nitrocellulose membrane (BIO-RAD). After blocking with 5% milk in Tris-buffered saline containing 0.1% Tween-20, nitrocellulose membranes were incubated overnight at 4°C in 0.1% Tween-20 containing 5% milk and a 1:1000 dilution of monoclonal antibody against Ser 473 of phospho-Akt (Cell Signaling Technology, Beverly, MA). Membranes were washed three times with 0.1% Tween-20 for 5 min before a 60-min incubation with a 1:10,000 dilution of horseradish peroxidase-labeled antimouse immunoglobulin G (Santa Cruz Biotechnology, Santa Cruz, CA) in 0.1% Tween-20 containing 5% milk. Bound antibody was detected by enhanced chemiluminescence (Amersham Pharmacia, Piscataway, NJ) on radiograph film. Ponceau staining of nitrocellulose membranes was used to verify equal protein loading. To determine total Akt, the membrane was stripped with restore stripping buffer (Pierce, Rockford, IL) and reprobed with polyclonal goat Akt antibody (Santa Cruz Biotechnology). Thereafter, the membrane was exposed to the identical interventions described above. Phosphorylated densities were normalized against the concentrations of total Akt. Quantitative analysis of the band densities was performed using AlphaImager 2000 software (Alpha Innotech Corporation, San Leandro, CA).

Table 1. Systemic Hemodynamics

	No.	Baseline	LAD Occlusion	Reperfusion (min) 2	60	120	180
HR (beats/min)							
CON	8	269 ± 10	246 ± 6	244 ± 8*	235 ± 8*	227 ± 6*	213 ± 8*
10s POST	8	264 ± 7	248 ± 3*	232 ± 5*	216 ± 9*	210 ± 6*	209 ± 4*
20s POST	8	245 ± 9	234 ± 13	231 ± 5	223 ± 6	214 ± 7*	211 ± 6*
ISO 0.5	8	266 ± 8	250 ± 4	243 ± 7*	235 ± 10*	213 ± 8*	209 ± 6*
ISO 1.0	8	268 ± 6	253 ± 7	251 ± 4	236 ± 7*	231 ± 12*	221 ± 13*
WOR	6	272 ± 11	264 ± 16	252 ± 12	237 ± 11*	219 ± 7*	208 ± 10*
DMSO	8	274 ± 8	249 ± 9*	239 ± 8*	232 ± 9*	218 ± 7*	210 ± 9*
WOR + ISO 1.0	6	268 ± 8	256 ± 3	248 ± 5	238 ± 7	225 ± 5*	201 ± 16*
WOR + 20s POST	6	267 ± 13	247 ± 7*	242 ± 7*	239 ± 8*	207 ± 8*	185 ± 11*
10s POST + ISO 0.5	8	269 ± 7	261 ± 10	249 ± 9*	239 ± 7*	232 ± 8*	223 ± 8*
WOR + 10s POST + ISO 0.5	6	263 ± 8	245 ± 10	258 ± 12	243 ± 10	225 ± 12*	216 ± 13*
MAP (mmHg)							
CON	8	86 ± 2	61 ± 6*	57 ± 5*	63 ± 6*	69 ± 5*	64 ± 5*
10s POST	8	96 ± 5	75 ± 5*	61 ± 4*	69 ± 4*	69 ± 2*	71 ± 4*
20s POST	8	85 ± 2	66 ± 5*	60 ± 2*	68 ± 5*	65 ± 4*	73 ± 4*
ISO 0.5	8	81 ± 5	62 ± 7*	50 ± 5*	65 ± 6*	60 ± 7*	61 ± 6*
ISO 1.0	8	87 ± 5	71 ± 4*	56 ± 4*	68 ± 4*	67 ± 4*	71 ± 3*
WOR	6	83 ± 6	86 ± 6	66 ± 6*	68 ± 3*	63 ± 5*	54 ± 5*
DMSO	8	93 ± 3	70 ± 5*	63 ± 5*	67 ± 5*	70 ± 5*	62 ± 7*
WOR + ISO 1.0	6	83 ± 4	80 ± 6	55 ± 8*	71 ± 4	69 ± 5	65 ± 8
WOR + 20s POST	6	86 ± 4	83 ± 3	71 ± 5*	70 ± 3*	64 ± 5*	63 ± 4*
10s POST + ISO 0.5	8	95 ± 3	84 ± 2	56 ± 4*	81 ± 5	82 ± 5	82 ± 5
WOR + 10s POST + ISO 0.5	6	73 ± 7	71 ± 9	64 ± 5	72 ± 5	70 ± 6	70 ± 6
RPP ($min^{-1} \cdot mmHg \cdot 10^3$)							
CON	8	26.1 ± 0.8	17.5 ± 1.7*	16.8 ± 1.3*	17.6 ± 1.5*	17.9 ± 1.2*	15.8 ± 1.2*
10s POST	8	28.3 ± 1.9	21.4 ± 1.4*	17.8 ± 1.0	17.5 ± 1.2*	17.1 ± 0.8*	17.5 ± 1.0*
20s POST	8	23.7 ± 1.1	18.9 ± 2.1*	17.7 ± 1.4*	18.3 ± 1.4*	16.5 ± 1.0*	17.9 ± 1.1*
ISO 0.5	8	24.7 ± 1.8	18.6 ± 1.8*	15.5 ± 1.8*	19.0 ± 1.8*	16.3 ± 1.8*	16.2 ± 1.5*
ISO 1.0	8	26.9 ± 1.2	20.6 ± 1.2*	18.5 ± 1.1*	19.5 ± 1.2*	18.2 ± 1.2*	18.1 ± 0.5*
WOR	6	25.6 ± 1.3	25.4 ± 2.2	20.4 ± 1.5*	18.5 ± 1.0*	15.7 ± 1.2*	13.4 ± 1.5*
DMSO	8	28.2 ± 1.2	20.4 ± 1.8*	17.3 ± 1.8*	18.5 ± 2.1*	17.3 ± 1.4*	15.5 ± 1.8*
WOR + ISO 1.0	6	24.9 ± 1.6	22.5 ± 1.7	16.3 ± 1.9*	19.4 ± 1.5	17.6 ± 1.2*	15.8 ± 2.3*
WOR +20s POST	6	25.4 ± 1.7	22.1 ± 0.3	19.5 ± 0.8*	18.9 ± 1.1*	14.9 ± 1.4*	13.3 ± 1.3*
10s POST + ISO 0.5	8	28.8 ± 1.3	24.8 ± 1.2*	18.4 ± 1.3*	23.1 ± 1.6*	22.3 ± 1.1*	21.3 ± 1.1*
WOR + 10s POST + ISO 0.5	6	22.1 ± 1.6	19.6 ± 2.2	19.6 ± 1.6	19.6 ± 1.3	17.5 ± 1.3	16.6 ± 1.4*

Data are mean ± SEM.

CON = control; DMSO = dimethyl sulfoxide; HR = heart rate; ISO = isoflurane; LAD = left anterior descending artery; MAP = mean arterial pressure; POST = postconditioning ischemia; RPP = rate-pressure product; WOR = wortmannin.

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

Statistical Analysis

Statistical analysis of data within and between groups was performed with analysis of variance for repeated measures followed by the Student-Newman-Keuls test. Changes were considered statistically significant when $P < 0.05$. All data are expressed as mean ± SEM.

Results

Eighty-seven rabbits were instrumented to obtain 80 successful experiments. One rabbit was excluded because of technical problems during instrumentation. Six rabbits were excluded because intractable ventricular fibrillation occurred during LAD occlusion (one control, one 0.5 MAC isoflurane, one 1.0 MAC isoflurane, two 10 s postconditioning, and one 20 s postconditioning experiment).

Systemic Hemodynamics

There were no differences in baseline hemodynamics between groups (table 1). Coronary artery occlusion significantly ($P < 0.05$) decreased mean arterial pressure and rate-pressure product in rabbits that did not receive wortmannin. Decreases in heart rate, mean arterial pressure, and rate-pressure product were observed during reperfusion in all experimental groups. There were no differences in hemodynamics among groups before, during, or after LAD occlusion.

Myocardial Infarct Size

Body weight, LV mass, AAR weight, and the ratio of AAR to LV mass were similar between groups (table 2). Three cycles of 20 s but not 10 s of postconditioning ischemia during early reperfusion reduced infarct size (20 ± 3% and 34 ± 3% of the LV AAR, respectively) as compared with control (41 ± 2%; fig. 2). Brief exposure

Table 2. Left Ventricular Area at Risk

	No.	Body Weight (g)	LV (g)	AAR (g)	AAR/LV (%)
CON	8	2758 ± 77	3.31 ± 0.17	1.23 ± 0.14	37 ± 3
10s POST	8	2599 ± 105	3.67 ± 0.18	1.21 ± 0.17	32 ± 4
20s POST	8	2613 ± 91	3.39 ± 0.22	1.24 ± 0.16	36 ± 3
ISO 0.5	8	2602 ± 57	3.78 ± 0.11	1.23 ± 0.13	33 ± 4
ISO 1.0	8	2567 ± 87	3.33 ± 0.16	1.13 ± 0.14	34 ± 4
WOR	6	2457 ± 56	3.71 ± 0.13	1.29 ± 0.13	35 ± 4
DMSO	8	2601 ± 97	3.98 ± 0.28	1.42 ± 0.12	36 ± 2
WOR + ISO 1.0	6	2393 ± 74	3.18 ± 0.30	1.35 ± 0.18	42 ± 4
WOR + 20s POST	6	2543 ± 89	3.28 ± 0.03	1.22 ± 0.09	37 ± 3
10s POST + ISO 0.5	8	2602 ± 56	3.76 ± 0.16	1.20 ± 0.17	32 ± 4
WOR + 10s POST + ISO 0.5	6	2437 ± 61	3.79 ± 0.19	1.58 ± 0.18	41 ± 4

Data are mean ± SEM.

AAR = area at risk; CON = control; DMSO = dimethylsulfoxide; ISO = isoflurane; POST = postconditioning ischemia; WOR = wortmannin.

to 1.0 but not 0.5 MAC isoflurane during early reperfusion also reduced infarct size (21 ± 2% and 43 ± 3%, respectively). Wortmannin and dimethyl sulfoxide alone did not affect infarct size (37 ± 2% and 43 ± 2% respectively). Wortmannin eliminated the protection produced by three cycles of 20 s of postconditioning ischemia and 1.0 MAC isoflurane (43 ± 2% and 43 ± 4%, respectively). The combination of 10 s postconditioning ischemia and 0.5 MAC isoflurane markedly decreased infarct size (17 ± 5%). This protective effect was also abolished by pretreatment with wortmannin (44 ± 2%).

Phosphorylation of Akt

The phosphorylation state of Akt after ischemia is illustrated by a representative Western blot (fig. 3, *upper panel*). Total Akt expression was comparable in all samples. The densities of phosphorylated Akt were normalized against these total Akt concentrations. Brief exposure to 1.0 MAC isoflurane or to the combination of postconditioning ischemia (10 s) and 0.5 MAC isoflurane during early reperfusion significantly ($P < 0.05$) increased (37 ± 6% and 35 ± 5%, respectively) the phosphorylation of Akt Ser 473 (fig. 3, *lower panel*) compared with control. Wortmannin inhibited phosphorylation of Akt in the absence and presence of isoflurane with and without postconditioning ischemia to a degree comparable to sham experiments performed in the absence of ischemia and reperfusion or administration of isoflurane (not shown).

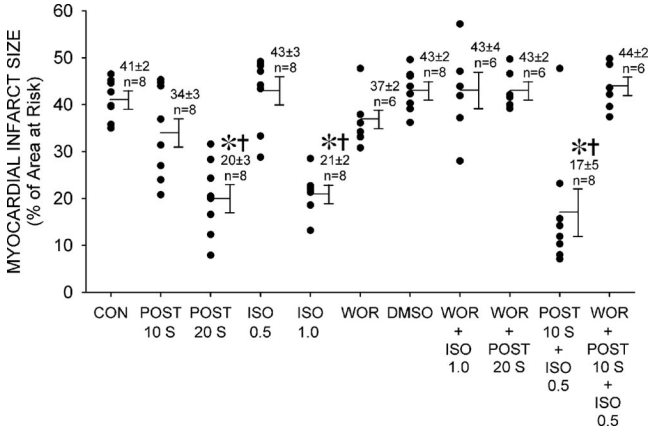

Fig. 2. Myocardial infarct size expressed as a percentage of the left ventricular area at risk for infarction in rabbits receiving 0.9% saline (CON), 3 cycles (10 or 20 s each) of postconditioning ischemia (POST), 0.5 or 1.0 minimum alveolar concentration isoflurane during reperfusion (ISO), wortmannin (WOR), dimethyl sulfoxide (DMSO), the combination of WOR + ISO, WOR + POST, POST + ISO, or WOR + POST + ISO. *Significantly ($P < 0.05$) different from CON; †significantly ($P < 0.05$) different from the same respective group in the presence of wortmannin.

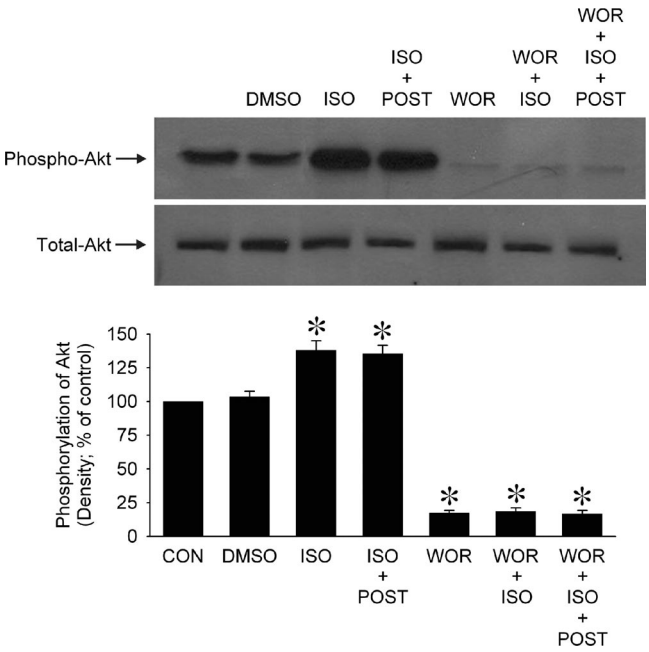

Fig. 3. Western blot analysis of phosphorylation of Akt at Ser 473 (*top lanes*) and total Akt (*bottom lanes*) in rabbits randomly assigned to receive 0.9% saline (CON), dimethyl sulfoxide (DMSO), wortmannin (0.6 mg/kg, intravenously), 1.0 minimum alveolar concentration isoflurane administered for 3 min before and 2 min after reperfusion in the presence and absence of wortmannin pretreatment, or the combination of postconditioning ischemia (10 s) and 0.5 minimum alveolar concentration isoflurane in the presence and absence of wortmannin pretreatment. Histograms depicting the relative density of phospho-Akt in each experimental group are shown in the *lower panel*. *Significantly ($P < 0.05$) different from CON.

Discussion

The results of the current investigation confirm and extend previous findings,[7–9] indicating that brief episodes of ischemia occurring during early reperfusion after coronary artery occlusion exert myocardial protection *in vivo*. The reductions in infarct size observed in the current investigation with three cycles of postconditioning ischemia (20 s) were very similar to those that we have previously reported during ischemic preconditioning in rabbits.[11,20] A major difference in ischemic preconditioning as compared with postconditioning appears to be the duration of ischemia, with the latter requiring much shorter durations of ischemic stimuli. This observation suggests that potential differences in the mechanisms responsible for these protective processes may exist. The current results also confirm previous observations[7–9] indicating that postconditioning requires a minimum time threshold of brief ischemic episodes because three cycles of 10 s of postconditioning ischemia during early reperfusion did not salvage myocardium from irreversible injury. The current investigation further demonstrates that brief exposure to 1.0 but not 0.5 MAC isoflurane during the final 3 min of coronary occlusion and the first 2 min of reperfusion produces protection against ischemia and reperfusion injury. In addition, the results indicate that administration of 0.5 MAC isoflurane (a concentration that did not produce cardioprotection alone) was capable of reducing the time threshold required for ischemic postconditioning. Finally, the current findings demonstrate for the first time that activation of the PI3K signaling pathway directly mediates the protective effects of both ischemia-induced and anesthetic-induced postconditioning *in vivo* and that isoflurane increases the phosphorylation of Akt.

Mechanisms responsible for anesthetic preconditioning have been examined in recent years, and many of the intracellular signaling elements responsible for this phenomenon have been identified.[11] Substantially less attention has been directed toward exploration of the potential beneficial effects of volatile agents when administered solely during reperfusion, but some experimental evidence has indicated that volatile anesthetics are capable of exerting protective effects under these conditions. Halothane prevented reoxygenation-induced hypercontracture of cardiac myocytes *in vitro*, a phenomenon postulated as an important cause of myocyte necrosis during early reperfusion.[12] Desflurane and sevoflurane, but not isoflurane, reduced myocardial infarct size when administered during the first 15 min of reperfusion in rabbits.[14] In contrast, the current results demonstrate that 1.0 MAC isoflurane reduces infarct size when administered 3 min before and 2 min after the onset of reperfusion. These results are supported by the findings of another previous study indicating that isoflurane enhances the functional recovery of isolated rat hearts when administered solely during reperfusion.[13] The beneficial effects of sevoflurane during reperfusion have also been shown to be dose-dependent.[15] These previous observations with sevoflurane are supported by the current results with isoflurane.

The mechanisms responsible for the beneficial effects of volatile anesthetics during reperfusion have yet to be firmly established. Halothane abolished reoxygenation-induced attenuation of sarcoplasmic reticulum-dependent oscillations of intramyoplasmic calcium concentration in isolated cardiac myocytes.[12] These findings suggested that volatile anesthetics may be preventing intracellular calcium overload during early reperfusion. Isoflurane and sevoflurane reduced postischemic adhesion of polymorphonuclear leukocytes.[21] Neutrophils are an important source of oxygen-derived free radicals during reperfusion that are known to be critical mediators of postischemic injury.[22,23] Thus, previous studies have implicated anesthetic-induced attenuation of well-known but relatively nonspecific mechanisms of reperfusion injury, but these studies have yet to suggest that an endogenous intracellular signal transduction pathway may also play a role in the protective effects of volatile anesthetics during reperfusion.

The current study demonstrates for the first time that postconditioning by ischemia and isoflurane are mediated by activation of PI3K. Moreover, isoflurane applied briefly during early reperfusion increases the phosphorylation of Akt. Although hypoxia itself has been shown to activate the PI3K/Akt cell survival pathway,[24] anesthetic postconditioning further enhanced this effect. PI3K converts phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate.[25,26] Phosphatidylinositol-3,4,5-trisphosphate-stimulated phosphorylation of the serine-threonine kinase Akt by phosphoinositide-dependent kinase 1 subsequently inhibits formation of the proapoptotic proteins Bad, Bax, and caspase 9. Moreover, Akt has been shown to stimulate endothelial nitric oxide synthase[27] and increase the formation of its product nitric oxide. The protective actions of nitric oxide during ischemic postconditioning have been preliminarily suggested.[9] In addition, phosphoinositide-dependent kinase 1 is a potent activator of other protein kinases, including protein kinase C, that have been implicated in the protection of myocardium against ischemia and reperfusion injury produced by volatile anesthetics.[11] Thus, it appears that the PI3K signaling cascade may contribute to the recruitment of multiple, redundant endogenous cardioprotective pathways to reduce reperfusion injury. Further research will be required to identify signaling components downstream of phosphorylated Akt that may be involved in ischemic or isoflurane-induced protection against infarction during reperfusion.

Emerging evidence strongly suggests that the PI3K-Akt cascade signaling pathway mediates reperfusion inju-

ry.[26] Bradykinin and a nonselective adenosine receptor agonist produced cardioprotection when administered at the onset of reperfusion in rabbit hearts by signaling through PI3K, extracellular signal-regulated kinases, and nitric oxide.[28] Administration of insulin during reperfusion also attenuated postischemic damage through a PI3K/Akt-dependent mechanism.[29–31] Phosphorylation of endothelial nitric oxide synthase by Akt and the consequent increase in nitric oxide production contributed to the antiapoptotic effect of insulin as well.[32] Interestingly, PI3K also appears to play a role in preconditioning as well. Wortmannin abolished insulin-induced preconditioning, and this drug also modestly attenuated the protective effects of ischemic preconditioning.[33,34] PI3K was identified in another study as an upstream signaling component that activated protein kinase C and stimulated nitric oxide production during ischemic preconditioning.[35] Wortmannin also abolished phosphorylation of Akt during ischemic preconditioning.[34] Acetylcholine-induced preconditioning was abolished by wortmannin or the selective mitochondrial adenosine triphosphate-dependent potassium channel antagonist 5-hydroxydeconoate as well.[36] At least one study has suggested that delayed ischemic preconditioning may also be mediated by activation of PI3K-dependent signaling pathways because wortmannin abolished the remote protection of multiple brief coronary occlusions performed 24 h before prolonged ischemia in rabbits.[37] Whether the PI3K signaling cascade also mediates acute or delayed preconditioning by volatile anesthetics is unknown and represents an important objective of future investigations by our laboratory.

The current results must be interpreted within the constraints of several potential limitations. The PI3K-Akt signaling pathway has been clearly implicated as protective in apoptosis that is either triggered or accelerated during reperfusion.[26,38] The current results indicate that activation of PI3K mediates salvage of myocardium against infarction during postconditioning by ischemia or the volatile anesthetic isoflurane, but further investigations are needed to ascertain whether these beneficial actions also involve a decrease in apoptosis. Wortmannin has been shown to be a selective PI3K inhibitor at the dose used in the current investigation.[28,39] Our results with Western analyses also confirm this result. Nevertheless, the possibility that wortmannin may have inhibited other protein kinases involved in myocardial protection cannot be completely excluded from the analysis. Further, a direct participation of phosphatidylinositol phosphates in the regulation of ion channels such as adenosine triphosphate-regulated potassium channels has been suggested.[40] Myocardial infarct size is determined primarily by the size of the AAR and extent of coronary collateral perfusion. The AAR, expressed as a percentage of total LV mass, was similar between groups in the current investigation. Rabbits have also been shown to possess little if any coronary collateral blood flow.[41] Thus, it appears unlikely that differences in collateral perfusion between groups account for the observed results. However, coronary collateral blood flow was not specifically quantified in the current investigation. The reductions in myocardial infarct size produced by ischemic and anesthetic-induced postconditioning occurred independent of changes in major determinants of myocardial oxygen consumption. Nevertheless, the current results require qualification because coronary venous oxygen tension was not directly measured, and myocardial oxygen consumption was not calculated in the current investigation. Phosphorylation of Akt by isoflurane and its inhibition by the PI3K antagonist wortmannin provides strong supportive evidence for the involvement of PI3K in isoflurane-induced postconditioning. Nevertheless, the possibility that another unrelated protein kinase was responsible for phosphorylation of Akt cannot be entirely excluded based on our results.

In summary, the current results indicate that brief administration of 1.0 MAC isoflurane immediately before and during early reperfusion salvages myocardium from infarction. The results further demonstrate that administration of 0.5 MAC isoflurane, a concentration of this agent that does not provide cardioprotection alone, reduces the time threshold of brief ischemic stimuli required to produce postconditioning. These beneficial effects of isoflurane that occur during early reperfusion are mediated by activation of the PI3K signaling pathway. Additional research will be required to identify other signaling elements involved in postconditioning by anesthetics and clarify the mechanisms responsible for this interesting phenomenon.

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