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N-*Acetylcysteine Restores Isoflurane-induced Preconditioning against Myocardial Infarction during Hyperglycemia*

Franz Kehl, M.D., D.E.A.A., John G. Krolikowski, B.A.,† Dorothee Weihrauch, D.V.M., Ph.D.,‡ Paul S. Pagel, M.D., Ph.D.,§ David C. Warltier, M.D., Ph.D.,‖ Judy R. Kersten, M.D.#*

Background: **Hyperglycemia generates reactive oxygen species and prevents isoflurane-induced preconditioning. The authors tested the hypothesis that scavenging reactive oxygen species with *N*-acetylcysteine will restore protection against myocardial infarction produced by isoflurane *in vivo*.**

Methods: **Barbiturate-anesthetized dogs (n = 45) were instrumented for measurement of systemic hemodynamics. Myocardial infarct size and coronary collateral blood flow were measured with triphenyltetrazolium staining and radioactive microspheres, respectively. All dogs were subjected to a 60-min left anterior descending coronary artery occlusion followed by 3 h of reperfusion. Dogs were randomly assigned to receive an infusion of 0.9% saline or 15% dextrose in water to increase blood glucose concentrations to 600 mg/dl (hyperglycemia) in the absence or presence of isoflurane (1.0 minimum alveolar concentration) with or without pretreatment with *N*-acetylcysteine (150 mg/kg IV) in six experimental groups. Isoflurane was discontinued, and blood glucose concentrations were allowed to return to baseline values before left anterior descending coronary artery occlusion.**

Results: **Myocardial infarct size was 27 ± 2% (n = 8) of the left ventricular area at risk in control experiments. Isoflurane significantly ($P < 0.05$) decreased infarct size (13 ± 2%; n = 7). Hyperglycemia alone did not alter infarct size (29 ± 3%; n = 7) but abolished the protective effect of isoflurane (25 ± 2%; n = 8). *N*-Acetylcysteine alone did not affect infarct size (28 ± 2%; n = 8) but restored isoflurane-induced cardioprotection during hyperglycemia (10 ± 1%; n = 7).**

Conclusions: **Acute hyperglycemia abolishes reductions in myocardial infarct size produced by isoflurane, but *N*-acetylcysteine restores these beneficial effects. The results suggest that excessive quantities of reactive oxygen species generated during hyperglycemia impair isoflurane-induced preconditioning in dogs.**

MYOCARDIAL infarction is a major perioperative complication that is associated with significant morbidity and mortality.[1] Hyperglycemia related to or independent of diabetes mellitus increases the risk of adverse cardiovascular events,[2,3] but the mechanisms responsible for this increase in risk are incompletely understood. Signal transduction pathways required for endogenous myocardial protection against ischemic injury are impaired by hyperglycemia or diabetes. We have previously demonstrated that diabetes and hyperglycemia block ischemic preconditioning and abolish reductions of infarct size produced by activation of adenosine triphosphate–regulated potassium (K_{ATP}) channels.[4,5] Volatile anesthetic preconditioning (APC) is also markedly attenuated by hyperglycemia and diabetes.[6,7] Hyperglycemia generates large quantities of reactive oxygen species (ROS),[8] and such reactive intermediates have been strongly implicated in the pathogenesis of ischemia–reperfusion injury.[9] Whether hyperglycemia-induced ROS also adversely affects APC is unknown. We tested the hypothesis that the antioxidant *N*-acetylcysteine restores isoflurane-induced preconditioning during acute hyperglycemia in a canine model of experimental myocardial infarction.

Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional 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 Physiological Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996).

General Preparation

Implantation of instruments has been described in detail previously.[10] Briefly, mongrel dogs of either sex were anesthetized with sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg) and ventilated using positive pressure with an air–oxygen mixture after tracheal intubation. Arterial blood pH was maintained within a physiologic range by adjustment of tidal volume and respiratory rate. End-tidal concentrations of isoflurane were measured at the tip of the endotracheal tube by an infrared anesthetic analyzer that was calibrated with known standards before and during experimentation. The canine minimum alveolar concentration of isoflurane used in the present investigation was 1.28%.[11] Temperature was maintained with a heating blanket. A 7-French, dual micromanometer–tipped catheter was inserted into the aorta and left ventricle (LV) for measure-

* Research Fellow, † Research Scientist, ‡ Assistant Professor of Anesthesiology, § Professor and Director of Cardiac Anesthesia, ‖ Professor of Anesthesiology, Pharmacology and Toxicology, and Medicine (Division of Cardiovascular Diseases) and Vice Chairman for Research, Department of Anesthesiology, # Professor of Anesthesiology, Pharmacology and Toxicology, Medical College of Wisconsin and the Zablocki VA Medical Center, Milwaukee, Wisconsin.

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Address reprint requests to Dr. Kersten: Medical College of Wisconsin, MEB-M4280, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226. Address electronic mail to: jkersten@mcw.edu.

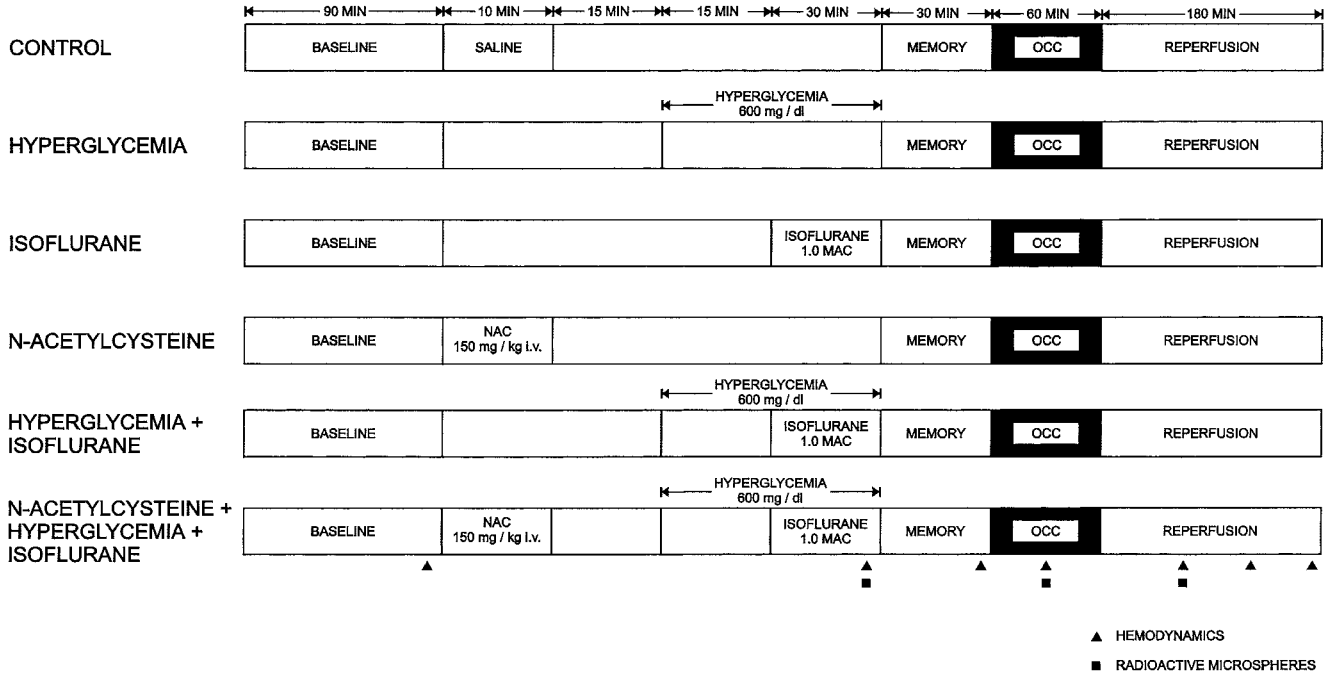

Fig. 1. Schematic diagram illustrating the experimental protocol. MAC = minimum alveolar concentration; NAC = *N*-acetylcysteine; OCC = occlusion.

ment of aortic and LV pressures and the maximum rate of increase of LV pressure ($+dP/dt_{max}$). Heparin-filled catheters were inserted into the left atrial appendage and the right femoral artery for administration of radioactive microspheres and withdrawal of reference blood flow samples, respectively. A catheter was also inserted into the right femoral vein for fluid or drug administration. A 1-cm segment of the left anterior descending coronary artery (LAD) was isolated immediately distal to the first diagonal branch, and a silk ligature was placed around the vessel for production of coronary artery occlusion and reperfusion. Hemodynamics were monitored continuously on a polygraph and digitized using a computer interfaced with an analog-to-digital converter.

Experimental Protocol

Baseline systemic hemodynamics were recorded 90 min after instrumentation was completed and calibrated. All dogs were subjected to a 60-min LAD occlusion followed by 3 h of reperfusion (fig. 1). Dogs were randomly assigned to receive 0.9% saline or 15% dextrose in water to increase blood glucose concentrations to 600 mg/dl in the absence or presence of isoflurane (1.0 minimum alveolar concentration) with or without *N*-acetylcysteine in six separate experimental groups. *N*-Acetylcysteine (150 mg/kg IV over a period of 10 min)[12] was infused 15 min before administration of dextrose. Isoflurane was administered for 30 min and discontinued 30 min before LAD occlusion.[13] Blood glucose concentrations were allowed to return to baseline values 30 min before LAD occlusion. Regional myocardial blood flow was measured at baseline, during LAD occlusion, and after 1 h of reperfusion. Dogs that developed intractable ventricular fibrillation and those with a subendocardial coronary collateral blood flow greater than 0.15 ml·min^{-1}·g^{-1} were excluded from the analysis.[14]

Measurement of Myocardial Infarct Size

At the end of each experiment, myocardial infarct size was measured as previously described.[15] Briefly, the LV area at risk for infarction (AAR) was separated from the normal area, and the two regions were incubated at 37°C for 20–30 min in 1% 2,3,5-triphenyltetrazolium chloride in 0.1 M phosphate buffer adjusted to pH 7.4. After overnight storage in 10% formaldehyde, infarcted and noninfarcted myocardial areas within the AAR were carefully separated and weighed. Infarct size was expressed as a percentage of the AAR.

Determination of Regional Myocardial Blood Flow

Carbonized plastic microspheres (15 ± 2 μm [SD] in diameter) labeled with ^{141}Ce, ^{103}Ru, or ^{95}Nb were used to measure regional myocardial perfusion as described previously.[10] Transmural tissue samples were selected from the ischemic region (distal to the LAD occlusion) and were subdivided into subepicardial, midmyocardial, and subendocardial layers of approximately equal thickness. Samples were weighed and placed in scintillation vials, and the activity of each isotope was determined. Similarly, the activity of each isotope in the reference blood flow sample was assessed. Tissue blood flow was calculated as $\dot{Q}_r \cdot C_m \cdot C_r^{-1}$, where $\dot{Q}_r$ = rate of with-

Table 1. Blood Glucose Concentrations

				Reperfusion, h	
	Baseline	Intervention	30-min CAO	1	3
CON	75 ± 4	62 ± 8	69 ± 5	66 ± 2	66 ± 5
NAC	79 ± 3	72 ± 7	76 ± 6	80 ± 5	78 ± 4
HYP	65 ± 5	577 ± 12*†	69 ± 13	76 ± 10	89 ± 8
ISO	72 ± 4	70 ± 5	69 ± 2	67 ± 4	63 ± 5
HYP + ISO	75 ± 4	584 ± 4*†	82 ± 13	59 ± 5	67 ± 7
NAC + HYP + ISO	73 ± 5	577 ± 9*†	149 ± 24*†	72 ± 11	73 ± 8

Data are mg/dl, mean ± SEM. Intervention = administration of saline, dextrose or *N*-acetylcysteine in the presence or absence of isoflurane.

* Significantly ($P < 0.05$) different from baseline. † Significantly ($P < 0.05$) different from the respective value in control experiments.

CAO = coronary artery occlusion; CON = control; HYP = hyperglycemia; ISO = isoflurane; NAC = *N*-acetylcysteine.

drawal of the reference blood flow sample (ml/min), C_m = activity (counts per minute per gram) of the myocardial tissue sample, and C_r = activity (counts per minute) of the reference blood flow sample. Transmural blood flow was considered to be the average of subepicardial, midmyocardial, and subendocardial blood flows.

Statistical Analysis

Statistical analysis of data within and between groups was performed with ANOVA for repeated measures followed by Student–Newman–Keuls test. Changes within and between groups were considered statistically significant at a value (two-tailed) of $P < 0.05$. All data are expressed as mean ± SEM.

Results

Forty-six dogs were instrumented to obtain 45 successful experiments. One dog in the isoflurane plus hyperglycemia group was excluded because blood glucose concentrations could not be maintained at 600 mg/dl. Blood glucose concentrations (table 1) were similar among groups at baseline. Blood glucose concentrations during dextrose administration were similar in the presence or absence of isoflurane or *N*-acetylcysteine. Blood glucose concentration returned to baseline values during reperfusion in each experimental group.

Hemodynamics and Collateral Blood Flow

There were no significant differences in hemodynamics between experimental groups at baseline (table 2). A transient increase in LV $+dP/dt_{max}$ was observed during hyperglycemia. *N*-Acetylcysteine alone did not alter hemodynamics. Isoflurane decreased heart rate, mean arterial and LV systolic pressures, and LV $+dP/dt_{max}$ in the presence or absence of hyperglycemia or *N*-acetylcysteine. Mean arterial and LV systolic pressures and LV $+dP/dt_{max}$ returned to baseline values 30 min after isoflurane was discontinued. LAD occlusion and reperfusion produced similar increases in LV end-diastolic pressure and decreases in LV $+dP/dt_{max}$ in all experimental groups. LAD occlusion decreased transmural myocardial perfusion in the ischemic region (table 3). There were no significant differences in coronary collateral blood flow between groups.

Myocardial Infarct Size

The LV AAR was similar between groups (control, 39 ± 2%; *N*-acetylcysteine, 41 ± 1%; isoflurane, 37 ± 1%; hyperglycemia, 37 ± 2%; isoflurane plus hyperglycemia, 37 ± 2%; *N*-acetylcysteine plus isoflurane plus hyperglycemia, 39 ± 3%). Myocardial infarct size expressed as a percentage of the AAR was 27 ± 2% (n = 8) in dogs receiving 0.9% saline. Isoflurane reduced infarct size to 13 ± 2% (n = 7) of the AAR (fig. 2). Hyperglycemia (n = 7) alone did not alter myocardial infarct size (29 ± 3%) but blocked the protective effect of isoflurane (25 ± 2%, n = 8). *N*-Acetylcysteine did not affect myocardial infarct size (28 ± 2%, n = 8) but restored the protective effect of isoflurane during hyperglycemia (10 ± 1%, n = 7).

Discussion

ROS have traditionally been viewed as toxic mediators of cellular injury. However, recent evidence indicates that small quantities of ROS act as important triggers of intracellular signaling. ROS generated by the mitochondrial electron transport chain during brief episodes of hypoxia or ischemia have been shown to activate signal transduction pathways that confer protection of ischemic myocardium.[16,17] Diazoxide, a selective mitochondrial K_{ATP} channel agonist, produces preconditioning by generating ROS. In contrast, the free radical scavengers *N*-2-mercaptopropionyl glycine and *N*-acetylcysteine reduce the intracellular concentration of ROS and abolish cardioprotection.[18–20] ROS also seem to be important triggers of APC. Volatile anesthetics cause profound reductions of myocardial infarct size and enhance the functional recovery of stunned myocardium.[13,21–23] These beneficial effects are blocked by antioxidant treatment.[23–25] Using confocal fluorescent microscopy with dihydroethidium staining, we recently demonstrated that isoflurane directly generates ROS. This effect is blocked by administration of *N*-2-mercaptopropionyl gly-

Table 2. Systemic Hemodynamics

					Reperfusion, h		
	Baseline	Intervention	Memory	30-min CAO	1	2	3
HR, beats/min							
CON	141 ± 5	137 ± 8	136 ± 7	133 ± 6	121 ± 6*	117 ± 7*	121 ± 7*
NAC	140 ± 7	130 ± 7	128 ± 6	123 ± 6*	126 ± 6	129 ± 6	128 ± 6
HYP	132 ± 7	128 ± 8	128 ± 8	128 ± 6	113 ± 6*	112 ± 5*	115 ± 6*
ISO	137 ± 4	109 ± 3*†	120 ± 4*	124 ± 4*	117 ± 6*	115 ± 7*	118 ± 9*
HYP + ISO	135 ± 4	111 ± 3*†	127 ± 5	131 ± 4	122 ± 3	122 ± 4	121 ± 5
NAC + HYP + ISO	123 ± 7	95 ± 4*†	108 ± 4†	108 ± 5†	104 ± 4*	100 ± 5*	104 ± 6*
MAP, mmHg							
CON	103 ± 6	102 ± 7	103 ± 5	96 ± 4	100 ± 5	105 ± 5	103 ± 5
NAC	107 ± 3	101 ± 4	103 ± 4	87 ± 4*	87 ± 7*	93 ± 6	95 ± 7
HYP	103 ± 4	100 ± 6	97 ± 4	82 ± 5*	92 ± 4	97 ± 5	94 ± 5
ISO	96 ± 6	60 ± 4*†	103 ± 2	96 ± 3	95 ± 2	95 ± 2	98 ± 2
HYP + ISO	95 ± 4	70 ± 4*†	95 ± 3	82 ± 5	91 ± 4	92 ± 5	91 ± 5
NAC + HYP + ISO	97 ± 3	61 ± 5*†	97 ± 4	90 ± 6	96 ± 3	97 ± 3	98 ± 4
LVSP, mmHg							
CON	114 ± 8	115 ± 10	114 ± 7	102 ± 6	100 ± 6	107 ± 6	108 ± 5
NAC	117 ± 3	113 ± 5	114 ± 5	92 ± 4*	90 ± 7*	98 ± 6*	98 ± 7*
HYP	112 ± 3	110 ± 6	107 ± 4	85 ± 6*	96 ± 5*	101 ± 5	98 ± 5
ISO	106 ± 5	67 ± 3*†	109 ± 3	101 ± 3	98 ± 2	100 ± 2	102 ± 2
HYP + ISO	107 ± 3	80 ± 4*†	103 ± 2	88 ± 6	95 ± 4	96 ± 4	95 ± 5
NAC + HYP + ISO	104 ± 4	66 ± 6*†	104 ± 4	92 ± 6	89 ± 4*	97 ± 5	98 ± 6
LVEDP, mmHg							
CON	6 ± 1	7 ± 1	6 ± 1	16 ± 2*	19 ± 2*	15 ± 3	16 ± 3*
NAC	5 ± 1	5 ± 1	5 ± 1	20 ± 4*	16 ± 3*	12 ± 3	11 ± 3
HYP	7 ± 1	10 ± 2	6 ± 2	11 ± 2*	13 ± 1*	13 ± 2*	12 ± 2*
ISO	5 ± 2	8 ± 1	7 ± 1	12 ± 2*	12 ± 1*	11 ± 2*	11 ± 1*
HYP + ISO	5 ± 1	9 ± 1*	4 ± 1	11 ± 3*	12 ± 2*	10 ± 1*	10 ± 1*
NAC + HYP + ISO	9 ± 2	11 ± 1	7 ± 1	17 ± 4*	16 ± 3	15 ± 2	14 ± 2
$+dP/dt_{max}$, mmHg/s							
CON	2,240 ± 160	1,900 ± 110	2,330 ± 160	1,840 ± 110*	1,520 ± 70*	1,500 ± 80*	1,350 ± 60*
NAC	1,910 ± 160	1,820 ± 140	1,800 ± 120†	1,330 ± 90*†	1,420 ± 110*	1,600 ± 90	1,530 ± 70*
HYP	1,710 ± 60	2,100 ± 160*	1,950 ± 90*	1,440 ± 120*	1,520 ± 80	1,480 ± 70	1,380 ± 60*
ISO	1,910 ± 170	970 ± 70*†	1,760 ± 150†	1,660 ± 120	1,510 ± 90*	1,480 ± 90*	1,470 ± 80*
HYP + ISO	1,750 ± 100	1,220 ± 40*†	1,680 ± 80†	1,340 ± 150*	1,460 ± 100*	1,380 ± 80*	1,310 ± 70*
NAC + HYP + ISO	1,870 ± 200	1,110 ± 90*†	1,930 ± 90	1,610 ± 90	1,390 ± 50*	1,400 ± 60*	1,400 ± 60*

Data are mean ± SEM. Intervention = administration of saline, dextrose, or *N*-acetylcysteine in the presence or absence of isoflurane.

* Significantly ($P < 0.05$) different from baseline. † Significantly ($P < 0.05$) different from the respective value in control experiments.

CAO = coronary artery occlusion; CON = control; $+dP/dt_{max}$ = maximal rate of increase of left ventricular pressure; HR = heart rate; HYP = hyperglycemia; ISO = isoflurane; LVSP and LVEDP = left ventricular systolic and end-diastolic pressures, respectively; MAP = mean aortic blood pressure; NAC = *N*-acetylcysteine.

cine, *N*-acetylcysteine, or the mitochondrial K_{ATP} channel antagonist 5-hydroxydecanoate.[25] These data provide strong support to the contention that small quantities of ROS are required for APC.

Large quantities of oxygen-derived free radicals are released during ischemia and reperfusion and cause direct lipid peroxidation, mitochondrial damage, and cell death.[9,26] Excessive amounts of ROS are also generated by the mitochondrial electron transport chain during hyperglycemia,[27] and these reactive intermediates may contribute to myocardial injury as well. We have previously demonstrated that hyperglycemia either independent of or secondary to diabetes mellitus attenuates APC in dogs.[6,7] An interaction between the dose of volatile anesthetic and severity of hyperglycemia was also observed. Higher blood glucose concentrations were necessary to block the protection of 1.0 compared with 0.5 minimum alveolar concentration isoflurane.[6] This observation led us to hypothesize that volatile anesthetics and glucose may exert opposing actions on K_{ATP} channel activity. The current results confirm and extend our previous findings and further suggest that the interaction between isoflurane and hyperglycemia is sensitive to the oxidation–reduction state within the cardiac myocyte.

The mitochondrial K_{ATP} channel plays a central role in both APC and ischemic preconditioning.[13,14,22,28] However, a direct cause-and-effect relationship between mitochondrial K_{ATP} channel opening and the production of ROS has not been demonstrated conclusively during APC or ischemic preconditioning. ROS enhance mitochondrial K_{ATP} channel activity but are also released when the channel is opened. Superoxide anion generated by xanthine oxidase activates mitochondrial K_{ATP} channels from bovine ventricular myocardium reconstituted in lipid bilayers.[29] The mitochondrial K_{ATP} channel agonist diazoxide also generates ROS measured with a

Table 3. Transmural Myocardial Perfusion in the Ischemic (LAD) Region

	Baseline	30-min CAO	1 h Reperfusion
CON	1.24 ± 0.14	0.07 ± 0.02*	1.68 ± 0.17*
NAC	0.76 ± 0.06	0.05 ± 0.01*	1.47 ± 0.14*
HYP	1.27 ± 0.16	0.05 ± 0.01*	2.00 ± 0.32*
ISO	0.78 ± 0.11	0.08 ± 0.01*	1.65 ± 0.33*
HYP + ISO	1.11 ± 0.10	0.05 ± 0.01*	1.80 ± 0.24*
NAC + HYP + ISO	0.77 ± 0.16	0.07 ± 0.02*	1.21 ± 0.16*

Data are ml · min^{-1} · g^{-1} mean ± SEM.

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

CAO = coronary artery occlusion; CON = control; HYP = hyperglycemia; ISO = isoflurane; LAD = left anterior descending coronary artery; NAC = *N*-acetylcysteine.

mitochondria-specific fluorescent probe.[19] However, it is possible that this action of diazoxide may not be a result of direct activation of the channels but instead may result from nonspecific uncoupling of electron transport. Interestingly, diazoxide markedly reduces ROS production in cardiac mitochondria subjected to hypoxia and reoxygenation.[30] Thus, the protective effects of mitochondrial K_{ATP} channel agonists may occur as a consequence of triggering by ROS that subsequently prevents the release of larger quantities of these reactive intermediates during reperfusion injury. Experiments conducted in isolated mitochondria demonstrate that a triggering quantity of ROS may exceed a critical threshold that results in a transition in mitochondrial inner membrane permeability and the subsequent release of a burst of ROS in a process that has been called "ROS-induced ROS release."[31] This transition in mitochondrial permeability precedes necrotic or apoptotic cell death, and glutathione is a primary defense against this event.[31,32] The current and previous results suggest that

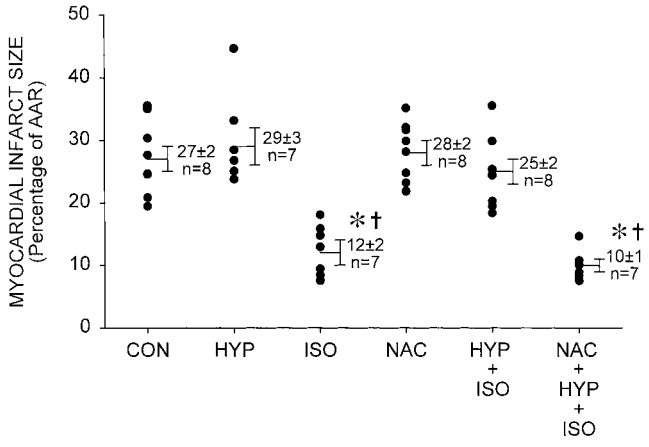

Fig. 2. Histogram illustrating myocardial infarct size as a percentage of the left ventricular area at risk (AAR) in dogs receiving 0.9% saline (*CON*) or 15% dextrose in water to increase blood glucose concentrations to 600 mg/dl (*HYP*) in the absence or presence of isoflurane (ISO; 1.0 minimum alveolar concentration [MAC]) and *N*-acetylcysteine (NAC; 150 mg/kg intravenously). *Significantly ($P < 0.05$) different from CON. †Significantly ($P < 0.05$) different from HYP + ISO.

hyperglycemia may accelerate, but volatile anesthetics or other mitochondrial K_{ATP} channel agonists may prevent this mitochondrial permeability transition in an oxidant-sensitive manner. This intriguing hypothesis remains to be specifically tested. There is also evidence to suggest that different ROS exert opposing actions on mitochondrial K_{ATP} channel activity, especially during conditions of high oxidative stress, such as hyperglycemia. Dismutation of superoxide anion leads to production of secondary ROS, including hydrogen peroxide, hydroxyl radical, and peroxynitrite,[33] and these radicals may differentially alter potassium channel activity. For example, superoxide anion and hydrogen peroxide enhance but peroxynitrite decreases calcium-activated potassium channel activity in rat cerebral arteries.[34] An important goal of future research will be to determine the actions of different species of free radicals on K_{ATP} channel activity and their impact on APC.

Nitric oxide is also a critical mediator of endogenous cardioprotective signal transduction. Nitric oxide directly activates mitochondrial K_{ATP} channels and potentiates agonist-induced increases in channel activity.[35] Diazoxide has been demonstrated to produce early and delayed preconditioning against infarction by activating mitochondrial K_{ATP} channels through a nitric oxide–dependent mechanism.[36] In contrast, hyperglycemia decreases the availability of nitric oxide by enhancing ROS production. Superoxide anion reacts with and inactivates nitric oxide and leads to the formation of peroxynitrite.[37,38] Many of the adverse consequences of hyperglycemia and diabetes are thought to result from decreased nitric oxide activity and increased generation of reactive intermediates. However, far less is known about the deleterious interactions among ROS, nitric oxide, and K_{ATP} channels during diabetes or how such interactions might affect APC.

The current results should be interpreted within the constraints of several potential limitations. First, ROS were not directly measured in the present investigation. However, we have shown previously that isoflurane directly produces ROS and that this effect is abolished by mitochondrial K_{ATP} channel antagonists and ROS scavengers, including *N*-acetylcysteine.[25] The ability of hyperglycemia to generate large quantities of ROS has also been well documented,[8] and using dihydroethidium staining, we have recently demonstrated that hyperglycemia dose-dependently increases ROS in canine myocardium.[39] *N*-Acetylcysteine was used to scavenge ROS. This drug is a sulfhydryl-containing glutathione precursor that exerts antioxidant effects by contributing to glutathione synthesis, serving as a glutathione peroxidase substrate, and directly scavenging several ROS through the actions of reduced glutathione.[40] The results indicate that hyperglycemia impairs APC. This effect is reversed by *N*-acetylcysteine, suggesting a role for ROS in this process. However, the identity of a specific ROS

involved in the interaction between hyperglycemia and APC was not determined in the present investigation, and it is possible that the beneficial effect of *N*-acetylcysteine occurred through an alternative pathway, such as inhibition of advanced glycation end products.[41] This mechanism is unlikely to account for the current experimental findings, however, because the formation of advanced glycation end products requires long-term (days to weeks) exposure to hyperglycemia.

The area of the LV at risk for infarction and coronary collateral blood flow are important determinants of the extent of myocardial infarction; however, no differences in these variables were observed among experimental groups that would account for the current findings. Isoflurane caused similar hemodynamic effects in the presence or absence of hyperglycemia and *N*-acetylcysteine. Hyperglycemia and *N*-acetylcysteine alone had few hemodynamic effects, yet their presence significantly influenced the protective effect of isoflurane. Although it is unlikely that the hemodynamic effects of isoflurane, *N*-acetylcysteine, or hyperglycemia were responsible for the observed differences in infarct size, coronary venous oxygen tension was not measured and myocardial oxygen consumption was not directly quantified in the present investigation. Thus, changes in myocardial metabolism during the administration of isoflurane in the presence or absence of hyperglycemia or *N*-acetylcysteine cannot be completely excluded from the analysis. Experiments were conducted in an established model of myocardial infarction using acutely instrumented, barbiturate-anesthetized dogs, but these results may not be directly comparable to those in anesthetized humans.

In summary, the current results confirm our previous findings indicating that hyperglycemia abolishes the myocardial protection produced by isoflurane and demonstrate that pretreatment with the antioxidant *N*-acetylcysteine before the onset of hyperglycemia restores the beneficial effects of isoflurane in dogs. The current results further suggest that cardioprotective signal transduction during administration of isoflurane is sensitive to the oxidation–reduction state within the cardiac myocyte. Defining the precise relationships between ROS, mitochondrial K_{ATP} channels, and nitric oxide and their role in APC represent important goals of future research.

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