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Morphine Enhances Pharmacological Preconditioning by Isoflurane

Role of Mitochondrial K_{ATP} Channels and Opioid Receptors

Lynda M. Ludwig, B.S., Hemal H. Patel, Ph.D.,* Garrett J. Gross, Ph.D.,† Judy R. Kersten, M.D.,‡ Paul S. Pagel, M.D., Ph.D.,§ David C. Warltier, M.D., Ph.D.‖*

Background: **Adenosine triphosphate–regulated potassium channels mediate protection against myocardial infarction produced by volatile anesthetics and opioids. We tested the hypothesis that morphine enhances the protective effect of isoflurane by activating mitochondrial adenosine triphosphate–regulated potassium channels and opioid receptors.**

Methods: **Barbiturate-anesthetized rats (n = 131) were instrumented for measurement of hemodynamics and subjected to a 30 min coronary artery occlusion followed by 2 h of reperfusion. Myocardial infarct size was determined using triphenyltetrazolium staining. Rats were randomly assigned to receive 0.9% saline, isoflurane (0.5 and 1.0 minimum alveolar concentration [MAC]), morphine (0.1 and 0.3 mg/kg), or morphine (0.3 mg/kg) plus isoflurane (1.0 MAC). Isoflurane was administered for 30 min and discontinued 15 min before coronary occlusion. In eight additional groups of experiments, rats received 5-hydroxydecanoic acid (5-HD; 10 mg/kg) or naloxone (6 mg/kg) in the presence or absence of isoflurane, morphine, and morphine plus isoflurane.**

Results: **Isoflurane (1.0 MAC) and morphine (0.3 mg/kg) reduced infarct size (41 ± 3%; n = 13 and 38 ± 2% of the area at risk; n = 10, respectively) as compared to control experiments (59 ± 2%; n = 10). Morphine plus isoflurane further decreased infarct size to 26 ± 3% (n = 11). 5-HD and naloxone alone did not affect infarct size, but abolished cardioprotection produced by isoflurane, morphine, and morphine plus isoflurane.**

Conclusions: **Combined administration of isoflurane and morphine enhances the protection against myocardial infarction to a greater extent than either drug alone. This beneficial effect is mediated by mitochondrial adenosine triphosphate–regulated potassium channels and opioid receptors *in vivo*.**

VOLATILE anesthetics and opioids protect myocardium against ischemia and reperfusion injury. Previous studies indicate that halothane,[1] enflurane,[1,2] isoflurane,[1] and sevoflurane[3] improve function of isolated rat and rabbit hearts subjected to global ischemia and reperfusion. Halothane[4,5] and isoflurane[6,7] enhance the functional recovery of stunned myocardium and reduce infarct size in dogs. Isoflurane also mimics ischemic preconditioning in human myocardium *in vitro*.[8] Stimulation of the δ_1-opioid receptor elicits a cardioprotective effect that is abolished by selective antagonists.[9–13] Activation of this receptor also appears to mediate both the acute and delayed phases of ischemic preconditioning.[14] Morphine, a μ receptor agonist with δ_1 receptor agonist properties, has been shown to mimic ischemic preconditioning in embryonic chick cardiac myocytes[15,16] and isolated rat hearts.[17]

The mitochondrial adenosine triphosphate–sensitive potassium (K_{ATP}) channel has been implicated as a primary mediator of ischemic, volatile anesthetic–, and opioid-induced preconditioning.[7,12,18,19] K_{ATP} channel antagonists abolish the cardioprotective effects of volatile anesthetics[6–8,20–22] and opioids.[9,12,13,15–17] Recently, our laboratory demonstrated that combined administration of isoflurane and selective δ_1-opioid receptor agonists potentiates K_{ATP} channel opening and enhances protection against myocardial ischemia and reperfusion injury.[23] This action is abolished by the nonselective K_{ATP} channel antagonist glyburide. This study provided the impetus to test the current hypothesis that morphine enhances isoflurane-induced protection against myocardial infarction. We further hypothesized that this protective effect is associated with an acute memory period and is mediated by activation of mitochondrial K_{ATP} channels and opioid receptors in rats.

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 (Milwaukee, Wisconsin). Furthermore, all conformed to the *Guiding Principles in the Care and Use of Animals*[24] of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals*.[25]

Drugs

Isoflurane was purchased from Abbott Laboratories (Chicago, IL). Morphine sulfate was purchased from Elkins-Sinn (Cherry Hill, NJ). Thiobutabarbital sodium, sodium 5-hydroxydecanoic acid (5-HD), and naloxone

* Graduate Student in Pharmacology and Toxicology, † Professor of Pharmacology and Toxicology, ‡ Professor of Anesthesiology and Pharmacology and Toxicology, § 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, the Medical College of Wisconsin.

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Address reprint requests to Dr. Warltier: Medical College of Wisconsin, MEB-M4280, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226. Address electronic mail to: cknapp@mcw.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

hydrochloride dihydrate were purchased from Sigma Research Biochemicals Incorporated (Natick, MA) and dissolved in saline.

General Preparation

Male Wistar rats weighing between 270 and 430 g were anesthetized with intraperitoneal thiobutabarbital sodium (100–150 mg/kg). Rats were adequately sedated to ensure that pedal and palpebral reflexes were absent throughout the experimental protocol. Heparin-filled catheters were inserted into the right jugular vein and the right carotid artery for fluid or drug administration and measurement of arterial blood pressure, respectively. A tracheotomy was performed, the trachea was intubated with a cannula connected to a rodent ventilator (model 683; Harvard Apparatus, South Natick, MA), and the lungs were ventilated with an air–oxygen mixture. A positive end-expiratory pressure of 5–10 cm H_2O was applied to minimize the development of atelectasis. Arterial blood gas tensions and acid–base status were monitored at regular intervals and maintained within a normal physiologic range (pH, 7.35–7.45; arterial carbon dioxide tension ($Paco_2$), 25–40 mmHg; and arterial oxygen tension (Pao_2), 90–150 mmHg) by adjusting the respiratory rate or tidal volume throughout the experiment. Body temperature was maintained at 36°C using a heating blanket. A left thoracotomy was performed in the fifth intercostal space, and the pericardium was opened. A 6-0 prolene ligature was placed around the proximal left descending coronary artery and vein in the area immediately below the left atrial appendage. The ends of the suture were threaded through a propylene tube to form a snare. Coronary artery occlusion was produced by clamping the snare onto the epicardial surface of the heart with a hemostat and was confirmed by the appearance of epicardial cyanosis. Reperfusion was achieved by unclamping the hemostat and loosening the snare and was verified by observing an epicardial hyperemic response. Hemodynamics were continuously recorded on a polygraph (model 7E; Grass Instruments, Quincy, MA) and digitized using a computer interfaced with an analog-to-digital converter. Rate–pressure product, an index of myocardial oxygen consumption, was calculated by multiplying the heart rate and mean arterial pressure.

Experimental Protocol

The experimental design used in the current investigation is illustrated in figure 1. All rats underwent 30 min of coronary artery occlusion followed by 2 h of reperfusion. Rats were randomly assigned to receive intravenous vehicle (0.9% saline), morphine (0.1 and 0.3 mg/kg), isoflurane (0.5 and 1.0 minimum alveolar concentration [MAC]), or the combination of morphine (0.3 mg/kg) and isoflurane (1.0 MAC). In eight additional groups of experiments, rats received 5-HD (10 mg/kg) or naloxone (6 mg/kg) in the presence or absence of isoflurane (1.0 MAC) and morphine

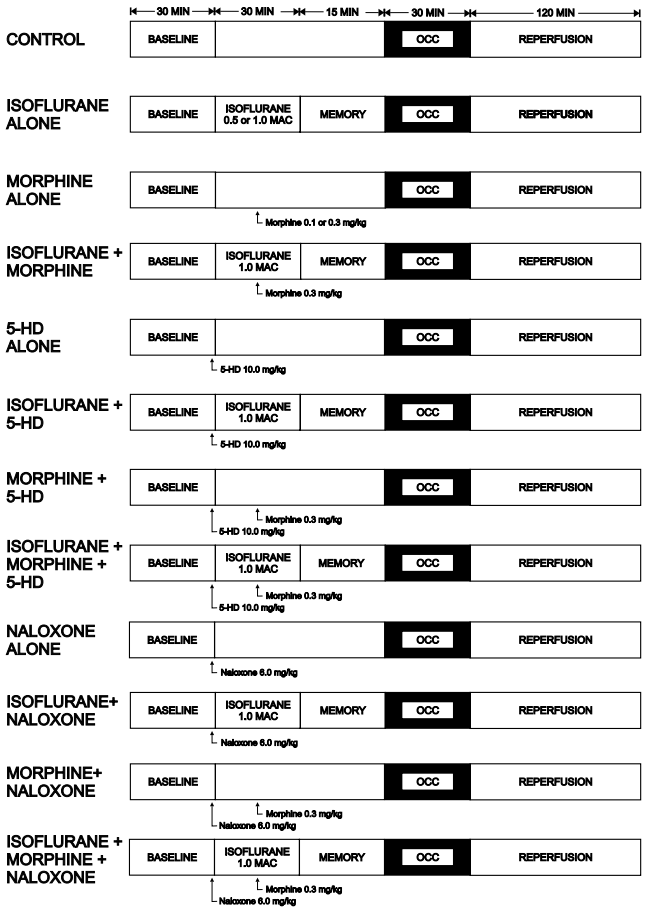

Fig. 1. Schematic illustration of experimental protocols. Isoflurane was administered for 30 min and discontinued 15 min (memory period) before coronary artery occlusion (OCC). Morphine was administered 30 min prior to the occlusion. 5-Hydroxydecanoic acid (5-HD) and naloxone were administered 50 min before occlusion.

(0.3 mg/kg) alone and in combination. Vehicle, morphine, 5-HD, and naloxone were administered intravenously. Morphine was given 30 min before occlusion. 5-HD and naloxone were given 50 min prior to occlusion. Isoflurane was administered *via* a vaporizer (model 100F; Ohio Medical Products, Madison, WI) for 30 min and discontinued 15 min (memory period) before coronary artery occlusion. End-tidal concentrations of isoflurane were measured using an infrared gas analyzer that was calibrated with known standards before and during experimentation. The MAC value of isoflurane used for rats in the current investigation was 1.4%.[26] Following discontinuation of the volatile anesthetic, end-tidal concentrations of isoflurane decreased to zero before coronary occlusion.

Determination of Infarct Size

Myocardial infarct size was measured as previously described.[27] Briefly, the coronary artery was reoccluded after the 2-h reperfusion period. Patent blue dye was administered intravenously to stain the normal region of the left ventricle (LV), and the heart was rapidly excised.

The LV was separated from the remaining tissue and cut into 5 or 6 cross-sectional pieces of 2 mm in thickness. The blue-stained LV normal zone was separated from the nonstained LV area at risk (AAR) and incubated at 37°C for 15 min in 1% 2,3,5-triphenyltetrazolium chloride in 0.1 M phosphate buffer adjusted to pH 7.4. Tissues were fixed overnight in 10% formaldehyde, and the infarcted tissue was carefully separated from the AAR using a dissecting microscope. Infarct size and AAR size were determined by gravimetric analysis. Infarct size was expressed as a percentage of the LV AAR.

Statistical Analysis

Statistical analysis of data within and between groups was performed with multiple analysis of variance for repeated measures followed by Student-Newman-Keuls test. Statistical significance was defined as $P < 0.05$. All values are expressed as mean ± SEM.

Results

One hundred thirty-one rats were instrumented to obtain 103 successful experiments. Twelve rats were excluded as a result of technical difficulties with the experimental preparation. Sixteen other rats developed malignant ventricular arrhythmias before completion of the experiment and were excluded from further analysis.

Systemic Hemodynamics

No significant differences in systemic hemodynamics or the rate-pressure product were observed between experimental groups under baseline conditions (table 1). Isoflurane significantly ($P < 0.05$) decreased heart rate, mean arterial pressure, and rate–pressure product in the presence or absence of morphine, 5-HD, or naloxone. Hemodynamics returned to baseline values 15 min after isoflurane had been discontinued (memory period) prior to coronary occlusion. Administration of morphine, 5-HD, or naloxone did not affect hemodynamics. Coronary artery occlusion and reperfusion produced similar decreases in heart rate, mean arterial pressure, and rate–pressure product between experimental groups.

Myocardial Infarct Size

The AAR mass (range, 0.25 ± 0.03 to 0.37 ± 0.03 g) and AAR as a percent of LV mass (range, 39 ± 2 to 56 ± 3%) were similar among groups. 5-HD, naloxone, isoflurane (0.5 MAC), and morphine (0.1 mg/kg) did not affect infarct size (54 ± 5% [n = 6], 53 ± 3% [n = 6], 51 ± 4% [n = 9], and 44 ± 4% of the LV AAR [n = 10], respectively) as compared to control experiments (59 ± 2% [n = 10]; fig. 2). In contrast, 1.0 MAC isoflurane and 0.3 mg/kg morphine reduced infarct size (41 ± 3% [n = 13] and 38 ± 2% [n = 10], respectively; fig. 2). The combination of morphine (0.3 mg/kg) and isoflurane (1.0 MAC) produced a marked reduction in infarct size (26 ± 3% [n = 11]; fig. 3). 5-HD eliminated the protection produced by isoflurane alone, morphine alone, or the combination of morphine and isoflurane (60 ± 4% [n = 6], 53 ± 4% [n = 6], and 52 ± 3% [n = 6], respectively; fig. 3). Naloxone also abolished reductions in myocardial infarct size produced by isoflurane alone, morphine alone, or the combination of morphine and isoflurane (56 ± 6% [n = 6], 53 ± 3% [n = 6], and 48 ± 4% [n = 7], respectively; fig. 4).

Discussion

A large body of evidence indicates that K_{ATP} channel opening is a critical element in the signal transduction responsible for anesthetic-induced protection against myocardial ischemic injury. We have previously demonstrated that reductions in myocardial infarct size produced by isoflurane in dogs are associated with an acute memory period and are abolished by glyburide.[7] Isoflurane also mimics preconditioning in rabbit hearts,[1,20,28] an action that is inhibited by 5-HD.[20] More recently, we used the selective sarcolemmal and mitochondrial K_{ATP} channel antagonists HMR-1098 and 5-HD, respectively, to demonstrate that both these channels play a role in the protective effects of desflurane in dogs.[21] A number of investigations have also implicated activation of K_{ATP} channels in opioid-induced protection against ischemic damage. The selective δ_1-opioid receptor agonist TAN-67 exerts a protective effect that is sensitive to inhibition by K_{ATP} channel antagonists.[9,12,13] Morphine reduces infarct size in the intact rat heart, and this beneficial effect is blocked by glyburide.[17] Morphine-induced preconditioning of isolated cardiac myocytes is inhibited by glyburide[16] and 5-HD.[15,16] Taken together, these data suggest that both volatile anesthetics and opioids protect myocardium against ischemia by activating K_{ATP} channels.

We recently demonstrated that isoflurane and the selective δ_1-opioid agonists TAN-67 and BW373U86 protect rat myocardium against infarction and potentiate the antiischemic effects of the mitochondrial K_{ATP} channel agonist diazoxide.[23] Administration of isoflurane in combination with these δ_1 agonists substantially enhances the protective effect of either drug alone. The current results confirm and extend these findings by demonstrating that the clinically relevant opioid morphine markedly enhances the reduction in myocardial infarct size produced by isoflurane. In contrast to the previous study, the current investigation demonstrates that isoflurane elicits protective effects following discontinuation for 15 min before coronary artery occlusion, suggesting that isoflurane-induced cardioprotection in the *in vivo* rat is associated with an acute memory phase. The current results also indicate that 5-HD and naloxone inhibit the

Table 1. Systemic Hemodynamics

						Reperfusion, h	
	Rats, n	Baseline	Isoflurane	Preocclusion	30 min CAO	1	2
Heart rate, beats/min	—	—	—	—	—	—	—
Control	10	380 ± 13	–	364 ± 15	369 ± 13	329 ± 10*	322 ± 10*
Isoflurane, 0.5 MAC	9	386 ± 12	339 ± 9*	360 ± 10*	370 ± 11*	324 ± 12*	333 ± 13*
Isoflurane, 1.0 MAC	13	382 ± 12	295 ± 6*	353 ± 8*	357 ± 9*	304 ± 11*	304 ± 6*
Morphine, 0.1 mg/kg	10	398 ± 13	–	372 ± 9*	366 ± 11*	330 ± 13*	314 ± 10*
Morphine, 0.3 mg/kg	10	357 ± 2	–	361 ± 11	346 ± 11	321 ± 16*	311 ± 15*
Isoflurane + Morphine	11	388 ± 13	300 ± 9*	369 ± 11	366 ± 12	314 ± 10*	314 ± 10*
5-HD	6	382 ± 15	–	370 ± 9	376 ± 12	337 ± 12*	336 ± 12*
Isoflurane + 5-HD	6	392 ± 20	286 ± 11*	347 ± 19*	358 ± 18*	300 ± 15*	305 ± 10*
Morphine+ 5-HD	6	386 ± 7	–	359 ± 13*	363 ± 16*	315 ± 15*	273 ± 15*
Isoflurane + Morphine+ 5-HD	6	390 ± 13	281 ± 15*	356 ± 17	365 ± 19	338 ± 16*	320 ± 16*
Naloxone	6	402 ± 13	–	374 ± 13*	378 ± 10*	363 ± 7*	346 ± 4*
Isoflurane + Naloxone	6	385 ± 9	289 ± 10*	358 ± 16*	348 ± 13*	328 ± 20*	307 ± 14*
Morphine+ Naloxone	6	389 ± 9	–	376 ± 10	378 ± 12	334 ± 12*	321 ± 16*
Isoflurane + Morphine+ Naloxone	7	404 ± 12	302 ± 14*	356 ± 23*	381 ± 13	334 ± 19*	324 ± 16*
MAP, mmHg	—	—	—	—	—	—	—
Control	10	122 ± 7	–	111 ± 7	107 ± 8	79 ± 4*	65 ± 6*
Isoflurane, 0.5 MAC	9	99 ± 8	76 ± 6*	90 ± 5	90 ± 5	55 ± 2*	56 ± 2*
Isoflurane, 1.0 MAC	13	110 ± 4	65 ± 2*	105 ± 4	101 ± 4	58 ± 4*	52 ± 5*
Morphine, 0.1 mg/kg	10	116 ± 4	–	116 ± 3	100 ± 6*	81 ± 5*	68 ± 4*
Morphine, 0.3 mg/kg	10	107 ± 7	–	110 ± 5	96 ± 6*	73 ± 8*	64 ± 8*
Isoflurane + MOR	11	110 ± 5	67 ± 6*	100 ± 5*	97 ± 5*	65 ± 5*	53 ± 3*
5-HD	6	108 ± 6	–	110 ± 5	105 ± 6	71 ± 6*	66 ± 6*
Isoflurane + 5-HD	6	115 ± 4	64 ± 3*	105 ± 8	98 ± 9*	71 ± 8*	58 ± 6*
Morphine + 5-HD	6	117 ± 6	–	119 ± 9	114 ± 10	74 ± 8*	54 ± 7*
Isoflurane + Morphine + 5-HD	6	113 ± 2	58 ± 6*	95 ± 4*	96 ± 4*	74 ± 7*	54 ± 8*
Naloxone	6	102 ± 5	–	104 ± 4	98 ± 5	66 ± 5*	55 ± 7*
Isoflurane + Naloxone	6	110 ± 6	71 ± 4*	110 ± 7	95 ± 11	75 ± 13*	61 ± 6*
Morphine + Naloxone	6	111 ± 5	–	111 ± 4	107 ± 7	68 ± 7*	55 ± 2*
Isoflurane + Morphine + Naloxone	7	115 ± 3	78 ± 5*	112 ± 5	108 ± 3	71 ± 5*	54 ± 4*
RPP, $min^{-1} \cdot mmHg \cdot 10^{-3}$)	—	—	—	—	—	—	—
Control	10	52.6 ± 3.8	–	46.3 ± 5.3	43.9 ± 4.2*	31.9 ± 2.3*	26.5 ± 2.8*
Isoflurane, 0.5 MAC	9	46.6 ± 3.8	32.6 ± 2.0*	40.1 ± 2.0*	39.2 ± 2.4*	24.7 ± 9.9*	25.1 ± 1.4*
Isoflurane, 1.0 MAC	13	37.8 ± 6.1	18.3 ± 2.8*	31.6 ± 6.1*	30.3 ± 4.9*	17.2 ± 3.0*	14.8 ± 2.6*
Morphine, 0.1 mg/kg	10	53.1 ± 2.7	–	50.4 ± 2.0	42.5 ± 3.0*	33.6 ± 2.4*	28.3 ± 1.4*
Morphine, 0.3 mg/kg	10	45.6 ± 3.0	–	47.6 ± 2.8	39.1 ± 2.8*	29.8 ± 3.4*	26.0 ± 3.2*
Isoflurane + Morphine	11	51.6 ± 4.0	27.0 ± 2.0*	44.7 ± 3.2*	42.8 ± 2.9*	27.7 ± 2.0*	23.5 ± 1.6*
5-HD	6	48.1 ± 3.0	–	48.7 ± 2.3	46.1 ± 2.5	31.5 ± 2.5*	30.0 ± 2.4*
Isoflurane + 5-HD	6	54.4 ± 3.7	25.5 ± 1.2*	45.4 ± 4.6*	42.5 ± 4.3*	27.8 ± 3.1*	24.0 ± 2.8*
Morphine+ 5-HD	6	54.1 ± 3.5	–	51.2 ± 4.5	48.4 ± 5.6	28.8 ± 3.2*	19.8 ± 2.7*
Isoflurane + Morphine + 5-HD	6	50.7 ± 1.8	21.9 ± 2.4*	41.0 ± 3.4*	41.0 ± 3.4*	31.6 ± 3.3*	22.1 ± 2.6*
Naloxone	6	50.0 ± 3.6	–	47.0 ± 3.0	43.3 ± 2.9*	30.9 ± 2.3*	25.8 ± 2.5*
Isoflurane + Naloxone	6	53.1 ± 3.0	27.9 ± 1.8*	48.6 ± 3.7	39.6 ± 5.1*	33.5 ± 6.3*	24.9 ± 2.8*
Morphine + Naloxone	6	52.1 ± 1.2	–	50.1 ± 1.5	47.5 ± 3.5	30.9 ± 2.5*	25.3 ± 1.4*
Isoflurane + Morphine + Naloxone	7	55.5 ± 2.3	30.7 ± 2.6*	47.9 ± 4.1*	47.0 ± 2.5*	30.4 ± 2.9*	24.3 ± 2.2*

Data shown as mean ± SEM.

*$P < 0.05$, significant difference compared with baseline.

5-HD, 5-hydroxydecanoic acid; CAO, coronary artery occlusion; MAP, mean arterial pressure; RPP, rate-pressure product.

protective effects of both morphine and isoflurane. These data imply that mitochondrial K_{ATP} channel activation and opioid receptor stimulation mediate these beneficial actions.

Multiple intracellular signaling pathways have been implicated in endogenous protection against ischemia, but all appear to act through the K_{ATP} channel as the end effector. Patch clamp recordings conducted in rabbit cardiac myocytes indicate that both adenosine and protein kinase C (PKC) synergistically enhance K_{ATP} channel activity.[29] Flavoprotein oxidation experiments also demonstrate that adenosine potentiates diazoxide-induced opening of mitochondrial K_{ATP} channels through PKC activation.[30] Similar signaling elements have been shown to elicit the protective effects of volatile anesthetics and opioids. We have shown that isoflurane[7] and desflurane[21] reduce myocardial infarct size in dogs by K_{ATP} channel activation. Isoflurane also activates adenosine receptors[31] and PKC[32] to enhance the functional recovery of stunned myocardium. Mitochondrial K_{ATP} channel activity is increased by sevoflurane in rat ventricular myocytes, and this effect is abrogated by either adenosine receptor antagonism or PKC inhibition.[33] Several reports indicate that myocardial protection pro-

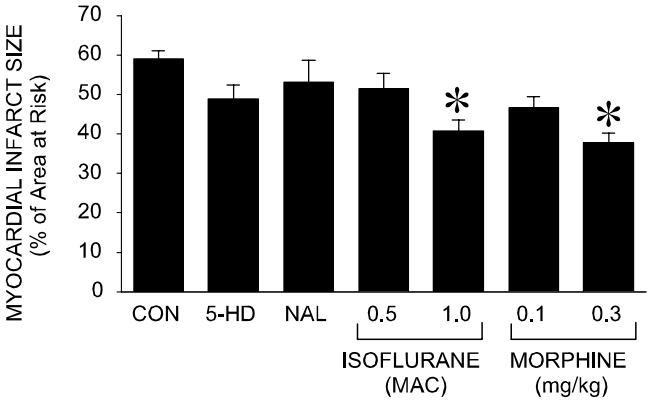

Fig. 2. Myocardial infarct size expressed as a percentage of the left ventricular area at risk in rats receiving saline (CON), 5-hydroxydecanoic acid (5-HD) alone, naloxone (NAL) alone, isoflurane, or morphine. *Significantly ($P < 0.05$) different from CON.

duced by morphine[15–17] and δ_1-opioid agonists[9,12,13] is abolished by K_{ATP} channel blockade. Recently, it was demonstrated that the δ_1 agonist TAN-67 causes PKC-δ isoform translocation to rat mitochondrial membranes and, further, that inhibition of PKC-δ translocation or blockade of δ_1-opioid receptors attenuates opioid-induced preconditioning.[11] In view of the previous data, the current results suggest that the combined administration of a volatile anesthetic and an opioid may activate signal transduction pathways that converge on the mitochondrial K_{ATP} channel to produce greater myocardial protection than that observed with either drug alone.

Despite this evidence suggesting that volatile anesthetics and opioids potentiate receptor-mediated events, it remains unclear whether these drugs also directly modulate intracellular signaling mediators. A recent study

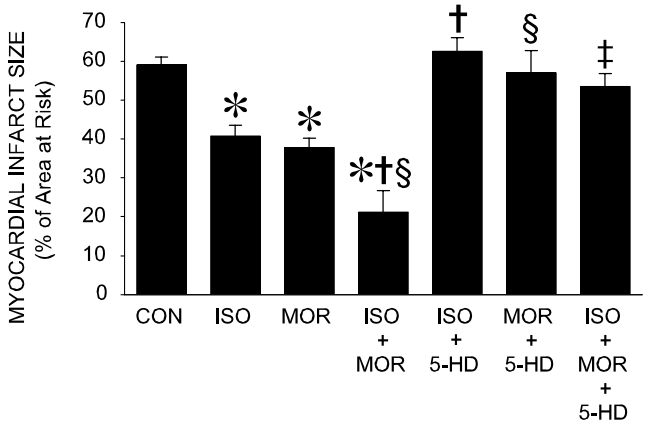

Fig. 3. Myocardial infarct size expressed as a percentage of the left ventricular area at risk in rats receiving saline (CON), 1.0 MAC isoflurane (ISO), 0.3 mg/kg morphine (MOR), the combination of 1.0 MAC isoflurane and 0.3 mg/kg morphine (ISO + MOR), and 5-hydroxydecanoic acid in the presence of isoflurane (ISO + 5-HD) or morphine (MOR + 5-HD) and in the presence of isoflurane and morphine (ISO + MOR + 5-HD). *Significantly ($P < 0.05$) different from CON. †Significantly ($P < 0.05$) different from ISO. §Significantly ($P < 0.05$) different from MOR. ‡Significantly ($P < 0.05$) different from ISO + MOR.

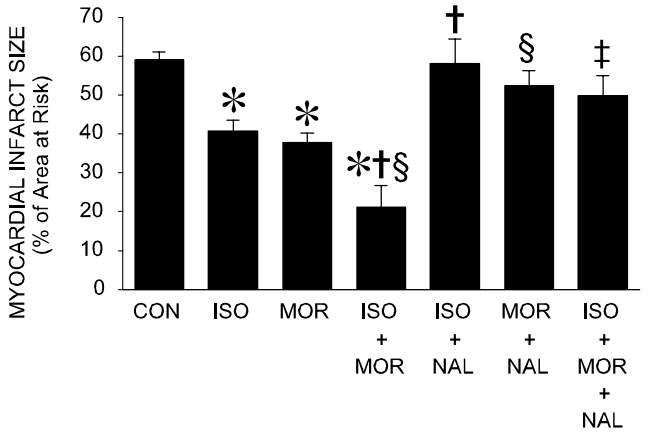

Fig. 4. Myocardial infarct size expressed as a percentage of the left ventricular area at risk in rats receiving saline (CON), 1.0 MAC isoflurane (ISO), 0.3 mg/kg morphine (MOR), the combination of 1.0 MAC isoflurane and 0.3 mg/kg morphine (ISO + MOR), and naloxone in the presence of isoflurane (ISO + NAL) or morphine (MOR + NAL) and in the presence of isoflurane and morphine (ISO + MOR + NAL). *Significantly ($P < 0.05$) different from CON. †Significantly ($P < 0.05$) different from ISO. §Significantly ($P < 0.05$) different from MOR. ‡Significantly ($P < 0.05$) different from ISO + MOR.

determined that isoflurane- and sevoflurane-induced increases in mitochondrial flavoprotein oxidation in isolated ventricular myocytes is inhibited by 5-HD.[34] Patch clamp recordings revealed that isoflurane exposure paradoxically inhibits sarcolemmal K_{ATP} channel opening in rabbit cardiac myocytes. However, subsequent elimination of isoflurane from the experimental preparation rendered the K_{ATP} channels less sensitive to adenosine triphosphate–induced closure of the channel, thereby increasing open probability.[35] We demonstrated that desflurane-induced decreases in myocardial infarct size are abolished by both 5-HD and the sarcolemmal K_{ATP} channel antagonist HMR-1098.[21] These data *in vitro* and *in vivo* support the contention that volatile anesthetics directly modulate K_{ATP} channel activity in both sarcolemmal and mitochondrial membranes. However, the preponderance of evidence collected to date indicates that the mitochondrial K_{ATP} channel is the predominant mediator of myocardial protection against ischemia. For example, the selective mitochondrial K_{ATP} channel agonist diazoxide reduces ventricular myocyte damage in a model of simulated ischemia, but the selective sarcolemmal K_{ATP} channel agonist P-1075 does not.[18] A recent investigation showed that isoflurane and sevoflurane preserve myocyte viability in a cellular model of ischemia, and this protective effect is attenuated by 5-HD but not HMR-1098.[33] Myocardial protection produced by δ_1 opioids is also inhibited by 5-HD but not HMR-1098.[12] The current results support the hypothesis that mitochondrial K_{ATP} channel activation is an essential signaling component in the enhanced protection produced by the combination of isoflurane and morphine, but the specific role of the sarcolemmal K_{ATP} channel in this pro-

cess was not defined in this study and will require additional investigation.

The current and previous[23] results demonstrate that opioid receptor activation mediates both isoflurane- and morphine-induced preconditioning. Morphine is highly selective for μ-opioid receptors, but myocardial protection produced by this drug is attenuated by the selective δ_1-opioid antagonist 7-benzylidenanoltroxone in isolated cardiac myocytes.[15,16] Thus, it is highly likely that morphine exerts protective effects through δ_1-opioid receptor activation. Previous results from our laboratory indicate that pertussis toxin abolishes cardioprotection produced by isoflurane, demonstrating that inhibitory guanine (G_i) nucleotide-binding proteins are linked to the signal transduction pathway that mediates anesthetic-induced preconditioning.[36] In addition, we have shown that adenosine receptor activation is associated with myocardial protection produced by volatile anesthetics.[31] A more recent investigation demonstrated that volatile anesthetic-induced preservation of ventricular myocyte viability during ischemia is also sensitive to adenosine receptor and G_i protein–mediated signaling blockade.[33] The current findings demonstrate that the nonselective opioid antagonist naloxone abolishes preconditioning by isoflurane, suggesting an important link between volatile anesthetics and another G protein–coupled family of receptors. Interestingly, volatile anesthetics have been shown to compete for the ligand-binding site of G protein–coupled receptors.[37] Recent investigations have also reported that isoflurane stimulates the production of reactive oxygen species that appear to play a crucial role in mediating myocardial protection.[38,39] In this regard, hydrogen peroxide has been shown to activate G_i and G_o proteins,[40,41] as well as various protein kinases that are involved in reducing cellular injury.[42–44] Thus, volatile anesthetics may stimulate the production of reactive oxygen species, which in turn may activate G proteins that are linked to cascades of signaling events that may ultimately activate mitochondrial K_{ATP} channels. It is also possible that volatile anesthetics may alter the pharmacodynamics of opioids *in vivo*. This intriguing finding requires additional study to clarify the apparent relation between volatile anesthetics and opioid receptors.

The current results must be interpreted within the constraints of several potential limitations. Although our findings indicate that isoflurane preconditions rat myocardium, a prior study using the isolated rat heart does not support this observation.[45] These contradictory data are likely explained by differences in experimental design, given that our current investigation used an *in vivo* model of regional ischemia and infarct size as the end point measured, whereas Martini *et al.*[45] demonstrated that isoflurane does not restore LV developed pressure or reduce creatine kinase in rat hearts subjected to global ischemia. Accordingly, myocardial infarct size is primarily determined by the size of the LV AAR and extent of coronary collateral perfusion. The AAR was similar between groups, and previous studies indicate that coronary collateral blood flow is minimal in rats.[46] Administration of isoflurane reduced many of the hemodynamic determinants of myocardial oxygen consumption, but mean arterial pressure returned to baseline values after isoflurane had been discontinued before coronary occlusion. It appears unlikely that transient alterations in systemic hemodynamics produced by isoflurane were responsible for the reductions in myocardial infarct size associated with administration of this volatile agent. However, myocardial oxygen consumption was not directly determined in the current investigation. Also, morphine alone produced comparable reductions in infarct size as compared to isoflurane in the absence of hemodynamic effects, similar to previous observations.[17] 5-HD and naloxone blocked reductions of infarct size during isoflurane and morphine but did not alter the hemodynamic effects of these drugs.

Other limitations of the study may be related to drug dose, specificity, and interaction. A previous investigation demonstrated that the efficacy of 5-HD was time-dependent in Wistar rats,[47] suggesting that 5-HD may exhibit variable pharmacokinetics among different species or animal strains. Because of this observation, higher doses of 5-HD and naloxone were used in these experiments to ensure effective antagonism of mitochondrial K_{ATP} channels and opioid receptors, respectively. Despite the possibility that higher drug doses may produce nonselective effects, 5-HD and naloxone alone had no effect on hemodynamic parameters or infarct size. In addition, barbiturates used to induce anesthesia in our animal model may potentially modulate the efficacy of volatile anesthetics to elicit protective effects. Recent investigations indicated that barbiturates prevent diazoxide-[48] and isoflurane-induced[34] mitochondrial K_{ATP} channel opening as determined by flavoprotein oxidation experiments in isolated ventricular myocytes. Nevertheless, similar antiischemic actions are produced by isoflurane in barbiturate-anesthetized rabbits[20,28] and dogs.[7]

In summary, the current results indicate that combined administration of isoflurane and morphine produces a marked reduction in myocardial infarct size that greatly exceeds the protection exerted by either drug alone in rats. The results also demonstrate that cardioprotection produced by isoflurane is associated with an acute memory period in rats, representing yet another *in vivo* animal model that may be utilized to examine the signaling pathways involved in mediating volatile anesthetic–induced preconditioning. The current results further indicate that 5-HD and naloxone abolish the protection produced by isoflurane and morphine. These findings strongly suggest that mitochondrial K_{ATP} channel opening and opioid receptor activation are crucial signaling elements in the enhanced cardioprotection conferred by

isoflurane and morphine *in vivo*. However, translation of experimental data about these beneficial actions into therapeutic modalities through a major clinical trial has yet to be resolved.

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