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Sarcolemmal and Mitochondrial Adenosine Triphosphate–dependent Potassium Channels

Mechanism of Desflurane-induced Cardioprotection

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Background: **Volatile anesthetic–induced preconditioning is mediated by adenosine triphosphate–dependent potassium (K_{ATP}) channels; however, the subcellular location of these channels is unknown. The authors tested the hypothesis that desflurane reduces experimental myocardial infarct size by activation of specific sarcolemmal and mitochondrial K_{ATP} channels.**

Methods: **Barbiturate-anesthetized dogs (n = 88) were acutely instrumented for measurement of aortic and left ventricular pressures. All dogs were subjected to a 60-min left anterior descending coronary artery occlusion followed by 3-h reperfusion. In four separate groups, dogs received vehicle (0.9% saline) or the nonselective K_{ATP} channel antagonist glyburide (0.1 mg/kg intravenously) in the presence or absence of 1 minimum alveolar concentration desflurane. In four additional groups, dogs received 45-min intracoronary infusions of the selective sarcolemmal (HMR 1098; 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or mitochondrial (5-hydroxydecanoate [5-HD]; 150 $\mu g \cdot kg^{-1} \cdot min^{-1}$) K_{ATP} channel antagonists in the presence or absence of desflurane. Myocardial perfusion and infarct size were measured with radioactive microspheres and triphenyltetrazolium staining, respectively.**

Results: **Desflurane significantly ($P < 0.05$) decreased infarct size to 10 ± 2% (mean ± SEM) of the area at risk as compared with control experiments (25 ± 3% of area at risk). This beneficial effect of desflurane was abolished by glyburide (25 ± 2% of area at risk). Glyburide (24 ± 2%), HMR 1098 (21 ± 4%), and 5-HD (24 ± 2% of area at risk) alone had no effects on myocardial infarct size. HMR 1098 and 5-HD abolished the protective effects of desflurane (19 ± 3% and 22 ± 2% of area at risk, respectively).**

Conclusion: **Desflurane reduces myocardial infarct size *in vivo*, and the results further suggest that both sarcolemmal and mitochondrial K_{ATP} channels could be involved. (Key words: Myocardial ischemia.)**

ADENOSINE triphosphate–dependent potassium (K_{ATP}) channels mediate the protective effects of ischemia-[1,2] and volatile anesthetic–induced[3–6] preconditioning. The protective effects of ischemia[7,8] and volatile anesthetics[3,4,6,9] are blocked by the nonspecific K_{ATP} channel antagonist glyburide.[10–12] Sarcolemmal and mitochondrial K_{ATP} channels have recently been identified, and the subcellular locations of the K_{ATP} channels involved in ischemic preconditioning have been characterized. Sarcolemmal K_{ATP} channels were initially linked to protection during ischemia,[13,14] but subsequent evidence suggested that these channels were not solely responsible for this process.[15,16] More recently, a role for mitochondrial K_{ATP} channels in ischemic preconditioning has also been suggested.[17,18] The subcellular K_{ATP} channel sites involved in anesthetic-induced preconditioning are unknown. We tested the hypothesis that desflurane reduces experimental myocardial infarct size by activation of sarcolemmal and mitochondrial K_{ATP} channels *in vivo* using, respectively, the site-specific K_{ATP} channel antagonists HMR 1098 [1-[[5-[2-(5-chloro-*o*-anisamidoethyl]-2-methoxyphenyl]sulfonyl]-3-methylthiourea, sodium salt; fig. 1] and sodium 5-hydroxydecanoate (5-HD).[17–23]

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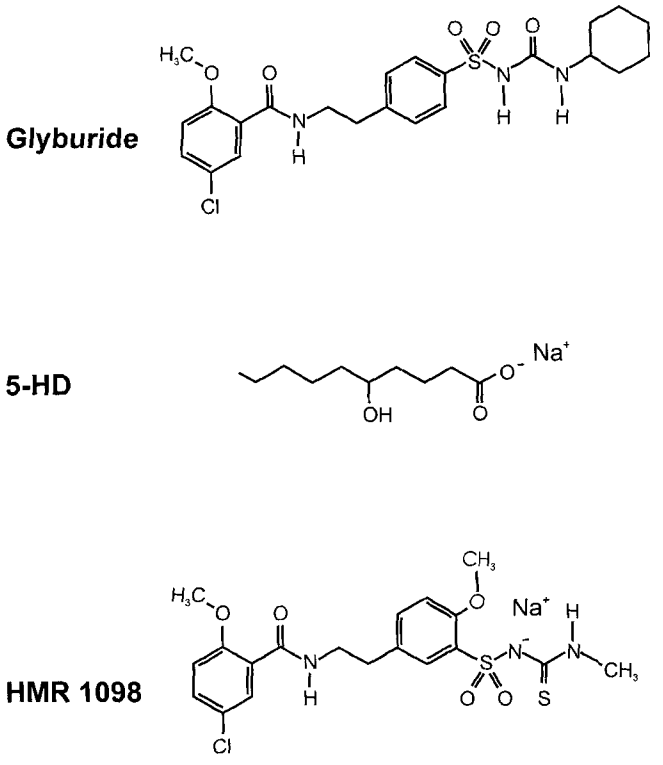

Fig. 1. Chemical structures of glyburide, 5-hydroxydecanoate (5-HD), and HMR 1098.

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. All procedures were in conformity with the *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society[24] and were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*.[25]

Surgical Preparation

The experimental methods have been previously described in detail.[9] Briefly, mongrel dogs (weight = 26 ± 1 kg, mean ± SEM) were anesthetized with sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg) and ventilated with an air and oxygen mixture (fraction of inspired oxygen = 0.25) after tracheal intubation. Tidal volume and respiratory rate were adjusted to maintain arterial blood gas tensions within a physiologic range. After calibration, a double pressure transducer-tipped catheter was inserted into the aorta and left ventricle (LV) *via* the left carotid artery to measure aortic and LV pressures, respectively. The maximum rate of increase of LV pressure ($+dP/dt_{max}$) was obtained by electronic differentiation of the LV pressure waveform. The femoral artery and vein were cannulated for the withdrawal of reference blood flow samples and fluid administration, respectively. A thoracotomy was performed at the left fifth intercostal space. A heparin-filled catheter was inserted into the left atrial appendage for administration of radioactive microspheres. A 1.0-cm segment of the left anterior descending (LAD) coronary artery was dissected immediately distal to the first diagonal branch, and a silk ligature was positioned around this vessel for production of coronary artery occlusion and reperfusion. Regional myocardial perfusion was measured in the ischemic (LAD) and normal (left circumflex coronary artery) zones using radioactive microspheres.[9] Myocardial infarct size was determined with triphenyltetrazolium chloride staining at the completion of each experiment.[26] End-tidal concentrations of desflurane were measured at the tip of the endotracheal tube by an infrared anesthetic gas analyzer that was calibrated with known standards before and during experimentation. The canine minimum alveolar concentration value of desflurane used in the present investigation was 7.2%.[27] Hemodynamic data were continuously monitored throughout the experiment, recorded on a polygraph, and digitized using a computer interfaced with an analog-to-digital converter.

Experimental Protocol

The experimental design used in the present investigation is illustrated in figure 2. Ninety minutes after completion of the surgical preparation, dogs were randomly assigned to one of eight experimental groups. All dogs underwent a 60-min LAD occlusion followed by 3-h reperfusion. In four groups of experiments, dogs received 0.9% saline (control) or the nonspecific K_{ATP} channel antagonist glyburide (0.1 mg/kg intravenously) in the presence and absence of 1.0 minimum alveolar concentration desflurane (end-tidal concentration). These experiments tested the hypothesis that desflurane reduces myocardial infarct size by K_{ATP} channel activation. To determine further whether the myocardial protection produced by desflurane was related to sarcolemmal or mitochondrial K_{ATP} channels, four additional

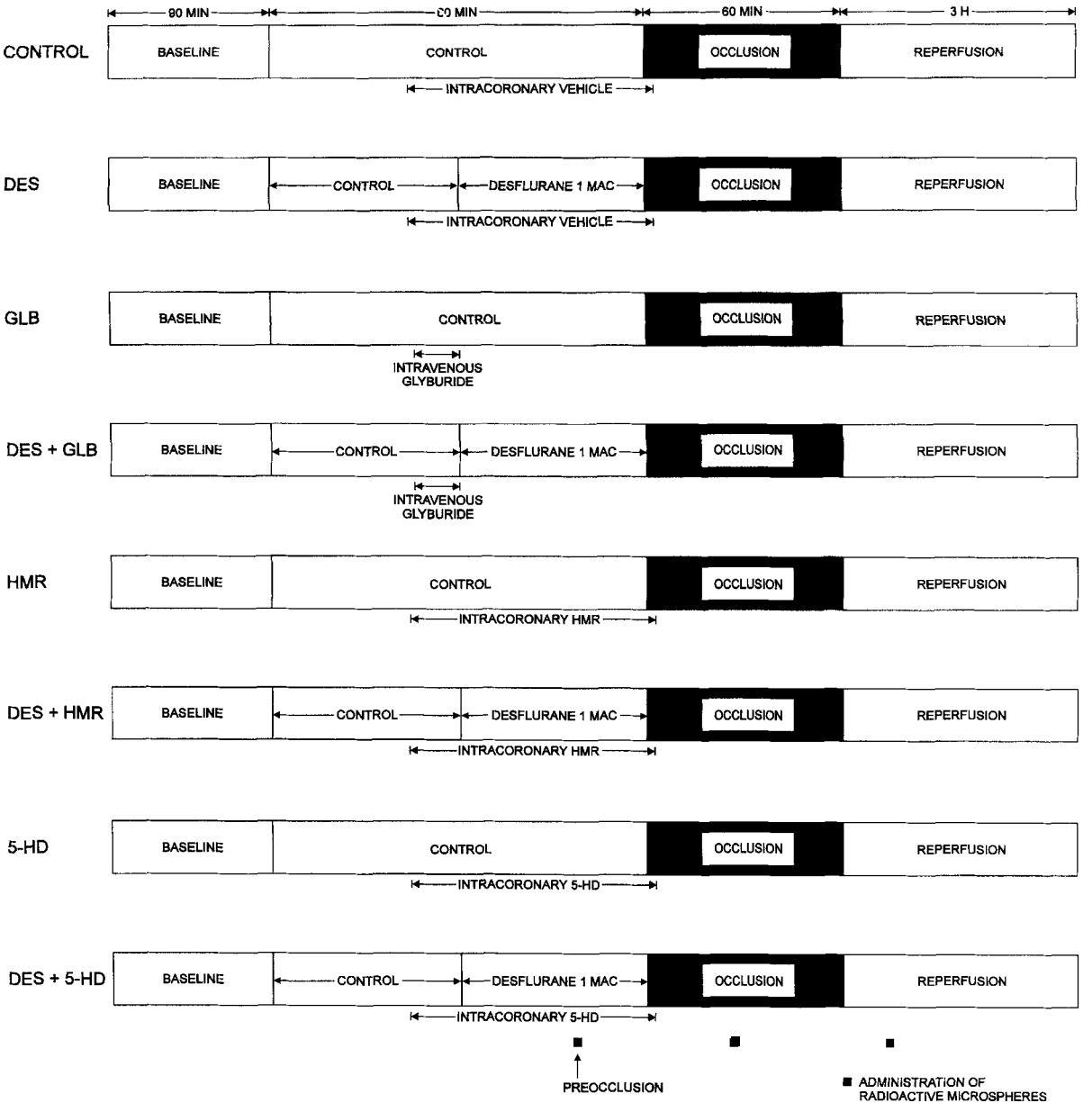

Fig. 2. Schematic illustration of the experimental protocol used in the present investigation. DES = desflurane; GLB = glyburide; 5-HD = 5-hydroxydecanoate.

groups of dogs were pretreated with intracoronary infusions of HMR 1098 (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in 10 ml 0.9% saline over 45 min, a dose comparable to that used previously *via* an intravenous route,[19]) or 5-HD (150 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in 10 ml 0.9% saline over 45 min, a dose previously used to block ischemic preconditioning in dogs[1]) in the presence or absence of 1.0 minimum alveolar concentration desflurane. Infusions of HMR 1098 and 5-HD were initiated 10 min before, continued during, and discontinued 5 min after the administration of desflurane.

Statistical Analysis

Statistical analysis of data within and between groups was performed with multiple analysis of variance for repeated measures with *post hoc* analysis by Student-Newman-Keuls test. Changes within and between

Table 1. Systemic Hemodynamics

	Baseline	Preocclusion	30 min CAO	Reperfusion 1 h	Reperfusion 2 h	Reperfusion 3 h
HR (beats/min)						
CON	137 ± 5	132 ± 5	129 ± 5	130 ± 8	129 ± 8	126 ± 7
DES	131 ± 4	111 ± 4*	123 ± 3	121 ± 6	130 ± 6	132 ± 7
GLB	127 ± 4	124 ± 4	124 ± 4	128 ± 6	126 ± 5	129 ± 5
DES + GLB	138 ± 4	116 ± 4*	134 ± 6	136 ± 6	142 ± 5	143 ± 7
HMR	134 ± 6	130 ± 8	132 ± 7	130 ± 5	126 ± 5	127 ± 5
DES + HMR	137 ± 7	111 ± 4*	130 ± 5	134 ± 5	133 ± 5	131 ± 6
5-HD	140 ± 7	137 ± 8	144 ± 13	141 ± 7	144 ± 6	143 ± 7
DES + 5-HD	130 ± 6	110 ± 4*§	124 ± 5	118 ± 4*	117 ± 5*§	123 ± 5
MAP (mmHg)						
CON	97 ± 4	94 ± 3	89 ± 5	102 ± 8	107 ± 6	103 ± 6
DES	94 ± 5	64 ± 4*†	85 ± 4	91 ± 4	100 ± 1	106 ± 1*
GLB	87 ± 3	92 ± 4	87 ± 4	104 ± 6	108 ± 7*	101 ± 9
DES + GLB	96 ± 4	63 ± 6*†‡	85 ± 3	98 ± 4	100 ± 3	98 ± 3
HMR	85 ± 5	84 ± 1	77 ± 3	90 ± 6	96 ± 6	97 ± 6
DES + HMR	91 ± 4	59 ± 2*†‖	80 ± 4*	95 ± 3	96 ± 2	96 ± 3
5-HD	92 ± 6	95 ± 6	80 ± 10	97 ± 2	104 ± 5	101 ± 5
DES + 5-HD	97 ± 6	64 ± 4*†§	80 ± 6	91 ± 7	92 ± 6	93 ± 6
RPP (beats/min^{-1} · mmHg^{-1} · 10^{-3})						
CON	14.5 ± 0.8	13.7 ± 0.6	12.2 ± 0.7	14.0 ± 1.2	14.5 ± 1.1	13.6 ± 0.7
DES	13.8 ± 1.0	8.3 ± 0.6*†	11.4 ± 0.8*	11.9 ± 0.9	13.8 ± 0.7	14.8 ± 0.8
GLB	12.2 ± 0.6	12.7 ± 0.7	11.6 ± 0.8	14.2 ± 1.1	14.6 ± 1.1	13.6 ± 1.5
DES + GLB	15.0 ± 0.9	9.0 ± 1.1*†‡	12.7 ± 0.9	14.9 ± 1.2	15.5 ± 1.0	15.5 ± 1.2
HMR	12.8 ± 1.3	12.1 ± 0.9	11.1 ± 0.9	12.1 ± 0.9	12.6 ± 1.0	12.8 ± 1.0
DES + HMR	14.2 ± 1.2	7.9 ± 0.4*†‖	11.6 ± 0.9*	13.8 ± 0.9	14.0 ± 0.7	13.8 ± 1.0
5-HD	14.4 ± 1.5	14.6 ± 1.6	13.0 ± 2.9	14.5 ± 0.9	15.7 ± 1.1	15.3 ± 1.2
DES + 5-HD	14.0 ± 0.9	8.5 ± 0.7*†§	11.0 ± 1.1*	11.7 ± 1.1*	11.3 ± 1.1*	12.3 ± 1.1*
LVSP (mmHg)						
CON	108 ± 5	104 ± 3	95 ± 4	107 ± 8	113 ± 6	109 ± 5
DES	102 ± 4	72 ± 3*†	94 ± 5	96 ± 4	105 ± 2	110 ± 2
GLB	98 ± 5	104 ± 5	96 ± 5	112 ± 6	117 ± 7*	112 ± 7
DES + GLB	109 ± 4	78 ± 7*†‡	95 ± 3	107 ± 5	109 ± 5	107 ± 5
HMR	100 ± 5	97 ± 2	88 ± 3	96 ± 6	101 ± 5	102 ± 5
DES + HMR	103 ± 5	72 ± 2*†‖	87 ± 4*	105 ± 5	107 ± 4	109 ± 4
5-HD	105 ± 7	109 ± 6	90 ± 10	105 ± 3	112 ± 6	107 ± 5
DES + 5-HD	109 ± 5	75 ± 4*†§	88 ± 7*	100 ± 7	102 ± 6	103 ± 5
LVEDP (mmHg)						
CON	6 ± 1	6 ± 1	15 ± 2*	18 ± 2*	15 ± 3*	16 ± 3*
DES	7 ± 1	9 ± 1*	12 ± 1*	11 ± 1*	11 ± 1*	12 ± 1*
GLB	7 ± 1	10 ± 1†	17 ± 2*	17 ± 2*	17 ± 2*	17 ± 2*
DES + GLB	9 ± 1	11 ± 1†	20 ± 2*	19 ± 3*	19 ± 2*	19 ± 2*
HMR	10 ± 1	9 ± 1	16 ± 2*	16 ± 3*	16 ± 2*	16 ± 3*
DES + HMR	7 ± 1	9 ± 1	19 ± 2*	17 ± 1*	16 ± 1*	16 ± 2*
5-HD	8 ± 1	8 ± 1	15 ± 1*	15 ± 2*	15 ± 1*	16 ± 1*
DES + 5-HD	7 ± 1	9 ± 1	14 ± 2*	16 ± 1*	19 ± 1*	17 ± 1*
+dP/dt$_{max}$ (mmHg/s)						
CON	1870 ± 220	1860 ± 240	1450 ± 140	1530 ± 100	1540 ± 110	1350 ± 100*
DES	1700 ± 120	960 ± 60*†	1480 ± 80	1330 ± 80*	1430 ± 70	1430 ± 100
GLB	1720 ± 140	1720 ± 160	1550 ± 130	1590 ± 100	1520 ± 90	1500 ± 140
DES + GLB	1530 ± 120	970 ± 110*†‡	1590 ± 60	1750 ± 130	1730 ± 100	1660 ± 80
HMR	1540 ± 110	1480 ± 90	1280 ± 60*	1320 ± 130*	1260 ± 80*	1200 ± 70*
DES + HMR	1810 ± 120	1020 ± 50*	1460 ± 60*	1700 ± 80	1660 ± 60	1540 ± 100*
5-HD	1810 ± 150	1930 ± 150	1510 ± 200	1610 ± 90	1540 ± 80	1490 ± 70
DES + 5-HD	1630 ± 120	990 ± 80*†§	1320 ± 90*	1420 ± 90*	1330 ± 100*	1350 ± 60*

Data are mean ± SEM.

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

† Significantly ($P < 0.05$) different from the corresponding value in dogs receiving saline (CON).

‡ Significantly ($P < 0.05$) different from the corresponding value in dogs receiving glyburide alone (GLB).

§ Significantly ($P < 0.05$) different from the corresponding value in dogs receiving 5-hydroxydecanoate alone (5-HD).

‖ Significantly ($P < 0.05$) different from the corresponding value in dogs receiving HMR 1098 alone (HMR).

CAO = coronary artery occlusion; HR = heart rate; MAP = mean aortic blood pressure; RPP = rate-pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressures, respectively; +dP/dt$_{max}$ = maximal rate of increase of left ventricular pressure; CON = control; DES = desflurane; GLB = glyburide; 5-HD = 5-hydroxydecanoate.

groups were considered statistically significant at $P < 0.05$. All data are expressed as mean ± SEM.

Results

Eighty-eight dogs were instrumented, and 63 successful experiments were completed. Four dogs were excluded because of intractable ventricular fibrillation during LAD occlusion or reperfusion (one control, one desflurane, one glyburide, one 5-HD). Fourteen dogs were excluded from analysis because transmural coronary collateral blood flow exceeded 0.2 ml · min^{-1} · g^{-1} (four desflurane, one glyburide, four 5-HD, one HMR 1098, one desflurane + 5-HD, three desflurane + HMR 1098). Four dogs were excluded because of technically difficult intracoronary catheter insertion (one desflurane, one 5-HD, one HMR 1098, one desflurane + HMR 1098). Two dogs were excluded because of the presence of heart worms (one desflurane, one HMR 1098). One dog was excluded because of profound hypotension throughout the experiment (HMR 1098).

Systemic Hemodynamics

No differences in baseline systemic hemodynamics were observed between experimental groups (table 1). Glyburide produced no hemodynamic effects. Desflurane caused significant ($P < 0.05$) decreases in heart rate, mean arterial and LV systolic pressures, rate-pressure product, and LV $+dP/dt_{max}$, and an increase in LV end-diastolic pressure. Desflurane produced similar hemodynamic effects in the presence and absence of glyburide. Intracoronary administration of HMR 1098 or 5-HD alone did not cause hemodynamic effects. The cardiovascular actions of desflurane were not affected by HMR 1098 or 5-HD pretreatment. LAD occlusion increased LV end-diastolic pressure in all groups, and there were no hemodynamic differences between groups during coronary artery occlusion or reperfusion.

Regional Myocardial Perfusion

Transmural myocardial blood flow in the ischemic (LAD) region is summarized in table 2. There were no intergroup differences in myocardial blood flow before or during LAD occlusion or reperfusion.

Myocardial Infarct Size

The area at risk was similar between groups (control, 44 ± 3%; desflurane, 42 ± 3%; glyburide, 43 ± 2%; desflurane + glyburide, 46 ± 1%; HMR, 46 ± 2%; desflurane + HMR, 46 ± 2%; 5-HD, 49 ± 2%; desflurane + 5-HD, 43 ± 1% of the LV). Desflurane significantly reduced myocardial infarct size to 10 ± 2% of the area at risk (fig. 3) compared with control experiments (25 ± 3%). Glyburide abolished the protective effects of desflurane (25 ± 2%) but had no effect on infarct size when administered alone (24 ± 2%). HMR 1098 and 5-HD did not affect infarct size (21 ± 4% and 24 ± 2%, respectively; figs. 4 and 5) but blocked the protective effects of desflurane (19 ± 3% and 22 ± 2%, respectively).

Table 2. Transmural Myocardial Blood Flow (ml · min^{-1} · g^{-1}) in the Ischemic Region

	Preocclusion	Coronary Artery Occlusion	Reperfusion
CON	0.90 ± 0.09	0.07 ± 0.01*	1.58 ± 0.23*
DES	0.98 ± 0.17	0.09 ± 0.02*	1.61 ± 0.22*
GLB	1.09 ± 0.21	0.08 ± 0.01*	1.85 ± 0.12*
DES + GLB	0.74 ± 0.11	0.07 ± 0.01*	1.65 ± 0.19*
HMR	1.04 ± 0.07	0.10 ± 0.02*	1.34 ± 0.28
DES + HMR	0.86 ± 0.04	0.07 ± 0.01*	1.71 ± 0.18*
5-HD	1.01 ± 0.15	0.09 ± 0.01*	1.70 ± 0.31*
DES + 5-HD	0.81 ± 0.15	0.06 ± 0.02*	1.50 ± 0.24*

Data are mean ± SEM.

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

CON = control; DES = desflurane; GLB = glyburide; HMR = HMR 1098; 5-HD = 5-hydroxydecanoate.

Discussion

Experimental evidence accumulated in recent years indicates that K_{ATP} channels play a central role in volatile

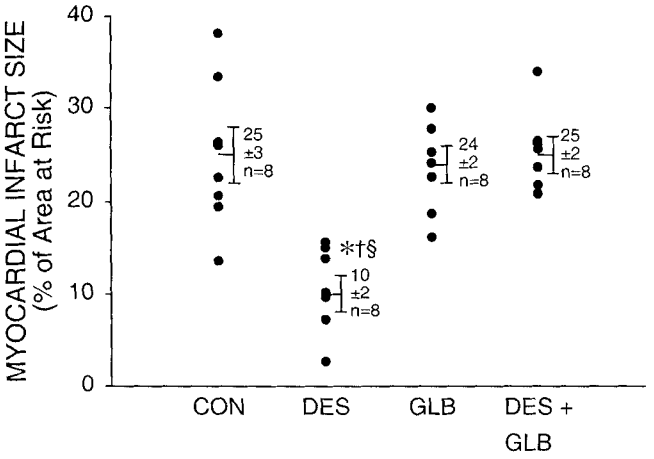

Fig. 3. Myocardial infarct size expressed as a percentage of the area at risk in dogs receiving saline (CON) and glyburide (GLB) in the presence and absence of 1.0 minimum alveolar concentration desflurane (DES). *Significantly ($P < 0.05$) different from CON. †Significantly ($P < 0.05$) different from GLB. §Significantly ($P < 0.05$) different from DES + GLB.

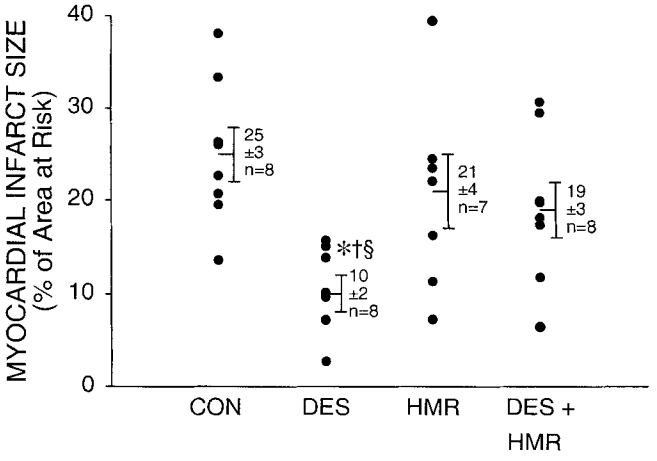

Fig. 4. Myocardial infarct size expressed as a percentage of the area at risk in dogs receiving saline (CON) and HMR 1098 in the presence and absence of 1.0 minimum alveolar concentration desflurane (DES). *Significantly ($P < 0.05$) different from CON. †Significantly ($P < 0.05$) different from HMR 1098. §Significantly ($P < 0.05$) different from DES + HMR 1098.

anesthetic-induced preconditioning.[3,5,6] Isoflurane and sevoflurane have been shown to reduce reversible and irreversible ischemic injury by activating K_{ATP} channels.[3,5,6] Isoflurane also produces an acute memory phase of myocardial protection by a K_{ATP} channel-mediated mechanism, an action similar to that observed with a brief ischemic stimulus.[3] We have recently demonstrated that sevoflurane reduces the time threshold of ischemic preconditioning *in vivo*,[6] demonstrating the additive actions of a brief ischemic episode and a volatile

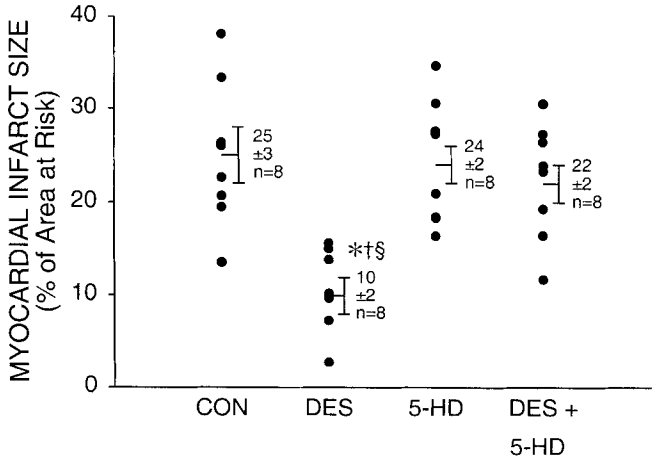

Fig. 5. Myocardial infarct size expressed as a percentage of the area at risk in dogs receiving saline (CON) and 5-hydroxydecanoate (5-HD) in the presence and absence of 1.0 minimum alveolar concentration desflurane (DES). *Significantly ($P < 0.05$) different from CON. †Significantly ($P < 0.05$) different from 5-HD. §Significantly ($P < 0.05$) different from DES + 5-HD.

anesthetic agent at the K_{ATP} channel. The present results with desflurane confirm and extend findings with other volatile anesthetics and indicate that this agent also exerts cardioprotective effects against irreversible ischemic injury. These beneficial effects were blocked by glyburide, indicating that desflurane-induced myocardial protection ultimately occurs through K_{ATP} channels. Furthermore, the reduction in infarct size produced by desflurane also occurred independent of alterations in systemic hemodynamics and transmural coronary collateral blood flow.

Adenosine triphosphate-dependent potassium channels are clearly involved in anesthetic-induced myocardial protection, but the subcellular location of these channels has not been defined. Noma[28] originally suggested that sarcolemmal K_{ATP} channel opening may hyperpolarize the cardiac myocyte in the presence of reduced intracellular concentrations of ATP during ischemia. Such sarcolemmal hyperpolarization reduces myocyte action potential duration[15,29,30] and partially inhibits voltage-dependent calcium (Ca^{2+}) channel activity. Subsequent reductions in myocardial contractility[31] and intracellular Ca^{2+} overload[30] may preserve intracellular energy stores[32] for vital processes during ischemia and reperfusion. Sarcolemmal K_{ATP} channel opening would maintain the normal function of the sodium (Na^+)-Ca^{2+} exchanger, further reducing intracellular Ca^{2+} accumulation.[30] Sarcolemmal K_{ATP} channels may also exert protective effects independent of action potential duration,[33,34] because protective effects of K_{ATP} channel openers have been observed without concomitant changes in action potential duration[15,16] and in unstimulated[35,36] and electrically inactive[33] cardiac myocytes. Furthermore, the specific sarcolemmal K_{ATP} channel subunit confers protection to transfected nonmyocyte cells in the absence of a developed action potential.[33] Alternatively, mitochondrial K_{ATP} channels[37] have recently been proposed as a site of action for K_{ATP} channel openers,[17,38,39] and these channels may play a central role in ischemic preconditioning.[18,21] Diazoxide, a selective mitochondrial K_{ATP} channel opener, reduced myocardial injury in isolated rat hearts subjected to ischemia and reperfusion.[21] This beneficial action was blocked by pretreatment with 5-HD. These data indicated that K_{ATP} channels located in the mitochondria may be involved in reducing ischemic injury. The precise mechanism through which mitochondrial K_{ATP} channels mediate such protective effects has yet to be determined. Opening of these channels causes transient mitochondrial K^+ uptake and matrix swelling, effects

that seem to favorably modulate mitochondrial metabolism.[38-40] The importance of sarcolemmal *versus* mitochondrial K_{ATP} channel opening during ischemic preconditioning is unresolved. Previous investigation is not unequivocal in favor of the sarcolemmal *versus* the mitochondrial K_{ATP} channel, and evidence for the involvement of both channels during ischemic preconditioning has recently been presented.[41,42]

The current investigation is the first to examine the subcellular K_{ATP} channel sites responsible for anesthetic-induced preconditioning. The results indicate that specific blockade of sarcolemmal K_{ATP} channels with HMR 1098 abolishes the protective actions of desflurane. This finding suggests that sarcolemmal K_{ATP} channel activation by volatile agents plays a role in reducing myocardial ischemic injury. In addition, 5-HD blocked the decrease in infarct size produced by desflurane, findings that also implicate a role for mitochondrial K_{ATP} channel activation in anesthetic-induced preconditioning. It is unknown if interactions exist between sarcolemmal and mitochondrial K_{ATP} channels during ischemic- or anesthetic-induced preconditioning.

The present findings must be interpreted within the constraints of several potential limitations. Desflurane-induced decreases in the rate-pressure product may have produced favorable alterations in myocardial oxygen supply-demand and contributed to a reduction in infarct size. However, K_{ATP} channel blockade with selective and nonselective antagonists completely abolished the protective effect of desflurane without affecting the hemodynamic actions of this agent. Volatile anesthetics also mediate protective effects during mechanical arrest produced by cardioplegia,[43] indicating that preferential alterations in myocardial metabolism are not solely responsible for the antiischemic actions of these drugs. Nevertheless, coronary venous oxygen tension was not measured, and myocardial oxygen consumption was not directly quantified in the present investigation; thus, favorable changes in myocardial metabolism during administration of desflurane cannot be completely excluded as a mechanism for the beneficial effect of this drug. HMR 1098 is the water-soluble salt of the selective sarcolemmal K_{ATP} channel antagonist HMR 1883.[19,20] Recent data using several different models suggest that HMR 1098 demonstrates a similar high degree of selectivity for sarcolemmal K_{ATP} channels (E. Marban, personal communication) at a concentration similar to that achieved in the current investigation (1 μM; calculated assuming coronary blood flow = 40 ml/min). However, the specificity of HMR 1098 has not been confirmed in canine myocardium; therefore, it is also possible that this drug abolished the protective effects of desflurane by an indirect mechanism or by blockade of mitochondrial K_{ATP} channels. 5-HD has been shown to abolish the cardioprotective effects of the selective mitochondrial K_{ATP} channel opener diazoxide[17,18,21,22] and to inhibit mitochondrial flavoprotein oxidation produced by the K_{ATP} channel agonist pinacidil while leaving sarcolemmal K_{ATP} current unaffected.[22] 5-HD has also been shown to antagonize the beneficial actions of the K_{ATP} channel agonist cromakalim without influencing action potential duration.[23] Although the specificity of 5-HD for mitochondrial K_{ATP} channels has not been confirmed in canine myocardium, these findings suggest that 5-HD preferentially blocks mitochondrial K_{ATP} channels at a concentration[1,17,22,23] similar to that achieved (450 μM) in the current investigation. It is unknown if higher concentrations of HMR 1098 and 5-HD may be incompletely selective for specific subcellular K_{ATP} channel locations. The actions of anesthetics to specifically enhance activation of sarcolemmal or mitochondrial K_{ATP} channels will require future investigation using patch clamp and flavoprotein fluorescence techniques. Interpretation of the present findings should also be qualified because only a single end-tidal concentration of desflurane was used. Higher inspired concentrations of desflurane may have produced more pronounced reductions in infarct size and may have altered the subcellular locus of action of this anesthetic.

In summary, the present results indicate that desflurane reduces experimental myocardial infarct size after prolonged coronary artery occlusion and reperfusion. Desflurane-induced cardioprotection is dependent on K_{ATP} channel activation, and selective antagonists of both sarcolemmal and mitochondrial K_{ATP} channels block reductions of infarct size afforded by this drug *in vivo*.

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