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Isoflurane Preconditions Myocardium against Infarction via *Release of Free Radicals*

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Background: **Isoflurane exerts cardioprotective effects that mimic the ischemic preconditioning phenomenon. Generation of free radicals is implicated in ischemic preconditioning. The authors investigated whether isoflurane-induced preconditioning may involve release of free radicals.**

Methods: **Sixty-one α-chloralose–anesthetized rabbits were instrumented for measurement of left ventricular (LV) pressure (tip-manometer), cardiac output (ultrasonic flowprobe), and myocardial infarct size (triphenyltetrazolium staining). All rabbits were subjected to 30 min of occlusion of a major coronary artery and 2 h of subsequent reperfusion. Rabbits of all six groups underwent a treatment period consisting of either no intervention for 35 min (control group, n = 11) or 15 min of isoflurane inhalation (1 minimum alveolar concentration end-tidal concentration) followed by a 10-min washout period (isoflurane group, n = 12). Four additional groups received the radical scavenger *N*-(2-mercaptoproprionyl)glycine (MPG; 1 mg · kg^{-1} · min^{-1}) or Mn(III)tetrakis(4-benzoic acid)porphyrine chloride (MnTBAP; 100 μg · kg^{-1} · min^{-1}) during the treatment period with (isoflurane + MPG; n = 11; isoflurane + MnTBAP, n = 9) or without isoflurane inhalation (MPG, n = 11; MnTBAP, n = 7).**

Results: **Hemodynamic baseline values were not significantly different between groups (LV pressure, 97 ± 17 mmHg [mean ± SD]; cardiac output, 228 ± 61 ml/min). During coronary artery occlusion, LV pressure was reduced to 91 ± 17% of baseline and cardiac output to 94 ± 21%. After 2 h of reperfusion, recovery of LV pressure and cardiac output was not significantly different between groups (LV pressure, 83 ± 20%; cardiac output, 86 ± 23% of baseline). Infarct size was reduced from 49 ± 17% of the area at risk in controls to 29 ± 19% in the isoflurane group ($P = 0.04$). MPG and MnTBAP themselves had no effect on infarct size (MPG, 50 ± 14%; MnTBAP, 56 ± 15%), but both abolished the preconditioning effect of isoflurane (isoflurane + MPG, 50 ± 24%, $P = 0.02$; isoflurane + MnTBAP, 55 ± 10%, $P = 0.001$).**

Conclusion: **Isoflurane-induced preconditioning depends on the release of free radicals.**

ISCHEMIC preconditioning, first described by Murry *et al.*,[1] markedly reduces myocyte death during prolonged periods of myocardial ischemia and has been reported in several mammalian species. Several nonischemic stimuli can precondition the heart, including pharmacologic challenge by adenosine,[2] opioids,[3] and several halogenated inhalational anesthetics,[4–6] including isoflurane.[4,7–13] In addition to animal studies, some work points to the existence of this strongest known endogenous protective mechanism against myocardial ischemia in human myocardium.[14,15] Preadministration of isoflurane 10 min before aortic cross-clamping and cardioplegic arrest during coronary artery bypass surgery has been shown to reduce myocardial damage in humans.[16]

Although the precise signaling pathway of this protective phenomenon is not fully understood, activation of mitochondrial or sarcolemmal adenosine triphosphate (ATP)-regulated potassium (K_{ATP}) channels is important for both ischemic and anesthetic-induced preconditioning.[17] Recent evidence suggests that opening of mitochondrial K_{ATP} channels during the preconditioning ischemia triggers the preconditioned state by generating free radicals, resulting in activation of several protein kinases and protection by an unknown end-effector mechanism.[18] Two very recent studies by McPherson and Yao[3,19] provided first evidence that opioid-induced preconditioning with morphine leads to activation of mitochondrial K_{ATP} channels, resulting in an increase of intracellular free radicals. However, it remains elusive whether release of free radicals is also critically important for isoflurane-induced myocardial preconditioning.

Therefore, the objective of the current study was to determine whether generation of free radicals is involved in isoflurane-induced cardioprotection. Specifically, we investigated whether the two structurally different antioxidant drugs, *N*-(2-mercaptoproprionyl) glycine (MPG) and Mn(III)tetrakis(4-benzoic acid)porphyrine chloride (MnTBAP), can block isoflurane-induced preconditioning in the rabbit heart *in vivo*.

Materials and Methods

The current study conforms to the Guiding Principles in the Care and Use of Animals as approved by the Council of the American Physiologic Society and was approved by the Animal Care Committee of the district of Düsseldorf (Düsseldorf, Germany).

General Preparation

The animal preparation has been described in detail previously.[20] Briefly, α-chloralose–anesthetized New Zealand White rabbits (mean weight, 2.98 ± 0.14 kg) were instrumented for measurement of aortic pressure (Statham transducer), cardiac output (ultrasonic flow probe), and left ventricular (LV) pressure (Millar tip

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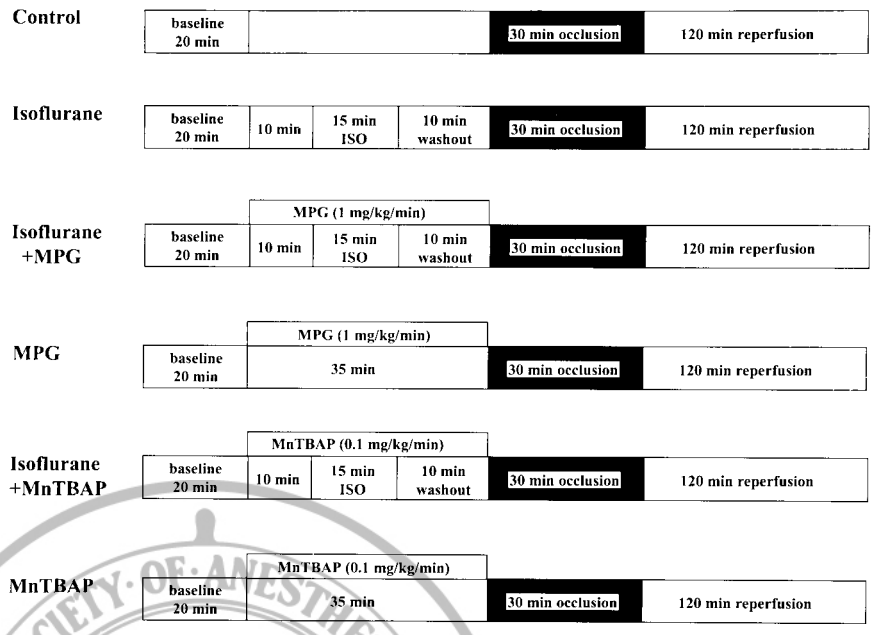

Fig. 1. Experimental protocol. ISO = isoflurane administration; MPG = *N*-(2-mercaptoproprionyl) glycine administration; MnTBAP = Mn(III)tetrakis(4-benzoic acid) porphyrine chloride administration.

manometer). A ligature snare was passed around a major coronary artery for later occlusion. The effectiveness of coronary artery occlusion was verified by the appearance of epicardial cyanosis and changes in surface electrocardiogram. Ventricular fibrillation during coronary artery occlusion was treated by electrical defibrillation (5 J). After coronary artery occlusion, the snare occluder was released, and reperfusion was verified by the disappearance of epicardial cyanosis. Temperature was measured inside the pericardial cradle and maintained between 38.3 and 38.7°C by adjusting a heating pad and an infrared lamp.

Experimental Protocol

The experimental protocol is shown in figure 1. Twenty minutes after completion of the surgical preparation, baseline measurements were performed. All rabbits in all groups underwent 30 min of coronary artery occlusion followed by 2 h of reperfusion.

Eleven rabbits underwent the ischemia–reperfusion procedure without further treatment (control group). Rabbits in the isoflurane group (n = 12) received isoflurane in an end-tidal concentration of 2% (corresponding to 1 minimum alveolar concentration in rabbits) for 15 min followed by a 10 min washout period. In a first set of experiments, we determined whether isoflurane-induced preconditioning depends on the release of free radicals using the radical scavenger MPG (1 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}). MPG was given for 10 min before isoflurane application, during isoflurane application, and during the washout phase in the isoflurane + MPG group (n = 11). To determine a potential effect of MPG itself on infarct size, another 11 rabbits received MPG (1 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}) for 35 min before the 30-min ischemia without isoflurane administration (MPG group). In a second set of experiments, we investigated the effects of a second chemically different antioxidant (MnTBAP). MnTBAP (100 μg $\cdot$ kg^{-1} $\cdot$ min^{-1}) was given for 35 min before the 30-min ischemia with (isoflurane + MnTBAP, n = 9) or without isoflurane administration (MnTBAP group, n = 7).

Infarct Size Assessment

After 2 h of reperfusion, the heart was arrested by injection of potassium chloride solution into the left atrium and quickly excised. The area at risk size was then determined by Evans blue staining of the nonischemic area, and infarct size within the area at risk was determined by triphenyltetrazolium chloride staining. The procedure has been described in detail previously.[20]

Data Analysis

Left ventricular pressure, its first derivative rate of pressure increase (dP/dt), aortic pressure, and stroke volume were recorded continuously on an ink recorder (Recorder 2800; Gould Inc., Cleveland, OH). The data were digitized using an analog-to-digital converter (Data Translation, Marlboro, MA) at a sampling rate of 500 Hz and processed later on a personal computer.

Hemodynamic Variables

Global systolic function was measured in terms of LV systolic pressure (LVSP) and maximum dP/dt (dP/dt$_{max}$). Global LV end-systole was defined as the point of minimum dP/dt (dP/dt$_{min}$), and LV end-diastole as the beginning of the sharp upslope of the LV dP/dt tracing. The time constant of decrease in LV isovolumic pressure (τ) was used as an index of LV relaxation. Cardiac output was calculated from stroke volume and heart rate, rate pressure product (RPP) from heart rate and LVSP, and systemic vascular resistance (SVR) from mean aortic

pressure and cardiac output, assuming a right atrial pressure of 0 mmHg in the open-chest preparation.

Statistical Analysis

Data are presented as mean and SD. Differences in hemodynamics were analyzed by two-way analysis of variance (ANOVA) for time and treatment (experimental group) effects. If an overall significance between groups was found in the first set of experiments, comparison was performed for each time point using one-way ANOVA followed by the Dunnett *post hoc* test with the isoflurane group as the reference group. Hemodynamic group effects in the second set of experiments were analyzed by one-way ANOVA followed by the Student *t* test for unpaired data with Bonferroni correction for multiple comparisons. If an overall significance within a group (time effect) was found, one-way ANOVA followed by the Dunnett *post hoc* test with the baseline value as the reference time point was used for the assessment of time effects in that group. In the first set of experiments, differences in infarct size were analyzed by ANOVA followed by the Dunnett *post hoc* test with the isoflurane group as the reference group. In the second set of experiments, differences in infarct size were analyzed by ANOVA followed by the Student *t* test with Bonferroni correction for multiple comparisons. The hemodynamic effects of MPG and MnTBAP administration were analyzed by the Student *t* test for paired observation. Changes within and between groups were considered statistically significant when the P value was < 0.05.

Results

A total of 66 animals were studied. Five animals died from ventricular fibrillation during coronary artery occlusion. In the remaining 61 animals, complete data sets were obtained (control group, n = 11; isoflurane group, n = 12; isoflurane + MPG group, n = 11; MPG group, n = 11; isoflurane + MnTBAP group, n = 9; MnTBAP group, n = 7).

Hemodynamic Function

Hemodynamic variables are summarized in figure 2 and table 1. During baseline recordings, there were no significant differences between groups in LVSP, cardiac output, heart rate, and calculated RPP.

Administration of MPG or MnTBAP had no influence on hemodynamics. LVSP, dP/dt_{max}, RPP, and SVR were reduced during isoflurane administration in the isoflurane group (LVSP by a mean of 39 ± 18%; dP/dt_{max} by a mean of 43 ± 24%; RPP by a mean of 30 ± 23%; SVR by a mean of 49 ± 12%), in the isoflurane + MPG group (LVSP by a mean of 41 ± 14%; dP/dt_{max} by a mean of 52 ± 24%; RPP by a mean 40 ± 20%, SVR by a mean of 38 ± 12%), and in the isoflurane + MnTBAP group (LVSP by a mean of 34 ± 24%; dP/dt_{max} by a mean of 39 ± 27%; RPP by a mean of 32 ± 26%; SVR by a mean of 29 ± 11%). After the 10-min washout period of isoflurane, all hemodynamic variables soon recovered and were not significantly different from baseline.

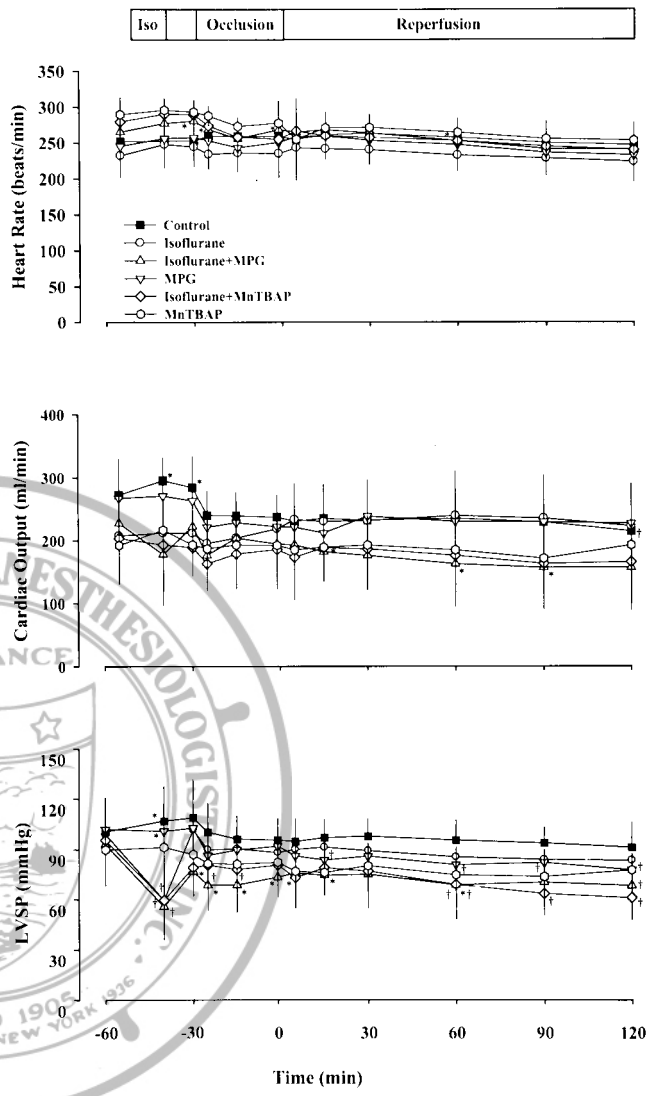

Fig. 2. Line plot showing the time course of heart rate, cardiac output, and left ventricular peak systolic pressure (LVSP) during experiments in the control, isoflurane, isoflurane + MPG, MPG, isoflurane + MnTBAP, and MnTBAP groups. Data are mean ± SD. MPG = *N*-(2-mercaptoproprionyl) glycine application; MnTBAP = Mn(III)tetrakis(4-benzoic acid)porphy-rine chloride application. †$P < 0.05$ *versus* baseline conditions; *$P < 0.05$ compared with the isoflurane group.

Coronary artery occlusion was accompanied by a reduction of LVSP (by a mean of 9 ± 24%) and dP/dt_{max} (by a mean of 9 ± 38%) in all groups (table 1 and fig. 2). RPP did not significantly differ between groups. With regard to LV relaxation, τ increased by 35 ± 53% and LV end diastolic pressure by a mean of 3 ± 2 mmHg during coronary artery occlusion (all values at 25 min of ischemia). After 2 h of reperfusion, LVSP was reduced by a mean of 22 ± 18% and dP/dt_{max} by 30 ± 26% of baseline

Table 1. Hemodynamic Variables

				Coronary Occlusion		Reperfusion		
	Baseline	ISO	Washout	5	25	5	30	120
LVEDP (mmHg)								
Control	2.6 ± 2.9	3.1 ± 4.5	2.4 ± 2.4	7.9 ± 4.1	7.1 ± 4.7	8.5 ± 5.2	7.3 ± 4.8	7.6 ± 5.4
ISO	1.8 ± 2.2	2.2 ± 1.9	3.2 ± 2.7	6.2 ± 5.8	7.4 ± 7.2	6.9 ± 6.9	5.3 ± 5.3	6.0 ± 7.7
ISO + MPG	3.7 ± 2.7	3.9 ± 4.5	5.3 ± 4.7	5.1 ± 5.0	5.3 ± 3.6	4.1 ± 3.3	3.5 ± 3.0	4.6 ± 2.7
MPG	4.0 ± 4.1	4.9 ± 2.8	6.2 ± 3.4	8.5 ± 6.7	9.6 ± 7.1	10.5 ± 7.0	8.2 ± 6.8	6.7 ± 4.9
ISO + MnTBAP	3.1 ± 3.9	2.9 ± 5.2	2.6 ± 4.0	4.7 ± 5.6	4.8 ± 5.9	4.8 ± 7.1	4.8 ± 7.2	2.6 ± 1.3
MnTBAP	2.2 ± 0.9	2.6 ± 1.7	2.2 ± 2.3	2.3 ± 1.6	4.4 ± 3.2	4.1 ± 4.0	3.2 ± 2.3	2.3 ± 1.8
dP/dtmax (mmHg)								
Control	4583 ± 1528	5037 ± 1764*	5107 ± 1801	4209 ± 1494	4223 ± 976	3032 ± 681	3909 ± 705	3398 ± 1241
ISO	4566 ± 1329	2328 ± 1147†	4766 ± 985	3347 ± 752†	3619 ± 750	2992 ± 789†	3266 ± 1024†	2656 ± 917†
ISO + MPG	4027 ± 1086	1858 ± 1222†	3537 ± 1155	2584 ± 960†	3120 ± 944	2674 ± 709	2681 ± 1021	2434 ± 958†
MPG	4967 ± 1312	5510 ± 2086*	5648 ± 2029	3961 ± 1142	4069 ± 907	3074 ± 899	3926 ± 822	3189 ± 664
ISO + MnTBAP	4133 ± 1395	2288 ± 763†	3416 ± 1512	2921 ± 901	3145 ± 924	2337 ± 757	2755 ± 652	2150 ± 657†
MnTBAP	3901 ± 1319	4215 ± 975*	3886 ± 563	3547 ± 675	3733 ± 830	3059 ± 1411	3353 ± 871	3175 ± 682
SVR (mmHg · min · l^{-1})								
Control	332 ± 62*	331 ± 69*	356 ± 65	374 ± 87	364 ± 102	346 ± 86	352 ± 91	332 ± 81
ISO	465 ± 160	219 ± 43†	454 ± 223	406 ± 158	405 ± 185	375 ± 139	343 ± 114	303 ± 48
ISO + MPG	385 ± 97	243 ± 72†	271 ± 63*	324 ± 94	316 ± 101	336 ± 95	326 ± 126	385 ± 157
MPG	341 ± 66	333 ± 60*	344 ± 70	350 ± 84	360 ± 65	338 ± 46	326 ± 53	297 ± 62
ISO + MnTBAP	318 ± 213	229 ± 43	347 ± 86	418 ± 126	353 ± 92	337 ± 76	341 ± 81	301 ± 74
MnTBAP	412 ± 65	379 ± 68*	403 ± 68	399 ± 88	392 ± 105	365 ± 84	365 ± 51	344 ± 56
RPP (mmHg · min^{-1} · 10^3)								
Control	25.3 ± 6.2	27.0 ± 6.6*	27.5 ± 6.6	26.2 ± 6.0	25.8 ± 5.1	24.4 ± 5.4	25.8 ± 3.7	22.9 ± 4.4
ISO	22.9 ± 4.0	15.6 ± 4.5†	24.7 ± 4.3	21.0 ± 4.0	20.7 ± 3.7	21.9 ± 3.1	21.6 ± 4.5	18.9 ± 7.5
ISO + MPG	24.3 ± 3.5	15.0 ± 6.7†	21.1 ± 4.6	18.1 ± 4.7	19.8 ± 4.8	19.8 ± 4.9	19.6 ± 7.0	16.6 ± 6.3
MPG	25.1 ± 5.2	26.3 ± 6.8*	26.9 ± 6.3	22.1 ± 6.7	23.2 ± 4.4	22.3 ± 3.8	22.1 ± 4.6	18.3 ± 3.0
ISO + MnTBAP	26.7 ± 5.9	17.4 ± 6.1†	22.9 ± 4.7	22.0 ± 4.1	20.1 ± 5.5†	19.4 ± 4.3†	19.8 ± 2.6†	14.7 ± 3.3†
MnTBAP	26.2 ± 7.8	27.1 ± 4.9*	25.6 ± 3.8	23.5 ± 2.2	22.8 ± 3.4	19.3 ± 5.6	21.7 ± 2.1	19.7 ± 3.1
τ (ms)								
Control	12.1 ± 2.6	13.1 ± 2.0	13.1 ± 3.4	15.3 ± 3.7	16.0 ± 2,9	17.9 ± 4.0†	16.7 ± 3.0	18.3 ± 4.8†
ISO	12.4 ± 2.8	14.5 ± 4.5	14.3 ± 4.2	17.6 ± 5.4	17.1 ± 5.0	15.3 ± 4.6	15.6 ± 4.4	18.1 ± 7.8
ISO + MPG	13.9 ± 3.7	19.4 ± 9.5	16.6 ± 6.9	16.8 ± 4.0	14.2 ± 3.1	13.6 ± 3.4	14.7 ± 4.3	18.4 ± 8.3
MPG	15.1 ± 2.7	14.6 ± 2.4	15.3 ± 2.4	18.1 ± 3.7	18.3 ± 4.5	18.0 ± 4.2	17.4 ± 4.8	17.4 ± 3.9
ISO + MnTBAP	12.4 ± 3.0	14.6 ± 5.5	12.9 ± 4.4	15.4 ± 4.8	18.3 ± 8.1	15.9 ± 6.4	14.3 ± 6.1	14.0 ± 3.4
MnTBAP	12.4 ± 2.4	11.7 ± 1.8	10.9 ± 2.2	14.0 ± 1.4	14.2 ± 1.8	13.8 ± 2.9	12.7 ± 2.3	11.6 ± 1.5

Data are mean ± SD.

LVEDP = left ventricular end-diastolic pressure; dP/dtmax = maximum rate of increase in left ventricular pressure; SVR = systemic vascular resistance; RPP = rate pressure product; τ = time constant of decrease in isovolumic left ventricular pressure. ISO = isoflurane; MPG = *N*-(2-mercaptoproprionyl) glycine; MnTBAP = Mn(III)tetrakis(4-benzoic acid)porphyrine chloride.

† $P < 0.05$ compared with baseline; * $P < 0.05$ compared with the isoflurane group.

values, still reflecting impaired myocardial contractile function in all groups at the end of the experiments. As a consequence of a reduction in heart rate and LVSP, RPP was reduced by a mean of 32 ± 17%. τ remained increased by a mean of 19 ± 28% at the end of the experiments.

Infarct Size

Mean LV dry weight was 0.68 ± 0.19 g, with no significant differences between groups (data from individual groups are given in table 2). The ischemic-reperfused area (area at risk) was 0.33 ± 0.21 g, and the area at risk constituted 46 ± 20% of the left ventricle, with no significant differences between groups. Isoflurane preadministration significantly reduced infarct size from 49 ± 17% of the area at risk (control group) to 29 ± 19% ($P = 0.04$, isoflurane *vs.* control group; fig. 3). Pretreatment with the antioxidants MPG or MnTBAP alone had no effect on infarct size (MPG: 50 ± 14%, $P = 0.03$ *vs.* isoflurane group; MnTBAP: 56 ± 15%, $P = 0.005$ *vs.* isoflurane group) but blocked isoflurane-induced preconditioning, as evidenced by an infarct size of 50 ± 24% in the isoflurane + MPG group ($P = 0.02$ *vs.* isoflurane group) and 55 ± 10% in the isoflurane + MnTBAP group ($P = 0.001$ *vs.* isoflurane group).

Discussion

The main finding of our study was that the two structurally different antioxidants, MPG and MnTBAP, completely blocked the cardioprotective effect of isoflurane-induced preconditioning in the rabbit heart *in vivo*. Thus, release of free radicals is critically important for isoflurane-induced preconditioning.

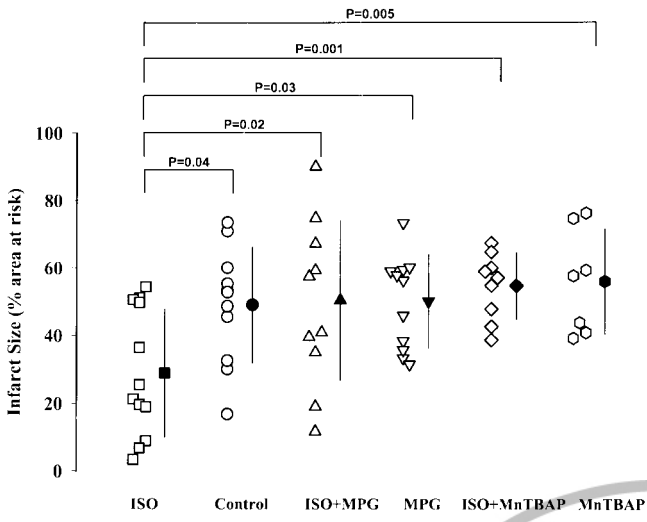

Fig. 3. Infarct size as a percentage of the area at risk in the ISO, control, ISO+MPG, MPG, ISO+MnTBAP, and MnTBAP groups. ISO = isoflurane; MPG = *N*-(2-mercaptopropriony1) glycine; MnTBAP = Mn(III)tetrakis(4-benzoic acid)porphyrine chloride. Open symbols = single data points, filled symbols = mean ± SD.

Our study confirms the results of several previous studies, that pretreatment with a clinically relevant dose of isoflurane (1.1–2% = 0.5–1 minimum alveolar concentration) protects the myocardium from a subsequent prolonged ischemia[8,10,11,13] and thus mimics the cardioprotective effects of ischemic preconditioning. In the current investigation, pretreatment with 2% end-tidal isoflurane for 15 min reduced infarct size by 42% in comparison with controls. In a former study performed in our laboratory using the same experimental animal model, anesthetic regimen, and duration of ischemia (30 min) and reperfusion (2 h),[20] one 5-min period of ischemic preconditioning reduced infarct size by 47%. Thus, we could confirm the results of Ismaeil *et al.*[10] and Piriou *et al.*,[11] who observed an approximately equivalent degree of protection offered by a 15-min preadministration of 1.1% end-tidal isoflurane to the protection induced by a 5-min period of preconditioning ischemia in the same experimental rabbit model.

We cannot rule out the effect of isoflurane-induced hypotension on the decrease of infarct size in the isoflurane group. However, it is unlikely that this decrease in blood pressure led to myocardial ischemia with consequent ischemic preconditioning, as we observed a similar reduction in blood pressure in animals treated with MPG + isoflurane or MnTBAP + isoflurane. The cardioprotective effect of isoflurane preadministration was completely blocked in these groups. In the expiratory gas, no measurable isoflurane could be detected 5 min after its discontinuation, and myocardial contractile function recovered during the 10-min washout period. Therefore, the cardioprotective effect of isoflurane pretreatment is not caused by a direct antiischemic effect.

All experiments were conducted during anesthesia with α-chloralose. This type of anesthesia maintains near-normal cardiovascular reflexes comparable with the awake state and is a classic anesthetic for physiologic and pharmacologic experiments.[21] An effect of α-chloralose on ischemic preconditioning has not been studied so far. However, we have shown previously that ischemic preconditioning can be elicited in the presence of α-chloralose.[20]

Mechanisms of Isoflurane-induced Preconditioning

Although the precise mechanism of this protective phenomenon is poorly understood, several important parts of the proposed signal transduction cascade have been identified and are identical to those involved in ischemic preconditioning. Some recent studies have addressed the role of adenosine receptors in isoflurane-induced preconditioning. Blocking A_1 receptors abolished isoflurane-induced cardioprotection against myocardial stunning[7] and against infarction in rabbits[10] and in human atrial trabecular muscles.[13] An investigation by Piriou *et al.*[11] suggested that mechanogated channels play a role in this phenomenon. Numerous reports support the central role of K_{ATP} channels in ischemic[22] and isoflurane-induced preconditioning.[7,9–11,13] Administration of a K_{ATP} channel blocker before or during isoflurane administration completely blocked the cardioprotection. Toller *et al.*[23] showed that isoflurane pretreatment reduces myocardial infarct size by activating inhibitory guanine nucleotide binding proteins and speculated that activation of these proteins couples A_1 receptors to K_{ATP} channels. All studies suggested that opening of K_{ATP} channels might be the end effector of isoflurane-induced preconditioning. However, a study by Pain *et al.*[18] in rabbit hearts revealed that opening of mitochondrial K_{ATP} channels may not be the final step in

Table 2. Weights and Area at Risk Size

	Control	ISO	ISO + MPG	MPG	ISO + MnTBAP	MnTBAP
Body weight (g)	3,003 ± 241	2,948 ± 189	2,993 ± 71	2,948 ± 89	2,999 ± 133	2,999 ± 38
LV weight (g)	0.79 ± 0.28	0.69 ± 0.16	0.55 ± 0.18	0.60 ± 0.09	0.75 ± 0.06	0.77 ± 0.15
Area at risk (g)	0.45 ± 0.33	0.37 ± 0.18	0.25 ± 0.18	0.26 ± 0.17	0.35 ± 0.10	0.28 ± 0.14
Area at risk/LV (%)	54.4 ± 29.7	50.4 ± 17.5	43.0 ± 15.1	42.4 ± 23.5	46.5 ± 12.2	35.9 ± 16.1
Infarct size (g)	0.19 ± 0.14	0.11 ± 0.08	0.14 ± 0.12	0.13 ± 0.09	0.19 ± 0.06*	0.14 ± 0.06

Data are mean ± SD.

* $P < 0.05$ compared with the isoflurane group.

LV = Left ventricle; ISO = isoflurane; MPG = *N*-(2-mercaptoproprionyl) glycine; MnTBAP = Mn(III)tetrakis(4-benzoic acid)porphyrine chloride.

the preconditioning cascade, but rather acts as a trigger for the preconditioned state through the generation of free radicals.[18] This theory is supported by many other studies that demonstrated a blockade of the cardioprotective effect of ischemic preconditioning by administrating radical scavengers such as superoxide dismutase or MPG during the preconditioning ischemia.[24–27] There are no studies available investigating the release of free radicals during isoflurane administration. However, two studies by McPherson and Yao[3,19] provided first evidence that also anesthetic-induced preconditioning with morphine leads to activation of mitochondrial K_{ATP} channels, resulting in an increase of intracellular free radical production. Furthermore, it has been shown that exposure to a low concentration of oxygen radicals can reproduce the beneficial effects of ischemic preconditioning.[27] Based on these findings, we hypothesized that the radical scavenger MPG might block isoflurane-induced preconditioning. In fact, administration of MPG ($1\ mg \cdot kg^{-1} \cdot min^{-1}$) for 10 min before or during the isoflurane inhalation and the 10-min washout period completely blocked the cardioprotective effects of isoflurane-induced preconditioning in a first set of experiments. This result was confirmed in a second set of experiments with the chemically different antioxidant MnTBAP. The differences in infarct size were not caused by differences in area at risk sizes, temperature, or hemodynamic parameters during ischemia and reperfusion.

N-(2-mercaptoproprionyl)glycine or MnTBAP were administered during the whole treatment period before the 30-min ischemia because it has been shown previously that the generation of free radicals is a trigger rather than a mediator of preconditioning-induced cardioprotection.[18] Consistent with other studies in rabbits using MPG[18,26,28] or MnTBAP,[18] both drugs itself had no effect on infarct size.

In contrast to the study by Kersten *et al.*,[7] we did not observe an improved functional recovery in the isoflurane group. The most likely reason for this finding is the duration of ischemia. Kersten *et al.* used four 5-min periods of coronary artery occlusion interspersed with 5 min of reperfusion to investigate the influence of isoflurane preadministration on myocardial stunning. In contrast, our study was designed to determine the mechanism of isoflurane-induced preconditioning against infarction as the classic end point to evaluate the cardioprotective effects of preconditioning; therefore, we used one 30-min period of ischemia. A study by Cohen *et al.*[29] demonstrated that a reduction of infarct size after preconditioning did not predict the extent of early functional improvement of reperfused hearts, but improvement of functional recovery became evident 2–4 days after the ischemia. Furthermore, the absolute difference in infarct size (in grams) between the isoflurane and the other groups is small in comparison with total LV mass, thereby reducing the influence of infarct size reduction on global myocardial function.

What is the source of the free radicals and what is the mechanism by which release of free radicals induces cardioprotection? Radicals are released from the mitochondria[30,31] as a consequence of K_{ATP} channel opening.[3,31,32] In contrast, K_{ATP} channel blockers prevent their release.[3,19,32] McPherson and Yao[3,19] demonstrated that stimulation of opioid receptors by morphine leads to activation of mitochondrial K_{ATP} channels followed by an increase of intracellular free radical production.[3,19] They suggested that this leads to a further amplified opening of K_{ATP} channels. Furthermore, it has been demonstrated that protein kinase C is activated by free radicals.[33] Activation of protein kinase C is an important step in the signal transduction cascade of both anesthetic-induced[4] and ischemic preconditioning.[34]

The current study now adds the finding that release of free radicals is also crucially involved in mediating the cardioprotection of isoflurane-induced preconditioning.

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