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LABORATORY INVESTIGATIONS

Effects of enflurane, isoflurane, sevoflurane and desflurane on reperfusion injury after regional myocardial ischaemia in the rabbit heart *in vivo*

B. PRECKEL, W. SCHLACK, T. COMFÈRE, D. OBAL, H. BARTHEL AND V. THÄMER

Summary

It is known that volatile anaesthetics protect myocardial tissue against ischaemic and reperfusion injury *in vitro*. In this investigation, we have determined the effects of the inhalation anaesthetics, enflurane, isoflurane, sevoflurane and desflurane, administered only during early reperfusion, on myocardial reperfusion injury *in vivo*. Fifty chloralose-anaesthetized rabbits were subjected to 30 min of occlusion of a major coronary artery followed by 120 min of reperfusion. Left ventricular pressure (LVP, tip-manometer), cardiac output (CO, ultrasonic flow probe) and infarct size (triphenyltetrazolium staining) were determined. During the first 15 min of reperfusion, five groups of 10 rabbits each received 1 MAC of enflurane (enflurane group), isoflurane (isoflurane group), sevoflurane (sevoflurane group) or desflurane (desflurane group), and 10 rabbits served as untreated controls (control group). Haemodynamic baseline values were similar between groups (mean LVP 106 (SEM 2) mm Hg; CO 281(7) ml min^{-1}). During coronary occlusion, LVP and CO were reduced to the same extent in all groups (LVP 89% of baseline; CO 89%). Administration of inhalation anaesthetics during early reperfusion further reduced both variables, but they recovered after discontinuation of the anaesthetics to values not different from control animals. Infarct size was reduced from 49 (5)% of the area at risk in the control group to 32 (3)% in the desflurane group ($P=0.021$), and to 36 (2)% in the sevoflurane group ($P=0.097$). In the enflurane group, infarct size was 39 (5)% ($P=0.272$). Isoflurane had no effect on infarct size (48 (5)%, $P=1.000$). The results show that desflurane and sevoflurane markedly reduced infarct size and therefore can protect myocardium against reperfusion injury *in vivo*. Enflurane had only a marginal effect and isoflurane offered no protection against reperfusion injury *in vivo*. These different effects suggest different protective mechanisms at the cellular level. (*Br. J. Anaesth*. 1998; **81**: 905–912).

Keywords: anaesthetics, volatile, enflurane; anaesthetics, volatile, isoflurane; anaesthetics, volatile, sevoflurane; anaesthetics, volatile, desflurane; heart, ischaemia; heart, myocardial function; rabbit

The term "lethal reperfusion injury" characterizes a situation in which myocardium losses viability by pathomechanisms that are triggered by restoration of oxygen and substrate supply after myocardial ischaemia.[1] It has been demonstrated that halothane administration only during the early reperfusion period can protect isolated cardiomyocytes and isolated rat hearts against reperfusion injury.[2,3] This protective effect of halothane was confirmed in an *in vivo* rabbit model of coronary artery occlusion and subsequent reperfusion, and was independent of the haemodynamic side effects of halothane.[4] Although several studies have examined the effects of the commonly used volatile anaesthetics, halothane, enflurane and isoflurane, in myocardial ischaemia,[5–7] little is known of the specific influence of these agents on myocardial reperfusion injury.[4] In addition, the influence of the new volatile anaesthetics, sevoflurane and desflurane, on reperfusion injury *in vivo* has yet to be described. In the isolated rat heart, improvement of myocardial function was observed after 30 min of ischaemia followed by 60 min of reperfusion if inhalation anaesthetics were administered during early reperfusion.[8,9] However, the time course of creatine kinase release (as a marker of cellular injury) and recovery of left ventricular (LV) developed pressure (as an index of myocardial function) varied depending on the inhalation anaesthetic used.[8,9]

We hypothesized that enflurane, isoflurane, sevoflurane and desflurane can protect the myocardium against reperfusion injury *in vivo* in a similar manner to halothane. Therefore, we designed this study to determine the effects of these volatile anaesthetics on reperfusion injury in the *in situ* rabbit heart with regional coronary artery occlusion and subsequent reperfusion. Baseline anaesthesia was maintained with continuous infusion of chloralose during the experiments and the volatile anaesthetics were administered only during the initial reperfusion period (15 min) to determine specific actions against reperfusion injury. Infarct size was determined by triphenyltetrazolium chloride (TTC) staining after 2 h of reperfusion.

Materials and methods

The study was performed in accordance with the regulations of the German Animal Protection Law and

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after obtaining permission from the Animal Care Committee of the District of Düsseldorf.

ANIMAL PREPARATION

The animal preparation has been described in detail previously.[4] In brief, 50 New Zealand White rabbits (body weight 2.8–4.4 (mean 3.6) kg) were anaesthetized with sodium thiopental (thiopentone) 15–30 mg kg^{-1}. After intubation of the trachea, anaesthesia was maintained by continuous infusion of α-chloralose 40 mg kg^{-1} h^{-1} and the lungs ventilated artificially. The rabbits were instrumented for measurement of aortic pressure (AOP, Statham transducer, PD23, Gould, Cleveland, OH, USA), LV pressure (Micro-Tip Pressure Transducer, Sensodyn S PO SF–1, Braun Melsungen AG, Melsungen, Germany) and cardiac output minus coronary flow volume (CO, 4S or 6S ultrasonic flow probe, T 208, Transonic Systems Inc., Ithaca, NY, USA). A snare was passed around a major coronary artery for later occlusion. Temperature was measured inside the pericardial cradle (GTH 1160, Digital Thermometer, Geisinger Electronic, Germany) and maintained at 38.5 ± 0.3°C by adjusting a heating pad and an infrared lamp.

EXPERIMENTAL PROCEDURE

Fifteen minutes after completion of the surgical preparation, baseline measurements were performed. In eight rabbits, the inhalation anaesthetics were given in a randomized order at concentrations of 0.5, 1.0 and 1.5 MAC, in order to assess the haemodynamic effects of the anaesthetics on normal rabbit myocardium in this experimental preparation under chloralose anaesthesia. After discontinuation of the volatile anaesthetic, at least 30 min were allowed until haemodynamic values recovered to baseline before the next agent was applied. For the ischaemia–reperfusion experiments, the prepared coronary artery was occluded after baseline measurements by tightening the snare. The effectiveness of this manoeuvre was verified by the appearance of epicardial cyanosis and changes in surface electrocardiogram. Ventricular fibrillation during coronary occlusion was treated by electrical defibrillation (5 J, DCS261 Defibrillator, Piekser, Ratingen, Germany). After 30 min of occlusion, the snare occluder was released. Reperfusion was verified by the disappearance of epicardial cyanosis. After 120 min 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 non-ischaemic area, and infarct size within the area at risk was determined by TTC staining. The procedure has been described in detail previously.[4]

Ten rabbits underwent the ischaemia–reperfusion procedure without further intervention (control group). Eight of these rabbits also served as controls in a previous study.[4] In 10 rabbits, enflurane, isoflurane, sevoflurane or desflurane (Vapor 19.3, Devapor, Drägerwerke AG, Lübeck, Germany) was added to the inspired gas starting 3 min before reperfusion to achieve a stable concentration at the beginning of the reperfusion period and was continued for the first 15 min of reperfusion. The volatile anaesthetics were titrated to an end-tidal concentration of 2.8% enflurane (enflurane group), 2.0% isoflurane (isoflurane group), 3.7% sevoflurane (sevoflurane group) and 8.9% desflurane (desflurane group) (Datex Capnomac Ultima, Division of Instrumentarium Corp., Helsinki, Finland), which corresponded to 1.0 MAC of the respective volatile anaesthetics in the rabbit.[10–12]

DATA ANALYSIS

LV pressure, its first derivative dP/dt, AOP and stroke volume were recorded continuously on an ink recorder (Recorder 2800, Gould Inc., Cleveland, OH, USA) and stored on a videotape recorder (SL-C 30 PS, Sony, Tokyo, Japan) using pulse code modulation (VPMD 8–12, Fa. Heim, Bergisch Gladbach, Germany) for later playback and analysis. The data were digitized using an analogue to digital converter (Data Translation, Marlboro, MA, USA) at a sampling rate of 500 Hz and processed later on a personal computer.

Haemodynamic variables

Global systolic function was measured in terms of LV peak systolic pressure (LVPSP) and maximum rate of pressure increase (dP/dtmax). Global LV end-systole was defined as the point of minimum dP/dt,[13] 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 diastolic function.[14] CO was calculated from stroke volume and heart rate, rate pressure product (RPP) from heart rate and LVPSP, and systemic vascular resistance (SVR) from mean AOP and CO, assuming a right atrial pressure of 0 mm Hg in the open-chest preparation.

Statistical analysis

Data are presented as mean (SEM). The effects of inhalation anaesthetics on normal myocardium were assessed by one-way analysis of variance (ANOVA) for repeated observations. In the ischaemia–reperfusion experiments, statistical analysis was performed by two-way ANOVA for time and treatment (experimental group) effects. If an overall significant effect between groups was found, comparison was made for each time using one-way ANOVA followed by the Dunnett's post-test, where appropriate. Differences in the influence of area at risk size on infarct size were determined by ANOVA for differences between regression slopes, followed by analysis of covariance.[15] Some of the control animals were from a previous study.[4] Therefore, the two treatment groups of the previous study were included in the statistical evaluation to prevent an increase in type I error by repeated use of the same controls.

Results

A total of 63 animals were used. Five animals died from ventricular fibrillation during coronary artery occlusion. In the remaining 58 animals, complete data sets were obtained (control group, $n = 10$; enflurane group, $n = 10$; isoflurane group, $n = 10$; sevoflu-

Table 1 Effects of increasing does of enflurane, isoflurane, desflurane, and sevoflurane on normal myocardium were tested during α-chloralose anaesthesia. LVPSP = left ventricular peak systolic pressure; CO = cardiac output; d*P*/d*t*max = maximum rate of left ventricular pressure increase; SVR = systemic vascular resistance

	Baseline	0.5 MAC	1.0 MAC	1.5 MAC
LVPSP (mm Hg)		(%)	(%)	(%)
Enflurane	106 (6.1)	87 (5.4)	70 (8.0)	45 (2.1)
Isoflurane	107 (6.6)	98 (3.3)	88 (6.1)	66 (6.3)
Desflurane	105 (5.1)	81 (5.0)	73 (10.3)	70 (8.9)
Sevoflurane	108 (5.4)	93 (4.1)	80 (7.5)	68 (10.5)
CO (ml min^{-1})		(%)	(%)	(%)
Enflurane	247 (16)	103 (6.9)	92 (8.5)	64 (5.1)
Isoflurane	238 (16)	103 (1.0)	10 0 (2.7)	97 (8.2)
Desflurane	236 (21)	103 (5.4)	98 (7.8)	10 2 (5.6)
Sevoflurane	273 (23)	95 (3.8)	93 (5.7)	90 (5.9)
d*P*/d*t*max (mm Hg s^{-1})		(%)	(%)	(%)
Enflurane	473 0 (685)	87 (8.7)	60 (12.2)	28 (3.7)
Isoflurane	475 8 (423)	10 1 (5.4)	85 (8.1)	56 (9.6)
Desflurane	505 3 (583)	88 (6.0)	66 (11.8)	57 (11.7)
Sevoflurane	524 6 (411)	90 (6.5)	71 (10.6)	48 (12.6)
SVR (mm Hg min litre^{-1})		(%)	(%)	(%)
Enflurane	396 (11)	82 (2.8)	68 (4.6)	56 (3.5)
Isoflurane	406 (30)	95 (3.3)	83 (6.4)	58 (6.6)
Desflurane	400 (28)	84 (3.3)	64 (9.4)	56 (7.7)
Sevoflurane	362 (36)	95 (2.7)	78 (6.8)	62 (10.9)

rane group, $n=10$; desflurane group, $n=10$; inhalation anaesthetic without coronary occlusion, $n=8$).

HAEMODYNAMIC FUNCTION

Effects of inhalation anaesthetics on normal myocardium

In eight rabbits, the effect of the inhalation anaesthetic on normal myocardium was tested in the experimental model of the present study (table 1). Two to three different anaesthetics were tested in each animal. There was a similar decrease in LVPSP, d*P*/d*t*max and SVR in all groups at 1.5 MAC. CO was maintained at lower concentrations until 1.0 MAC (the concentration used in the ischaemia–reperfusion experiments), but decreased at 1.5 MAC of enflurane.

Ischaemia–reperfusion experiments

The haemodynamic variables are summarized in figures 1 and 2 and table 2. During baseline recordings, groups were comparable in LVPSP and CO. By chance, heart rate was higher in the isoflurane, sevoflurane and desflurane groups compared with the control group (figs 1, 2). Consequently, RPP, as a major determinant of myocardial oxygen consumption, was comparable between the control group and the enflurane group and was higher in the isoflurane, sevoflurane and desflurane groups during baseline conditions.

In all groups, coronary occlusion was accompanied by a small reduction in LVPSP (15%), d*P*/d*t*max (23%) and CO (20%) (table 2; figs 1, 2). SVR remained unchanged. Diastolic function showed a prolongation of the time constant of decrease in isovolumic LV pressure (τ increased by 20%) and an increase in LVEDP (42%) during coronary artery occlusion (table 2) (all values at 25 min of coronary artery occlusion).

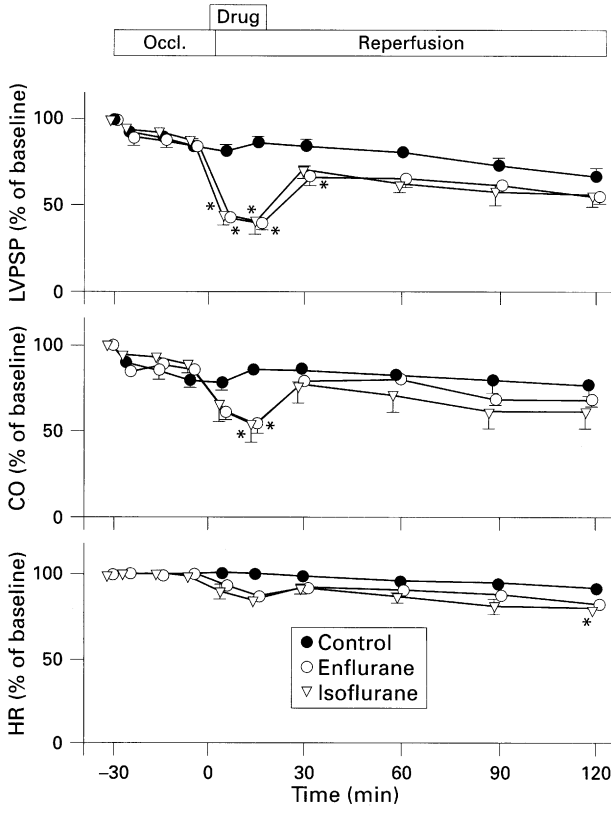

Figure 1 Line plot showing the time course of left ventricular peak systolic pressure (LVPSP), cardiac output (CO) and heart rate (HR) during experiments in the control, enflurane and isoflurane groups. Data are percentage changes (SEM) from baseline. Baseline LVPSP was 101 (SEM 5) mm Hg in the control group, 104 (6) mm Hg in the enflurane group and 118 (3) mm Hg in the isoflurane group. Baseline CO was 265 (20) ml min^{-1} in the control group, 285 (16) ml min^{-1} in the enflurane group and 286 (11) ml min^{-1} in the isoflurane group. Baseline HR was 239 (11) beat min^{-1} in the control group, 252 (6) beat min^{-1} in the enflurane group and 290 (5) beat min^{-1} in the isoflurane group. Occl. = Time of coronary occlusion; Drug = time of anaesthetic administration. *$P<0.05$ *vs* control group.

During the reperfusion period, the reduction in LVPSP (to 87% of baseline) and CO (to 85% of baseline) at 15 min of reperfusion remained unchanged in the untreated controls. Administration of 1.0 MAC of the volatile anaesthetics during the first 15 min of reperfusion caused a further decline in LVPSP to 39–54% of baseline values (all $P<0.05$ compared with the control group). Simultaneously, CO decreased in the enflurane group (54% of baseline values) and in the isoflurane group (53% of baseline values) (both $P<0.05$ compared with the control group). In the sevoflurane and desflurane groups, CO was reduced to a much smaller extent and was not significantly different from the control group (sevoflurane group 67% ($P=0.39$); desflurane group 79% of baseline values ($P=0.98$)). SVR was reduced in all treatment groups. With regard to diastolic function during reperfusion, the time constant of decrease in isovolumic LV pressure remained prolonged to the same extent as during the occlusion period in the control group, while administration of the inhalation anaesthetics further prolonged τ. At 15 min of reperfusion, d*P*/d*t*min was reduced significantly in all treatment groups, while there were no differences in LVEDP between treatment and control groups.

After discontinuation of the volatile anaesthetics,

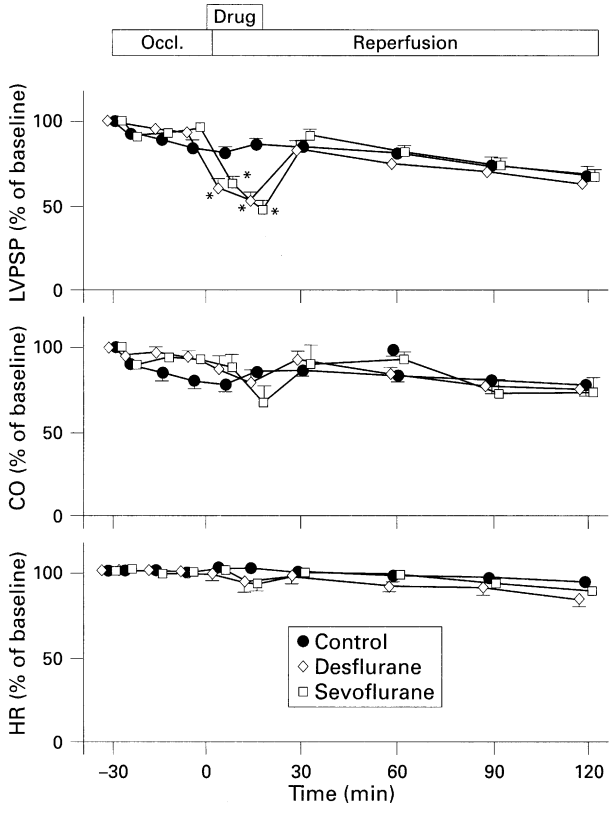

Figure 2 Line plot showing the time course of left ventricular peak systolic pressure (LVPSP), cardiac output (CO) and heart rate (HR) during experiments in the control, sevoflurane and desflurane groups. Data are percentage changes (SEM) from baseline. Baseline LVPSP was 115 (3) mm Hg in the sevoflurane group and 111 (2) mm Hg in the desflurane group (each $n=10$). Baseline CO was 269 (14) ml min^{-1} in the sevoflurane group and 275 (13) ml min^{-1} in the desflurane group. Baseline HR was 279 (7) beat min^{-1} in the sevoflurane group and 286 (7) beat min^{-1} in the desflurane group. Occl. = Time of coronary occlusion; Drug = time of anaesthetic administration. $*P<0.05$ *vs* control group.

haemodynamic variables recovered to values not different from controls, except for enflurane-treated animals, which had a lower LVPSP at 30 min of reperfusion (fig. 1).

After 120 min of reperfusion, LVPSP was reduced by approximately 35% of baseline in all groups and d*P*/d*t*max was reduced by 35–59% of baseline values at the end of the reperfusion period, still reflecting impaired myocardial function in all groups at the end of the experiments. Heart rate after 120 min of reperfusion was reduced by 8% of baseline values in the control group and by approximately 15% in the treatment groups, with no significant differences compared with controls except for the isoflurane group, in which heart rate was reduced by 20% of baseline ($P<0.05$). As a consequence of the reduction in heart rate and LVPSP, RPP was reduced by 36–50%, with no significant differences between the control and treatment groups. τ was prolonged to a greater extent in the enflurane group ($P<0.05$), while simultaneously, LVEDP increased by 75% in this group ($P=0.06$ compared with the control group).

INFARCT SIZE

Mean LV weight was 5.12 (0.13) g, with no differences between groups (data from individual groups are given in table 3). The ischaemic–reperfused area (area at risk) constituted 30.9 (1.1)% of LV. In the control group, infarct size was 48.8 (4.7)% of the area at risk (fig. 3). Infarct size was markedly reduced in the desflurane group (32.5 (3.3)% of the area at risk; $P=0.021$ *vs* control) and in the sevoflurane group (35.9 (1.9)%; $P=0.097$ *vs* control). In the enflurane-treated animals, infarct size was 38.7 (4.9)% ($P=0.272$ *vs* control). Isoflurane did not influence infarct size (47.5 (4.8)%; $P=1.000$ *vs* control). The relationship between the area at risk size and the amount of infarcted tissue is shown in figure 4. The slope of the regression line relating infarct size and area at risk size was markedly reduced in the sevoflurane (0.24 (0.10)) and desflurane (0.39 (0.18)) groups compared with the control group (0.78 (0.11)). In enflurane-treated hearts, the slope was 0.65 (0.15), and in isoflurane treated hearts it was 0.74 (0.20). Analysis of variance of multiple regressions followed by analysis of covariance showed no significant differences between groups ($P=0.139$).

Discussion

The main finding of our study was that inhalation of desflurane and sevoflurane during the first 15 min of reperfusion reduced infarct size after regional myocardial ischaemia in the rabbit heart *in vivo*. Enflurane offered only marginal protective effects and isoflurane had no effect on infarct size. Haemodynamic changes during reperfusion were smaller during administration of sevoflurane and desflurane compared with inhalation of enflurane or isoflurane.

CRITIQUE OF METHODS

Eight animals in the control group came from a previous study.[4] Infarct size in the *in situ* rabbit heart has been shown to vary over a wide range.[16 17] Control groups between studies should only be compared with each other if experimental conditions such as duration of coronary artery occlusion, pericardial temperature and anaesthetic regimens are similar, because all of these conditions may influence infarct size in rabbits.[18–20] We used chloralose for basal anaesthesia because it maintains near normal cardiovascular reflexes accompanied by a high sympathetic tone.[21] There are no studies investigating infarct size in rabbits using chloralose anaesthesia and the same duration of ischaemia and reperfusion. To prevent an increase in type I error by repeated use of the same controls, the two treatment groups of the previous study were included in the statistical evaluation. Therefore, the use of eight animals from a previous control group should not have produced false positive results. Group size was greater in this study than in our previous study because more animals were necessary to detect differences between groups if seven groups were studied.

To exclude the influence of the area at risk size on infarct size,[22] we analysed the relationship between the mass of the area at risk and infarct mass. From figure 4 it is apparent that desflurane and sevoflurane reduced the slope of the regression line compared with the control group, indicating myocardial protective actions on reperfusion injury. However, analysis

Table 2 Haemodynamic variables during ischaemia-reperfusion experiments. Data are mean (SEM). LVEDP = Left ventricular end-diastolic pressure; d*P*/d*t*max = maximum rate of left ventricular pressure increase; SVR = systemic vascular resistance; RPP = rate pressure product; τ = time constant of decrease in isovolumic left ventricular pressure. **P* < 0.05 compared with control group; †*P* < 0.05 compared with baseline conditions

		Coronary occlusion		Reperfusion			
	Baseline	5 min	25 min	5 min	15 min	30 min	120 min
LVEDP (mm Hg)		(%)	(%)	(%)	(%)	(%)	(%)
Control	7.7 (0.7)	145 (9)	142 (8)	148 (11)	146 (8)	134 (8)	107 (15)
Enflurane	7.7 (0.9)	169 (33)	149 (22)	131 (11)	137 (13)	142 (11)	175 (29)
Isoflurane	6.1 (0.5)	162 (12)	179 (17)	147 (8)	152 (15)	165 (19)	143 (14)
Desflurane	5.9 (0.5)	137 (9)	112 (18)	139 (12)	127 (10)	120 (10)	113 (7)
Sevoflurane	6.5 (0.5)	127 (8)	137 (10)	131 (9)	129 (9)	136 (9)	121 (8)
d*P*/d*t*max (mm Hg s^{-1})		(%)	(%)	(%)	(%)	(%)	
Control	4788 (353)	84 (3)	78 (6)	66 (3)†	72 (3)	70 (2)†	56 (7)†
Enflurane	5308 (334)	80 (4)	75 (5)†	29 (3)*†	26 (3)*†	51 (6)†	39 (4)†
Isoflurane	6341 (351)	93 (4)	86 (7)	32 (7)*†	29 (5)*†	58 (8)†	42 (9)†
Desflurane	5240 (267)	95 (3)	95 (5)	48 (5)†	41 (4)*†	73 (6)†	49 (5)†
Sevoflurane	5509 (219)	88 (6)	95 (3)	51 (6)†	37 (6)*†	84 (7)	53 (4)†
SVR (mm Hg min $litre^{-1}$)		(%)	(%)	(%)	(%)	(%)	
Control	336 (31)	105 (3)	99 (4)	99 (3)	96 (3)	94 (3)	79 (7)
Enflurane	306 (20)	104 (8)	98 (14)	48 (8)*†	40 (6)*†	71 (12)	54 (10)†
Isoflurane	374 (16)	103 (4)	100 (4)	45 (4)*†	45 (6)*†	78 (5)	57 (9)†
Desflurane	365 (27)	102 (3)	97 (4)	51 (4)*	46 (4)*	81 (4)	68 (6)
Sevoflurane	394 (19)	103 (6)	105 (3)	57 (3)*	49 (5)*	62 (3)	96 (23)
RPP (mm Hg min^{-1} 10^3)		(%)	(%)	(%)	(%)	(%)	
Control	24.5 (2.2)	92 (2)	84 (6)	82 (4)	87 (4)	83 (4)	63 (7)†
Enflurane	26.4 (1.7)	91 (5)	98 (!4)	48 (8)*†	39 (6)*†	71 (12)†	55 (10)†
Isoflurane	35.1 (0.9)	93 (2)*	97 (11)	45 (7)*†	37 (6)*†	71 (8)†	50 (7)†
Desflurane	31.8 (1.2)	96 (2)*	98 (8)	66 (11)†	56 (9)*†	86 (11)	56 (8)†
Sevoflurane	32.0 (1.2)	92 (4)*	96 (3)	64 (7)†	46 (7)*†	89 (5)	61 (7)†
τ (ms)		(%)	(%)	(%)	(%)	(%)	
Control	16.4 (1.6)	118 (6)	120 (7)	122 (8)	121 (5)	115 (4)	138 (15)†
Enflurane	15.2 (0.8)	128 (9)	124 (6)	227 (46)*†	197 (22)†	159 (25)	191 (12)*†
Isoflurane	12.4 (0.5)	123 (4)	140 (18)	204 (31)†	244 (35)*†	134 (15)	160 (12)†
Desflurane	12.6 (0.5)	118 (6)	118 (6)	140 (15)	160 (20)†	123 (10)	151 (8)†
Sevoflurane	13.6 (0.7)	124 (8)	115 (5)	136 (12)	231 (48)*†	104 (5)	137 (9)†

Table 3 Weights and area at risk size. Data are mean (SEM). LV = Left ventricle

	Control	Enflurane	Isoflurane	Sevoflurane	Desflurane
Body weight (kg)	3.69 (0.08)	3.50 (0.12)	3.65 (0.09)	3.43 (0.07)	3.89 (0.18)
LV weight (g)	5.23 (0.36)	4.97 (0.31)	5.15 (0.36)	4.67 (0.34)	4.99 (0.26)
Area at risk (g)	1.66 (0.17)	1.51 (0.15)	1.50 (0.12)	1.45 (0.10)	1.32 (0.11)
Area at risk/LV (%)	32.9 (3.34)	28.9 (2.02)	31.1 (2.22)	35.8 (2.32)	29.3 (1.74)
Infarct size (g)	0.85 (0.14)	0.62 (0.12)	0.76 (0.10)	0.52 (0.04)	0.43 (0.07)

of variance for differences between regression slopes did not confirm this as statistically significant with seven experimental groups.

Heart rate, and consequently RPP, were higher in the isoflurane, desflurane and sevoflurane groups compared with the control group during baseline measurements. Because d*P*/d*t* and τ are influenced by HR, comparison of these variables between groups is difficult. However, differences in HR should not be responsible for the reduction in infarct size in the sevoflurane and desflurane groups, because bradycardia but not tachycardia has been demonstrated to offer beneficial effects against myocardial ischaemia–reperfusion injury.[23 24]

INTERPRETATION OF RESULTS

Coronary artery reperfusion has become the treatment of choice in myocardial infarction. While there is no question that timely restoration of coronary blood flow is essential for the salvage of ischaemic myocardium, reperfusion of ischaemic myocardium after temporary coronary artery occlusion can initiate structural and biochemical changes that limit the amount of potentially salvageable myocardium (reperfusion injury).[1] The cause of this injury is apparently multifactorial.

A protective effect of inhalation anaesthetics on ischaemic–reperfused myocardium may be caused by negative inotropic and chronotropic actions. The inhalation anaesthetics, halothane, enflurane and isoflurane, have been shown to depress myocardial contractility in a dose-dependent manner. Under aerobic conditions, isoflurane may depress myocardial contractility less than halothane or enflurane[25 26] and halothane exerts most pronounced negative inotropic actions during post-ischaemic periods.[27] For the new anaesthetics, *in vivo* studies showed comparable cardiovascular effects of sevoflurane and isoflurane.[28–30] Desflurane had similar haemodynamic effects as isoflurane in patients with coronary artery disease, except for an increase in pulmonary artery pressure and pulmonary capillary wedge pressure.[31] In reperfused isolated rat hearts, the effects of isoflurane, enflurane and sevoflurane on cardiovascular haemodynamics were similar.[27] The aim of our study

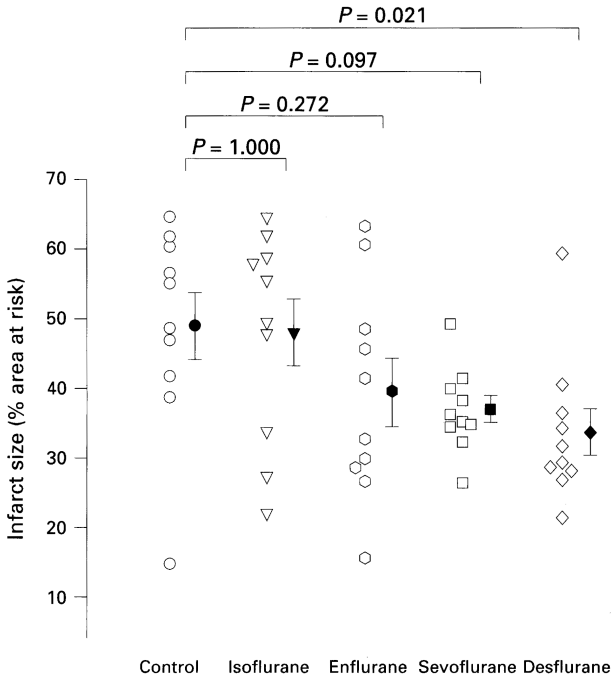

Figure 3 Infarct size as a percentage of the area at risk in the control, isoflurane, enflurane, sevoflurane and desflurane groups. Open symbols = single data points, filled symbols = mean (SEM).

was to investigate the effects of volatile anaesthetics on reperfusion injury *in vivo*. To exclude effects of volatile anaesthetics on the severity of ischaemia, we used α-chloralose for baseline anaesthesia and the volatile anaesthetics were administered only during early reperfusion to investigate specific actions against reperfusion injury. However, it is possible that alterations of systemic haemodynamics during the first 15 min of reperfusion also had effects on infarct size. Left ventricular unloading and a reduction in myocardial oxygen demand have been associated with beneficial effects against myocardial reperfusion injury.[32] LVPSP was reduced in all treatment groups, but was most pronounced in the enflurane and isoflurane groups. RPP is a variable of myocardial energy demand and oxygen consumption.[33] In our study, RPP decreased to a greater extent in the enflurane and isoflurane groups than in animals treated with sevoflurane or desflurane. However, reduction in infarct size in our study was greater using sevoflurane or desflurane than in animals which received enflurane or isoflurane. In the same experimental set-up, halothane offered pronounced protective effects against reperfusion injury even if the haemodynamic side effects were balanced by concomitantly infused norepinephrine (noradrenaline).[4]

It cannot be completely ruled out that favourable changes in myocardial inotropy and myocardial oxygen supply and demand relations during the first 15 min of reperfusion have contributed to protective effects against reperfusion injury. However, it is unlikely that they are solely responsible for the beneficial actions of sevoflurane and desflurane in this setting, and this supports the hypothesis that volatile anaesthetics also cause myocardial protection *via* other mechanisms. In isolated rat hearts perfused in a Langendorff mode, an experimental set-up where changes in haemodynamics play only a minor role, rapid recovery of post-ischaemic function was observed in desflurane-treated hearts.[8 9] After treatment with the other volatile anaesthetics, the hearts showed slower recovery of myocardial function, although all anaesthetics improved myocardial function after 60 min of reperfusion compared with control hearts.

With regard to lethal cellular injury, determined by creatine kinase release, these experiments showed that isoflurane had no effect on early lethal cellular injury compared with controls.[8 9] This is in accordance with our experiments *in vivo*, which showed no reduction in infarct size in isoflurane-treated animals compared with the control group. An increase in creatine kinase release after discontinuation of sevoflurane was observed in isolated rat hearts.[8 9] *In vivo*, there was a marked reduction in infarct size after inhalation of sevoflurane. These differences may suggest differences in early and late protection

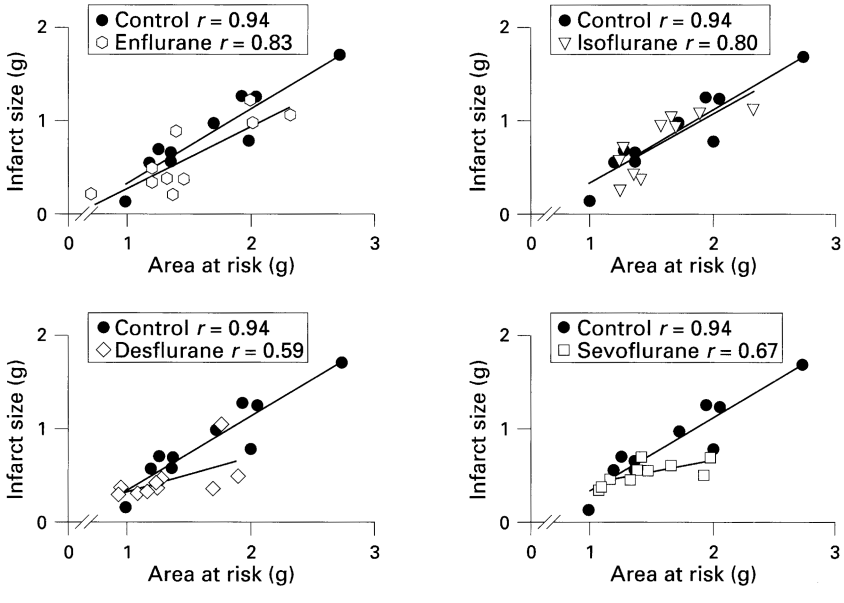

Figure 4 Scatterplot of the relationship between infarct size and size of the area at risk. Slopes of regression lines were smaller in the sevoflurane and desflurane groups compared with the control group. Control group, $y = 0.78$ (SEM 0.11) x–0.44 (0.19); sevoflurane group, $y = 0.24$ (0.10) x–0.17 (0.14); desflurane group, $y = 0.39$ (0.18) x–0.09 (0.25); enflurane group, $y = 0.65$ (0.15) x–0.37 (0.24); isoflurane group, $y = 0.74$ (0.20) x–0.39 (0.31).

against myocardial reperfusion injury by volatile anaesthetics.

Inhalation of anaesthetic was started 3 min before the end of coronary artery occlusion to ensure that a stable concentration of the anaesthetic was achieved at the start of reperfusion. Takahata, Ichihara and Ogawa demonstrated that sevoflurane attenuated ischaemic myocardial injury, an anti-ischaemic effect that could not be explained solely in terms of the haemodynamic actions of sevoflurane.[34] Isoflurane has been shown to exert protective effects if applied during ischaemia and reperfusion, despite maintenance of heart rate and arterial pressure at control values.[35] Because the rabbit has virtually no collateral circulation[36] and the volatile anaesthetics were administered for only 3 min during ischaemia, it is unlikely that a potential anti-ischaemic effect of volatile anaesthetics contributed to differences in infarct size. Volatile anaesthetics were titrated to achieve an end-tidal concentration corresponding to 1.0 MAC for rabbits. In clinical situations, comparison of volatile anaesthetics is performed using MAC values. Although the inhalation anaesthetics were administered 3 min before reperfusion, it is possible that steady state myocardial tissue concentrations had not been achieved. Differences in myocardial tissue concentrations during the early reperfusion period could not be excluded. However, the most lipophilic substance (halothane) and the least lipophilic substance (sevoflurane) caused similar reductions in infarct size.[4]

Apart from effects on systemic haemodynamics, other mechanisms have been proposed to offer potential protective effects against reperfusion injury. Beneficial alterations in intracellular calcium homeostasis,[37] actions on activated leucocytes and effects on oxygen-derived free radicals can attenuate reperfusion injury.[38] Volatile anaesthetics have been shown to influence, at least in part, sarcoplasmic reticulum function[39–41] and adhesion of leucocytes in the coronary system.[42] The protective effect of sevoflurane and desflurane observed in our study might be caused in part by actions on these cellular mechanisms. However, the exact mechanism of protection cannot be determined in the model used.

Because of increasing clinical use of thrombolysis, percutaneous balloon angioplasty and coronary bypass surgery, it is of great practical interest to determine if additional therapeutic intervention during the ischaemia–reperfusion period can lead to a reduction in ischaemia–reperfusion injury. Accumulated experimental evidence from *in vitro* and *in vivo* studies for protective effects of volatile anaesthetics should lead to clinical studies investigating effects of volatile anaesthetics on myocardial reperfusion injury.

In summary, we have shown that inhalation of sevoflurane and desflurane during the early reperfusion period reduced myocardial reperfusion injury after coronary artery occlusion *in vivo*, while enflurane showed only marginal protective effects and isoflurane offered no protection. In addition, the two new volatile anaesthetics altered systemic haemodynamics less than isoflurane and enflurane.

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