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Sevoflurane and Isoflurane Protect the Reperfused Guinea Pig Heart by Reducing Postischemic Adhesion of Polymorphonuclear Neutrophils

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Background: **Polymorphonuclear neutrophils (PMNs) contribute to reperfusion injury. Because volatile anesthetics can reduce PMN adhesion in the reperfused, nonworking heart, the authors analyzed whether this action of volatile anesthetics affects cardiac performance after ischemia and reperfusion and further clarified the underlying mechanism.**

Methods: **Isolated guinea pig hearts perfused with crystalloid buffer and performing pressure–volume work were used. Hearts were subjected to 15 min global ischemia and 20 min reperfusion. In the intervention groups an intracoronary bolus of 3×10^6 PMNs was applied in the second min of reperfusion, either in the absence or presence of 0.5 or 1 minimum alveolar concentration sevoflurane or isoflurane. The number of sequestered PMNs was calculated from the difference between coronary input and output (coronary effluent) of PMNs. Performance of external heart work, determined pre- and postischemically, served as criterion for recovery of myocardial function. Additionally, the expression of the integrin CD11b on the cell surface of PMN was measured before and after coronary passage.**

Results: **Injection of PMN in the reperfusion phase, but not under nonischemic conditions, reduced recovery of external heart work significantly (from 55 ± 7% to 19 ± 11%). Addition of sevoflurane or isoflurane in concentrations of 0.5 and 1 minimum alveolar concentration to the perfusate reduced postischemic PMN adhesion from 36 ± 8% to basal values (20 ± 7%) and prevented decline of cardiac function. CD11b expression on PMNs increased significantly during postischemic coronary passage under control conditions. Again, both anesthetics in both concentrations inhibited that activation.**

Conclusions: **Volatile anesthetics reduce PMN adhesion in the reperfused coronary system and thereby preserve cardiac function. Reduced expression of the adhesion molecule CD11b on PMNs in the presence of sevoflurane or isoflurane is, at least in part, responsible for the cardioprotective effect. (Key words: Adhesion molecule; endothelium; leukocyte.)**

SEVERAL causes for the reperfusion injury of the heart have been described, including the production of free radicals[1] or the retention of platelets[2] and polymorphonuclear neutrophils (PMNs)[1,3,4] in the coronary system. Close interaction of PMNs and endothelial cells can be mediated by two different pathways: capillary plugging, as a nonspecific physical process ensuing from narrowed capillary lumina after edema formation or reduced leukocyte elasticity,[5,6] and specific interaction of adhesion molecules, such as CD11b/CD18 with intercellular adhesion molecule 1.[7] The destructive potential of adherent and activated PMNs on the integrity of the endothelial barrier is mediated by their ability to form reactive oxygen species and to liberate proteolytic enzymes.[8]

In the clinical situation of cardiopulmonary bypass, activation of neutrophils is observed.[9,10] In the following reperfusion situation, these activated neutrophils can further aggravate the reperfusion injury. Kowalski *et al.*[11] demonstrated that sevoflurane, isoflurane, and halothane in concentrations of 1 and 2 minimum alveolar concentrations (MACs) each reduced PMN adhesion in the reperfused, nonworking heart. In that study, neither the effect of reduced PMN adhesion on myocardial performance after ischemia nor the underlying mechanism for reduced neutrophil adhesion were analyzed. Thus, we examined the effects of sevoflurane and isoflurane in the clinically relevant concentrations of 0.5 and 1 MAC on PMN adhesion in the reperfused myocardium, using a standardized model of isolated guinea pig hearts, performing pressure–volume work, and washed human PMN. Additionally, PMNs were immunocytometrically characterized (expression of CD11b) before and after

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coronary passage to detect a possible effect of anesthetics on PMN activation.

Material and Methods

Heart Preparation

The care of the animals was in full accordance with German animal-protection laws, and the experiments were officially approved by the institutional animal care committee.

Hearts were isolated from male guinea pigs (body weight 200–300 g) following cervical dislocation, without use of any anticoagulants or anesthetics. After median thoracotomy the beating hearts were arrested immediately by superfusion with ice-cold isotonic saline. The ascending aorta was cannulated and the hearts were excised. The isolated organs were perfused at 37°C using a modified Krebs-Henseleit buffer containing, in millimoles per liter, NaCl 126, $NaHCO_3$ 24, KCl 4.7, $MgSO_4$ 0.6, $CaCl_2$ 1.25, KH_2PO_4 1.2, pyruvate 0.3, and glucose 5.5, plus insulin 5 IU/L, and equilibrated with 94.4% O_2 and 5.6% CO_2 (pH 7.40 ± 0.05). Initially, perfusion was in a nonworking "Langendorff" mode to allow further preparation. The veins entering the right atrium were ligated and the pulmonary artery was cannulated to enable collection of the coronary venous effluent. The left atrium was cannulated through the pulmonary veins to allow filling and contraction of the left atrium.[12] During work phases the hearts performed pressure-volume work at a left atrial filling pressure of 12 cm H_2O and a mean aortic pressure of 80 cm H_2O.

The perfusion pressure in the nonworking mode (Langendorff mode) was continuously recorded in the aortic cannula with a pressure transducer (FMI GmbH, Egelsbach, Germany), and aortic and coronary flows were monitored with an ultrasonic flow meter (Transsonic Systems, Ithaca, NY). Heart rate was derived from the phasic perfusion pressure signal.

Preparation of PMNs

The use of human PMNs instead of guinea pig PMNs in our xenogenic model offered several advantages: Using human blood, we were able to reduce substantially the number of animals needed for the experiments, as no blood-donor animals were required. Additionally, a monoclonal antibody against the integrin CD11b is available for human PMNs but not for those of guinea pigs. Furthermore, in previous experiments we have found that PMNs from guinea pigs and humans show a quantitatively similar degree of adhesion in our model under both preischemic and postischemic conditions.[13]

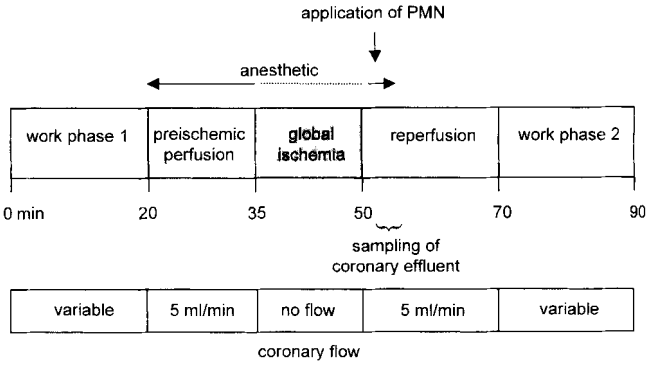

Fig. 1. Schematic presentation of the experimental protocol for working hearts. Hearts performed volume–pressure work for 20 min, both at the beginning and at the end of the protocol. After work phase W_1, coronary perfusion was changed to a volume-constant mode. The preischemic phase (5 ml/min) was successively followed by global ischemia (no-flow) and reperfusion (5 ml/min) phases. Isoflurane or sevoflurane in a concentration of 0.5 or 1 minimum alveolar concentration were applied in the preischemic perfusion phase and the first 5 min of the reperfusion phase. In the control group no anesthetics were applied. In the second min of reperfusion a bolus of 3 × 10^6 human polymorphonuclear neutrophils was applied into the coronary system. During bolus application (60 s) and the following 60 s the coronary effluent was sampled to determine the number of polymorphonuclear neutrophils leaving the coronary system.

PMNs were isolated from fresh venous blood of healthy volunteers, as described in detail previously.[14] Briefly, the blood was anticoagulated with 0.1% EDTA and centrifuged at 380*g* for 10 min. Plasma was discarded and the buffy coat carefully collected. The buffy coat was incubated with an iron-tagged monoclonal antibody against CD15 (Miltenyi, Bergisch Galdbach, Germany), an epitope specific for neutrophils, at 4°C for 15 min, and subsequently passed through a magnetized column. The column was flushed with phosphate-buffered saline (pH 7.4) to wash away unlabeled cells and, after removing the column from the magnetic field, PMNs were eluted with phosphate-buffered saline. The eluent was centrifuged at 380*g* for 10 min, and the resulting cell pellet resuspended in Tyrode's solution, counted, and adjusted to a final cell count of 3 x 10^6 cells/ml Tyrode's solution. The total time of preparation was less than 1 h.

Experimental Protocol for Working Hearts

The perfusion protocol is outlined in figure 1. Initially, hearts performed pressure-volume work (W_1) for 20 min. During this work phase the mean aortic pressure

and the left atrial filling pressure were held constant, whereas the coronary flow was autoregulated by the hearts. Thereafter, perfusion of the hearts was changed to a nonworking, constant coronary flow mode, during which—in contrast to the work phases—coronary perfusion pressure was variable. A coronary flow of 5 ml/min was established for 15 min. After that, hearts were subjected to global no-flow ischemia for 15 min. Myocardial reperfusion was established at constant coronary flow of 5 ml/min for the next 20 min, whereafter work (W_2) was performed again (20 min) under conditions identical to those of W_1. To rule out temperature effects in the nonworking Langendorff phases, hearts were immersed in 37°C Tyrode's solution.

Sevoflurane or isoflurane were added in concentrations of 0.5 MAC or 1 MAC (corresponding to 1 or 2 vol% sevoflurane[15] and 0.6 or 1.2 vol% isoflurane[16], respectively) to the oxygen-carbon dioxide gas mixture used to equilibrate a separate perfusate reservoir for the nonworking mode. This perfusate was used 15 min preischemically and for the first 5 min of the reperfusion phase. The addition of anesthetics to the perfusate was achieved by means of a calibrated vaporizer (Dräger, Lübeck, Germany), as described previously,[17] and was monitored by piezo electric gas detectors (Dräger). To ensure equilibration of volatile anesthetics with the liquid phase, application of anesthetics with the gas phase to the perfusate began 30 min before its use. To exclude negative inotropic effects of sevoflurane and isoflurane on cardiac performance during W_1 and W_2, no anesthetics were applied during the work phases. To speed up washout of volatile anesthetics from the heart until W_2, an anesthetic-free, oxygenated perfusate was used after the 5th min of reperfusion.

In the second min of reperfusion an optional 1-ml bolus of PMNs (3×10^6) suspended in Tyrode's solution was applied over 1 min into the coronary system *via* the aortic cannula with an infusion pump. To determine the rate of basal adhesion under nonischemic conditions, in one group of hearts the PMN bolus was applied in the 10th min of preischemic, volume-constant perfusion (see below).

Hearts were randomly allocated to the following experimental groups:

Time-control: perfusion with 5 ml/min coronary flow for 50 min without inducing ischemia, W_1 and W_2 being performed as in the other cases (n = 6)

Ischemia-control: no application of volatile anesthetic or PMNs (n = 9)

Ischemia + PMNs preischemic: application of PMNs in the 10th min of the preischemic perfusion (n = 6)

Ischemia + PMNs postischemic: application of PMNs in the second min of reperfusion (*n* = 10)

Ischemia + 1 MAC sevoflurane: application of 2 vol% sevoflurane to the perfusate, no infusion of PMNs (n = 8)

Ischemia + 1 MAC sevoflurane + PMNs: application of 2 vol% sevoflurane to the perfusate and infusion of PMNs in the second min of reperfusion (n = 6)

Ischemia + 0.5 MAC sevoflurane + PMN: application of 1 vol% sevoflurane to the perfusate and infusion of PMNs in the second min of reperfusion (n = 6)

Ischemia + 1 MAC isoflurane: application of 1.2 vol% isoflurane to the perfusate, no infusion of PMNs (n = 9)

Ischemia + 1 MAC isoflurane + PMNs: application of 1.2 vol% isoflurane to the perfusate and infusion of PMNs in the second min of reperfusion (n = 6)

Ischemia + 0.5 MAC isoflurane + PMNs: application of 0.6 vol% isoflurane to the perfusate and infusion of PMNs in the second min of reperfusion (n = 6)

At the end of W_1 and W_2 coronary flow, aortic flow, spontaneous heart rate, and ejection time of stroke volume were measured. External heart work (EHW) was calculated from these variables as the sum of pressure-volume work and acceleration work.[12] Because preload and afterload were held constant during phases of work, changes in cardiac performance were reflected largely as alterations in cardiac output. Recovery of EHW was defined as the ratio of the values obtained in W_2 and W_1 and expressed as a percentage.

Adhesion Measurements

Sequestration of PMNs into the coronary system was determined from the arteriovenous difference in absolute PMN count. Immediately before each intracoronary application of a bolus of PMNs, a test bolus of equal volume and duration (1 ml in 1 min) was sampled to determine the number of cells actually leaving the application syringe (PMN input). To quantify the number of PMNs leaving the coronaries (PMN output), coronary effluent was collected continuously during the minute of bolus application and in the following minute. Pilot studies had shown that only a negligible number of the applied PMNs (< 1%) emerged after such a 120-s sampling period. The test bolus and the coronary effluent were sampled into ice-cold 10% formaldehyde solution to fix cells for counting and flow cytometric analysis.

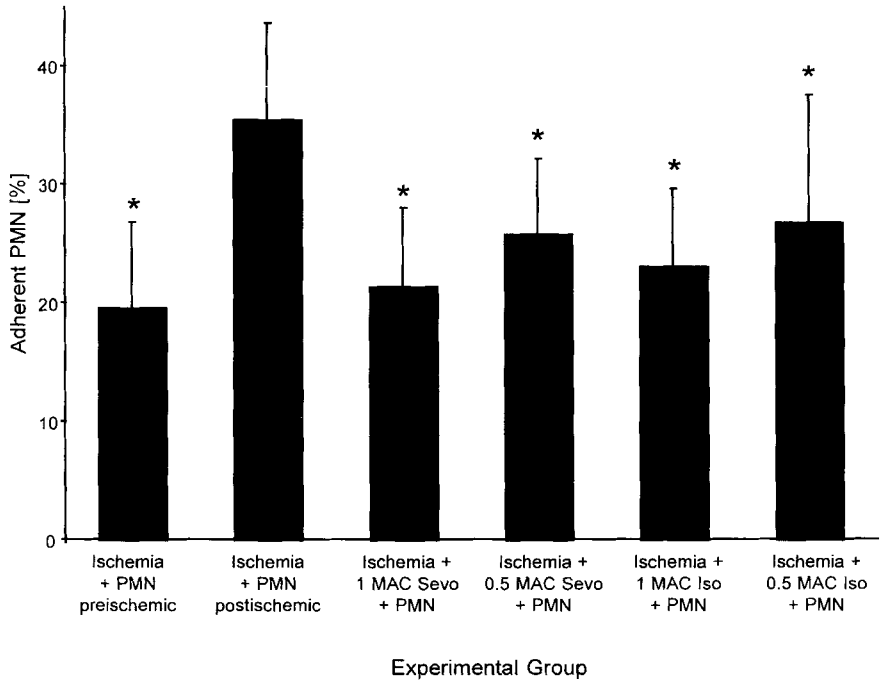

Fig. 2. Sequestration of polymorphonuclear neutrophils (PMNs) in the coronary system. Application of either isoflurane or sevoflurane in a concentration of 0.5 or 1 minimum alveolar concentration significantly reduced adhesion of PMNs in postischemic hearts to the level obtained under preischemic conditions. In the ischemia plus PMNs preischemic and ischemia plus PMNs postischemic groups, no anesthetics were applied. Data shown are means ± SD (n = 6–10). *$P < 0.05$ *versus* the ischemia plus PMNs postischemic group.

The percentage of PMNs adherent to the endothelium was calculated as follows:

$$\text{Adhesion (\%)} = \{1 - (\text{PMN output/PMN input})\} \times 100.$$

Flow Cytometry

Formalin-fixed PMNs of the test bolus and of the coronary effluent were analyzed in a FACScan flow cytometer (Becton Dickinson, San Jose, CA) for the expression of the integrin subunit CD11b using a fluorescent dye-labeled monoclonal antibody (Serotec, Oxford, UK). In general, samples of the test boli and the coronary effluent fixed in formalin were centrifuged at 1,200*g* for 15 min and the supernatant was discarded. PMNs were resuspended in 100 μl Cellwash (a phosphate-buffered saline solution; Becton Dickinson) and incubated 15 min after addition of 5 μl of the monoclonal antibody. Thereafter, 1 ml of Cellwash was added for dilution of the antibody and PMNs were centrifuged again at 1,200*g* for 15 min. The pellet was resuspended in 500 μl Cellwash and the cells were immediately analyzed in the flow cytometer for relative mean particle fluorescence intensity and forward scatter and sideward scatter distribution. Light is scattered as a cell passes through the laser beam. Forward scattered light is a measure of particle size and shape and is normally measured in the laser beam axis. Sideward scattered light is related to the granularity of the cells and measured at a 90-degree angle to the laser axis. To compensate for any day-to-day variation in instrument response, nonspecific relative mean particle fluorescence intensity of the corresponding negative control was subtracted from values measured with specific antibodies.

Statistical Analysis

Comparison of several groups was performed using one-way analysis of variance and the Student-Newman-Keul's test. Differences between data were considered significant at $P < 0.05$.

Results

Adhesion of PMN

Intracoronary adhesion of PMNs under nonischemic conditions amounted to 20% of the applied number (fig. 2), whereas in the second min of reperfusion the percentage of PMNs becoming adherent significantly increased to 36%. Application of sevoflurane or isoflurane in concentrations of 0.5 or 1 MAC reduced postischemic intracoronary sequestration of PMN back to the nonischemic basal level (fig. 2). Basal adhesion of PMN under nonischemic conditions is not significantly influenced by

Table 1. Time Course of Coronary Perfusion Pressure in Hearts Perfused at a Rate of 5 ml/min

Experimental Group	Preischemic Perfusion	Reperfusion 5 min	Reperfusion 15 min
Ischemia–control	42 ± 13	30 ± 7	46 ± 12
Ischemia + PMN preischemic	45 ± 8	32 ± 6	57 ± 13
Ischemia + PMN postischemic	37 ± 8	30 ± 5	47 ± 16
Ischemia + 1 MAC Sevo	37 ± 13	30 ± 6	41 ± 13
Ischemia + 1 MAC Sevo + PMN	49 ± 11	32 ± 4	58 ± 16
Ischemia + 0.5 MAC Sevo + PMN	44 ± 8	31 ± 4	53 ± 11
Ischemia + 1 MAC Iso	35 ± 11	27 ± 4	41 ± 12
Ischemia + 1 MAC Iso + PMN	34 ± 6	27 ± 4	43 ± 8
Ischemia + 0.5 MAC Iso + PMN	40 ± 10	25 ± 5	50 ± 10

No statistically significant differences were detectable between the groups at any time point. Especially, application of PMN in the 2nd min of reperfusion did not increase coronary perfusion pressure. Values, given in cmH_2O, are means ± SD (n = 6–10 each).

PMN = polymorphonuclear neutrophil; MAC = minimum alveolar concentration; Sevo = sevoflurane; Iso = isoflurane.

volatile anesthetics, even when applied at a concentration of 2 MAC.[11]

Coronary Perfusion Pressure

The time course of the coronary perfusion pressure during preischemic and postischemic volume-constant perfusion is outlined in table 1. No overt decrease of coronary perfusion pressure and thereby of coronary resistence (constant flow) was observed in the presence of the volatile anesthetics at the concentrations employed here. Coronary perfusion pressure did not change after application of the PMN bolus in the second min of reperfusion, and, thus, capillary plugging can be ruled out as a major cause of PMN sequestration. During early postischemic reperfusion there was always some reactive dilatation (decrease in coronary perfusion pressure). In the course of reperfusion coronary perfusion pressure rose continuously, exceeding preischemic levels after 15 min in all groups. However, no significant differences were detectable between groups at any time.

Hemodynamic Parameters and Recovery of EHW

The hemodynamic data of the working hearts are listed in detail in table 2. There were no significant differences of any of the measured parameters between groups in W_1. Coronary flow was consistently lower in W_2 than in W_1, whereas heart rate only tended to fall somewhat. The greatest effects, however, were seen for aortic flow and EHW. For both parameters, the values of the ischemia plus PMN postischemic group were significantly lower in W_2 compared with all other groups. The volatile anesthetics had no influence by themselves but served to overcome the postischemic losses of function induced by PMN applied in the second min of reperfusion.

The postischemic recovery of EHW is illustrated in figure 3. EHW of time-control hearts, *i.e.*, hearts that had not been subjected to ischemia, proved to be stable and in W_2 averaged about 95% of the value in W_1. EHW of ischemia-control hearts in W_2 was reduced to about 60% (fig. 3A). Neither the application of 1 MAC isoflurane or sevoflurane in the absence of PMNs nor the preischemic infusion of PMNs had an additional influence on functional recovery (fig. 3B). In contrast, application of PMNs in the second min of reperfusion significantly reduced recovery of EHW to a mere 20%: a loss of about 40% *versus* the ischemia-control group and of 75% in comparison with the time-control group. Application of sevoflurane and isoflurane at concentrations of both 0.5 and 1 MAC prevented the deterioration of heart performance otherwise seen after PMN application (fig. 3B).

Immunocytometric Characterization of PMN

The relative immunofluorescence intensity of CD11b before coronary passage (test bolus) ranged between 10 and 22 relative units but did not significantly differ between the groups. Because the expression of CD11b showed a marked variability between different cell preparations already in the test boli, the individual percentage change of CD11b after coronary passage was calculated for each experiment. That relative increase is depicted for all groups in figure 4. Whereas preischemic infusion of PMNs did not result in an activation, postischemic application of PMNs led to a significant increase of CD11b expression (fig. 4). Interestingly, application of sevoflurane and isoflurane significantly inhibited the rise in CD11b expression during postischemic coronary passage, both at 0.5 and 1 MAC concentration.

The forward scatter of PMNs, which is a measure of

Table 2. Preischemic (W_1) and Postischemic (W_2) Hemodynamic Parameters of Hearts Subjected to 15 min of Global Ischemia and 20 min of Reflow with or without Application of PMN in the 2nd min of Reperfusion

Experimental Group		Coronary Flow (ml/min)	Aortic Flow (ml/min)	Heart Rate (min^{-1})	EHW (mJ/min)
Time–control	W_1	9.1 ± 0.6	49.8 ± 5.6	246 ± 12	400 ± 40
	W_2	8.2 ± 0.6*	47.1 ± 7.4*	235 ± 10	370 ± 50*
Ischemia–control	W_1	9.9 ± 2.4	49.6 ± 5.6	246 ± 18	400 ± 50
	W_2	7.5 ± 2.9*	27.4 ± 8.0*†	237 ± 20	240 ± 70*†
Ischemia + PMN preischemic	W_1	8.1 ± 1.1	50.8 ± 15	250 ± 12	400 ± 10
	W_2	5.1 ± 0.6†	31.4 ± 4.2*†	244 ± 12	240 ± 30*†
Ischemia + PMN postischemic	W_1	8.5 ± 1.6	45.2 ± 11	245 ± 17	370 ± 80
	W_2	4.0 ± 1.0†	7.6 ± 6.7†	217 ± 28	70 ± 50#
Ischemia + 1 MAC Sevo	W_1	10.5 ± 1.9	54.9 ± 7.4	247 ± 13	450 ± 50
	W_2	6.6 ± 0.9*	29.4 ± 11*†	244 ± 11	250 ± 80*†
Ischemia + 1 MAC Sevo + PMN	W_1	8.0 ± 1.6	54.3 ± 8.1	251 ± 13	420 ± 70
	W_2	5.0 ± 1.5†‡	29.4 ± 9.5*†	239 ± 13	230 ± 70*†
Ischemia + 0.5 MAC Sevo + PMN	W_1	8.2 ± 1.5	50.3 ± 11	248 ± 18	400 ± 80
	W_2	4.8 ± 0.8†‡	22.6 ± 9.3*†	238 ± 17	180 ± 70*†
Ischemia + 1 MAC Iso	W_1	9.4 ± 1.3	50.8 ± 6.9	242 ± 11	410 ± 50
	W_2	5.9 ± 1.0†	25.1 ± 7.6*†	239 ± 12	210 ± 60*†
Ischemia + 1 MAC Iso + PMN	W_1	9.3 ± 0.9	48.8 ± 6.7	244 ± 15	390 ± 50
	W_2	5.4 ± 0.5†	21.5 ± 8.8*†	229 ± 19	180 ± 60*†
Ischemia + 0.5 MAC Iso + PMN	W_1	8.9 ± 1.2	55.2 ± 11	258 ± 9	430 ± 80
	W_2	6.0 ± 1.4†	29.5 ± 14*†	253 ± 19*	240 ± 10*†

Isoflurane or sevoflurane, each in a concentration of 0.5 and 1 MAC, were applied to some hearts 15 min preischemically plus the initial 5 min of the reperfusion phase. Values are means ± SD; n = 6–10 each.

EHW = external heart work; PMN = polymorphonuclear neutrophil; MAC = minimum alveolar concentration; Sevo = sevoflurane; Iso = isoflurane.

* $P < 0.05$ *versus* ischemia + PMN postischemic group.

† $P < 0.05$ *versus* time–control group.

‡ $P < 0.05$ *versus* ischemia–control group.

shape and volumes change of cells,[18] increased in all groups during coronary passage, although the sideward scatter, which is a parameter of cellular granule content,[18] decreased (table 3). Thus, application of volatile anesthetics did not influence the average shape change and secretion of granula of PMNs seen in PMNs collected after coronary passage.

Discussion

As previously demonstrated, volatile anesthetics did not influence the basal adhesion of PMNs under nonischemic conditions, even at a concentration of 2 MAC.[11] In the reperfusion phase adhesion was almost doubled, but application of sevoflurane or isoflurane at concentrations of 0.5 and 1 MAC equally reduced postischemic PMN sequestration to or near to basal levels. Application of 2 MAC had no further effect.[11] Obviously, in the range investigated, there was no direct dose–response relationship for the applied concentrations of anesthetics and PMN adhesion, we rather observed an all-or-nothing relation. Thus, both sevoflurane and isoflurane selectively reduce postischemic PMN adhesion at concentrations relevant for the clinical situation.

Reduced PMN sequestration in the reperfusion phase was associated with significantly improved recovery of EHW in comparison to the ischemia plus PMNs group. Our finding that reduced neutrophil adhesion occurs in parallel to improved heart recovery after ischemia is in accordance to results obtained by others.[3,19] Cardioprotective properties of sevoflurane and isoflurane have been described by several groups, including metabolic[20–22] or, additionally, functional improvement after ischemia[23–26], although without considering involvement of PMN. Our finding that 1 MAC sevoflurane or isoflurane in the absence of PMNs did not improve recovery of EHW in comparison to the ischemia-control group does not exclude a direct cardioprotective effect of volatile anesthetics, as described by others. The experimental setup and protocol (duration of ischemia, mode of reperfusion, species, *etc.*) may have an important influence on the direct cardioprotective properties of volatile anesthetics.

The inhibitory effect of volatile anesthetics on PMN

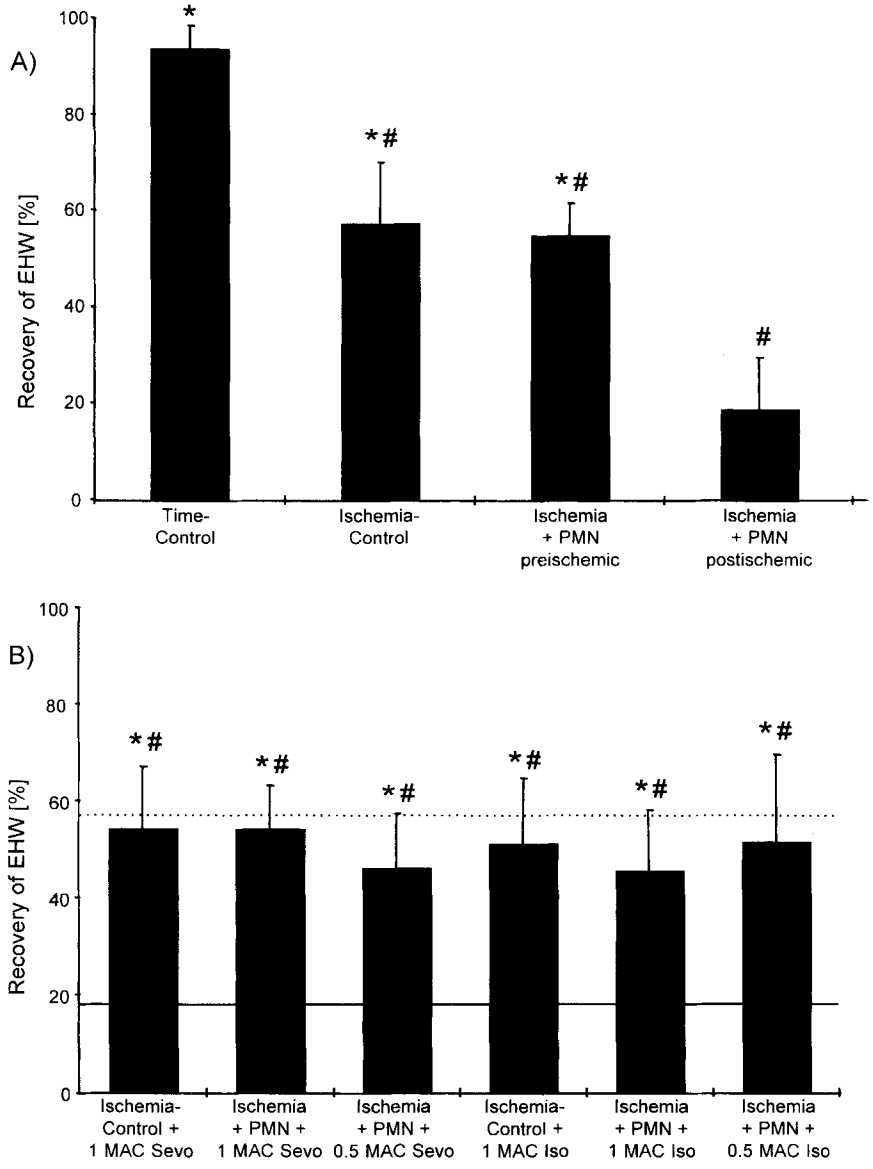

Fig. 3. Recovery of external heart work (W_2 *vs.* W_1). (*A*) shows the control groups without anesthetics, (*B*) the intervention groups receiving sevoflurane or isoflurane. The dashed line in the lower panel indicates the recovery of external heart work (EHW) of the ischemia-control group, the complete line that of the ischemia plus polymorphonuclear neutrophils (PMNs) postischemic group. Time-control hearts were not subjected to ischemia. All other groups were subjected to 15 min of global no-flow ischemia, which caused loss of recovery. After application of 3 × 10^6 PMNs in the second min of reperfusion, recovery of EHW was significantly decreased in comparison with the ischemia-control group, but not when infusion of PMNs occurred in the presence of either sevoflurane or isoflurane. Minimum alveolar concentrations (MAC) of 0.5 and 1 volatile anesthetic were about equally protective. Application of anesthetics (1 minimum alveolar concentration) under control conditions had no impact on recovery of EHW. Values are means ± SD, n = 6–10. *$P < 0.05$ *versus* the ischemia plus PMN postischemic group; #$P < 0.05$ *versus* the time-control group.

adhesion could be mediated by "nonspecific" effects on the myocardium or the microvasculature or by "specific" effects on the integrin-mediated adhesion event. As an example of the former, sevoflurane and isoflurane might influence the ischemic alterations of the heart and thereby protect the myocardium in the reperfusion phase. Two facts argue against this possibility. First, in previous studies we demonstrated that sevoflurane and isoflurane had no impact on the production of lactate by isolated hearts after ischemia.[11,27] Second, in the present study no increased recovery of heart function was detectable under control conditions (no PMN infusion) in the presence of 1 MAC of sevoflurane or isoflurane in comparison with the ischemia-control group, as one would have to expect for an ischemia-mitigating effect of volatile anesthetics.

CD11b is expressed on the cell surface of PMNs and is up-regulated after activation. It forms a heterodimer with CD18, and this complex binds to intercellular adhesion molecule 1 which is expressed on the endothelium, thereby mediating the sticking of activated PMNs.[28] Coapplication of PMNs and a monoclonal antibody against CD18 reduced PMN adhesion to about 10% under pre- and postischemic conditions in our model.[13] Part of this remaining

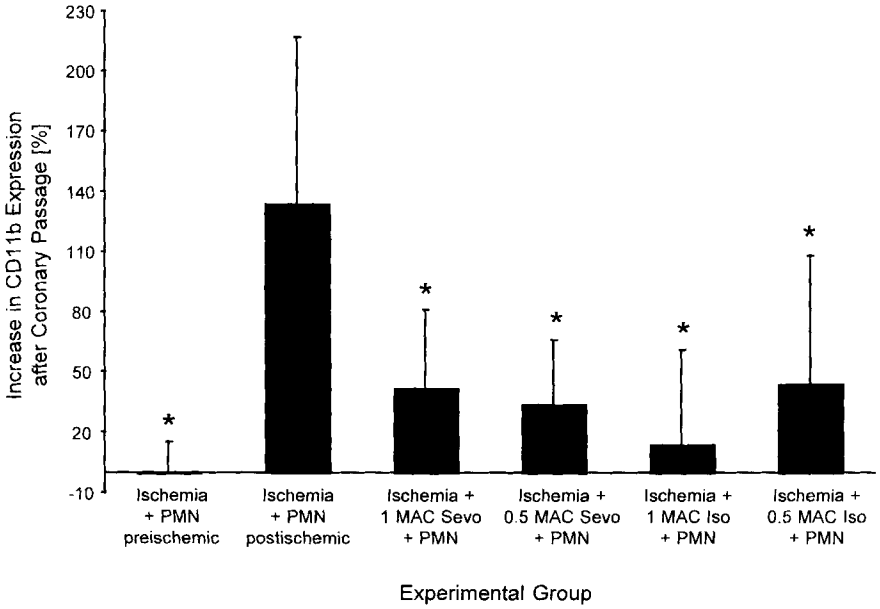

Fig. 4. Increase of CD11b expression of polymorphonuclear neutrophils (PMNs) after coronary passage. The percent increase of CD11b expression of PMNs after coronary passage in comparison with the PMNs of the test bolus is illustrated in this figure. Under preischemic conditions no increase of CD11b expression was detectable. In contrast, PMNs applied in the reperfusion phase experienced a significant increase of CD11b. The presence of sevoflurane or isoflurane in the perfusate (0.5 and 1 minimum alveolar concentration) inhibited the activation of PMNs about equally. *$P < 0.05$ *versus* the ischemia plus PMNs postischemic group.

10% of sequestered PMNs might be the consequence of mechanical trapping in the coronary system. However, the coronary perfusion pressure showed no increase after PMN application, unlike what has been observed after capillary plugging with 10-μm diameter microspheres.[13] Thus, formation of PMN aggregates and any substantial capillary plugging can be excluded in our experimental setting. Reduced mechanical trapping of PMNs caused by vasodilation of the microvasculature in the presence of volatile anesthetics as a relevant factor for the reduction of PMN adhesion can be also largely ruled out at 0.5 and 1 MAC (table 1). For these reasons, the majority of PMNs, especially under postischemic conditions, probably become adherent as the result of specific, integrin-mediated interaction with the coronary endothelium. Sevoflurane and isoflurane probably interfere with the integrin-mediated binding of PMNs. This could be caused by inhibition of endothelial activation (favoring rolling, production of PMN activators, *etc.*) or of stimulation of PMNs (CD11b/CD18 upregulation, *etc.*).

Table 3. Changes (Relative Value in the Coronary Effluent minus Relative Value in Test Bolus) of the Forward and Sideward Scatter of PMN after Coronary Passage, Measured by Flow Cytometry

Experimental Group	Forward Scatter	Sideward Scatter
Ischemia + PMN preischemic	10 ± 2	−6 ± 2
Ischemia + PMN postischemic	9 ± 7	−4 ± 4
Ischemia + 1 MAC Sevo + PMN	11 ± 7	−6 ± 4
Ischemia + 0.5 MAC Sevo + PMN	15 ± 5	−6 ± 3
Ischemia + 1 MAC Iso + PMN	7 ± 9	−3 ± 6
Ischemia + 0.5 MAC Iso + PMN	11 ± 3	−7 ± 3

For neither of the two parameters significant differences were detectable between groups. Values are means ± SD; n = 6–10 each.

PMN = polymorphonuclear neutrophil; MAC = minimum alveolar concentration; Sevo = seroflurane; Iso = isoflurane.

Coronary passage of PMNs induced a shape change (polarization) and a slight degranulation of PMNs[18] in all groups, including PMNs applied under preischemic conditions. For these changes, therefore, the coronary passage itself, but not reperfusion conditions, seems to suffice as trigger. Polarization and degranulation of PMN normally occur after their activation. In the ischemia plus PMNs postischemic group the most activated PMNs become stuck in the coronary system and therefore cannot be analyzed in the coronary effluent. Accordingly, PMNs applied postischemically show about the same extent of polarization and degranulation after coronary passage as those applied preischemically. This is corroborated by the fact that PMNs pretreated with anti-CD18, which inhibits adhesion of the activated PMNs, show a significantly increased degranulation after coronary passage.[13] In contrast, the additional PMN emerging from hearts receiving the volatile anesthetics were not activated to any greater extent than the preischemic controls. Thus, one may conclude that volatile anesthetics mitigate PMN adhesion. Significantly increased expression of CD11b after coronary passage was only observed when PMNs were applied in the postisch-

emic phase. Thus, special stimuli present in the early reperfusion, such as free radicals, cytokines, or platelet-activating factor, appear to be responsible for the increased CD11b expression on the cell surface. Accordingly, sevoflurane and isoflurane at 0.5 and 1 MAC could interfere with the activation cascade for CD11b of PMNs. Several studies have shown interference of volatile anesthetics with intracellular messenger systems, such as cyclic adenosine monophosphate,[29] cyclic guanosine monophosphate,[30] or inositol triphosphate signal cascades.[31] Additionally, an endothelial site of action could reduce postischemic activation, because immunomodulatory effects of volatile anesthetics on cytokines have been described.[32] Two arguments for PMNs as the target of the inhibitory effect of anesthetics have been found. Kowalski *et al.*[11] demonstrated that even the application of sevoflurane to the perfusate directly before PMN application reduced the amount of adherent neutrophils after ischemia. In *in vitro* experiments on endothelial cell cultures, Möbert *et al.*[33] found that the pretreatment of PMNs, but not of endothelial cells, with volatile anesthetics reduced the amount of adherent PMN.

The results of our study cannot be directly transferred to the clinical situation, as our experimental model has inherent limitations. For instance, nonhuman hearts were perfused with a crystalloid buffer, not with whole blood. The latter may have direct effects on cardiac performance.[34,35] Furthermore, a possible modulation of PMN reactivity by other blood constituents (*e.g.*, platelets) or plasma components cannot be assessed. Finally, different shear rates in the coronary system may have essential influence on PMN adhesion and may be different in human hearts during reperfusion in the presence of erythrocytes.

In conclusion, cardiac dysfunction after global ischemia was significantly more pronounced after intracoronary application of PMN in the reperfusion phase. The presence of 0.5 or 1 MAC sevoflurane and isoflurane in the perfusate significantly reduced the amount of adherent PMNs and prevented PMN-induced deterioration of cardiac function. Attenuation of the increased expression of CD11b on PMNs during postischemic coronary passage seems to be at least one mechanism relevant to this cardioprotective effect. Our observation of improved postischemic recovery of EHW resulting from inhibited PMN activation and adhesion is a further important aspect in favor of cardioprotective properties of volatile anesthetics.

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