

Intermittent Pharmacologic Pretreatment by Xenon, Isoflurane, Nitrous Oxide, and the Opioid Morphine Prevents Tumor Necrosis Factor α -induced Adhesion Molecule Expression in Human Umbilical Vein Endothelial Cells

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Background: The barrier properties of the endothelium are of critical importance during pathophysiologic processes. These barrier properties depend on an intact cytoskeleton and are regulated by cell adhesion molecules. Tumor necrosis factor α (TNF- α) is known to induce cell adhesion molecule expression. In myocardium, the protective effect by xenon and isoflurane preconditioning was found to be linked to the cytoskeleton. The authors investigated the impact of different anesthetics and morphine on TNF- α -induced endothelial cell adhesion molecule expression.

Methods: Human umbilical vein endothelial cells were isolated from three different preparations. Cells were either left untreated or pretreated with xenon, nitrous oxide, isoflurane (each 0.43 minimum alveolar concentration), or morphine (100 ng/ml) and stimulated with 10 ng/ml TNF- α . Reverse-transcription polymerase chain reaction and fluorescence-activated cell sorting of intracellular cell adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin were performed. Transcriptional activity of nuclear factor κ B was assessed by infrared electrophoretic mobility shift assay.

Results: Tumor necrosis factor α significantly induced messenger RNA (mRNA) and protein expression of cell adhesion molecules as well as transcriptional activity of nuclear factor κ B. All four agents prevented TNF- α -induced mRNA and protein expression of intracellular cell adhesion molecule 1. Vascular cell adhesion molecule 1 expression was only blocked by the inhalational anesthetics and not by morphine. None of the four agents had an effect on TNF- α induced E-selectin expression. TNF- α -induced transcriptional activity of nuclear factor κ B was decreased by all four agents.

Conclusion: These results suggest a protective effect of anesthetics on TNF- α -induced endothelial cell damage.

ONE major target of damage during ischemia-reperfusion is the endothelium, and the barrier properties of the endothelium are critically important during different

pathophysiologic processes. In health, the luminal endothelial cell surface is a relatively nonadhesive conduit for the macromolecular constituents of the blood. During ischemia-reperfusion, various adhesive interactions between endothelial cells and the constituents of the blood are changed to recruit circulating leukocytes to sites of inflammation. These processes are mainly regulated by changes in the cytoskeleton and the regulation of the expression of cell adhesion molecules (CAMs), such as intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin.¹

An increased release of proinflammatory cytokines, such as tumor necrosis factor α (TNF- α), proceeds this process and increases the expression of CAMs during ischemia-reperfusion or inflammatory processes. The regulation of CAM expression occurs at the transcriptional level and is mediated, especially for ICAM-1, *via* the proinflammatory transcription factor nuclear factor κ B (NF- κ B).²

During the past decade, inhalational anesthetics, *i.e.*, halogenated fluorocarbons such as isoflurane, as well as the noble gas xenon, have been recognized to induce preconditioning,^{3,4} and we previously showed that this effect is mediated by phosphorylation and translocation of protein kinase C- ϵ from the cytosol to membranes and p38 mitogen-activated protein kinase.⁵ Downstream of protein kinase C and p38 mitogen activated protein kinase, the activation of small heat shock protein 27 and colocalization of heat shock protein 27 with the actin cytoskeleton link pharmacologic preconditioning by anesthetics to the cytoskeleton of the cardiomyocyte.⁶

So far, not much is known about the effects of anesthetic preconditioning on the endothelium. It was shown that polymorphonuclear neutrophil adhesion, which contributes to the endothelial damage during ischemia-reperfusion, can be influenced by volatile anesthetics.^{7–9} Zahler *et al.*¹⁰ suggested that the phenomenon of ischemic preconditioning of the cardiomyocytes might be transferable to the endothelium. They showed that transient exposition of human umbilical vein endothelial cells (HUVECs) to hydrogen peroxide for 5 min reduced the TNF- α -induced expression of ICAM-1 and E-selectin. It was shown that preconditioning of rat aortic endothelial cells by anoxia and reoxygenation leads to a decreased expression of ICAM-1. This effect was mediated *via* protein kinase C,¹¹ which is a key mediator

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of anesthetic-induced preconditioning of the heart *in vivo*.⁵ A continuous pretreatment of HUVECs (60 min) with 1 minimum alveolar concentration (MAC) of the volatile anesthetic desflurane might suppress the expression of CAMs.¹² Interestingly, de Rossi *et al.*¹³ showed that also the chemically inert gas xenon can reduce the cell surface expression of L-selectin and P-selectin glycoprotein ligand 1 in isolated neutrophils.

These data suggest that anesthetics coming from different drug classes might share the property to differentially regulate cytokine-induced CAM expression on the endothelium.

Therefore, we hypothesized that the anesthetics nitrous oxide, xenon, and isoflurane and the analgetic opioid morphine might influence the TNF- α -induced expression of the three adhesion molecules ICAM-1, VCAM-1, and E-selectin. Moreover, we assumed that the four anesthetic-related agents might have different effects on the expression of the respective adhesion molecule on endothelial cells.

Materials and Methods

The hospital's medical ethics committee was consulted, but according to German regulations, no formal approval is required for research with noninvasive interventions, such as working with human umbilical cords.

All experiments with gases were performed in a specialized gas chamber under temperature control. Six Petri dishes containing cells can be placed on the tray in the center of the gas chamber. The respective gases or gas mixtures are administered from below the dishes and equally distributed by using a fan inside the chamber. Their concentrations and compositions can be monitored at the outlet of the chamber by a gas analyzer (Capnomatic Ultima; Datex, Helsinki, Finland). We worked with a standard mixture of xenon (70%, 25% O₂, 5% N₂), which was provided by Linde Gas AG (Pullach, Germany) and accompanied by a certificate of analysis for xenon verifying the composition of the mixture. Moreover, we measured the oxygen content given on the bottle (25%) at the outlet of the gas chamber to have an indirect proof for the validity of the mixture. In the bottom of the chamber, a temperature plate is installed, which is connected to an outside thermometer and a temperature-controlling device that exactly controlled and regulated the temperature within the chamber.

Materials

Xenon and nitrous oxide were purchased from Linde Gas AG. Isoflurane (Forene[®]) was from Abbott (Wiesbaden, Germany). If not mentioned otherwise, all materials were purchased from either Sigma (Taufkirchen, Germany) or Merck-Eurolab (Munich, Germany).

Isolation and Passaging of HUVECs

Human umbilical vein endothelial cells were prepared by digestion of umbilical veins with 0.1 g/l collagenase A (Roche, Mannheim, Germany) and were grown in endothelial cell growth medium (Promocell, Heidelberg, Germany) supplemented with penicillin (100 U/ml)-streptomycin (100 ng/ml). To compensate for interindividual differences and to obtain the necessary yield of cells, cells of at least two umbilical cords were combined for each cell preparation (primary culture), and each experiment was performed with cells from three different preparations (three different primary cultures). Each primary cell culture flask containing a confluent monolayer of HUVECs on 25 cm² was disseminated into a 75-cm² cell culture flask (splitting 1:3). This procedure was repeated after confluency of passage 1 until passage 2 or 3. Depending on the method that was subsequently used (fluorescence-activated cell sorting, reverse-transcription polymerase chain reaction, electrophoretic mobility shift assay), cells were disseminated in different tissue culture plates to facilitate stimulation and subsequent assembling of the cells for the measurement. Each experiment was performed at least three times in triplicate. Therefore, our sample size is given by the amount of primary cell culture preparations we worked with.

For passaging of HUVECs, medium was removed and the cells were washed three times with ice-cold phosphate-buffered saline (PBS). Afterward, HUVECs were incubated with 2 ml trypsin-EDTA solution for 2 min at 37°C. The cells were gradually detached, and the digestion of trypsin was stopped with media 199 containing 10% fetal calf serum. After centrifugation at 218g, 4°C, for 10 min, the supernatant was discarded and the pellet was resuspended in endothelial cell growth medium (Promocell) and penicillin (100 U/ml)-streptomycin (100 ng/ml). Experiments were performed with cells of passage 2 or 3. HUVECs were identified using an anti-serum against the von Willebrand factor (Serotec LTD, Wiesbaden, Germany). Fluorescence-activated cell sorting analysis of von Willebrand factor staining revealed that the cell preparation was 92% pure.

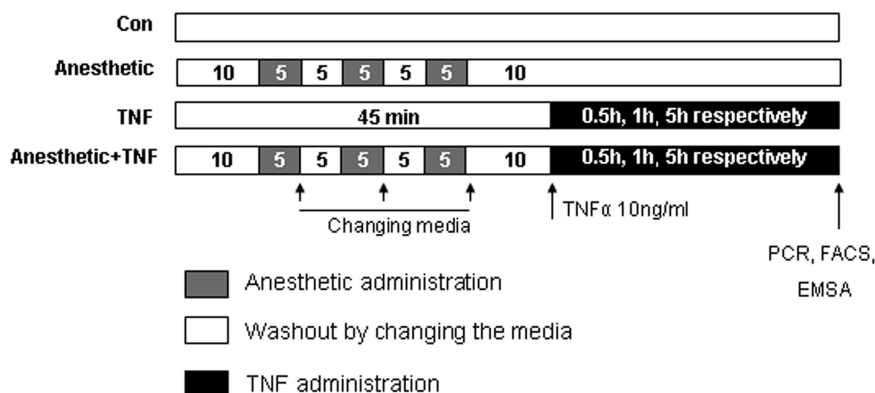
Experimental Protocol

The experimental protocol is shown in figure 1.

After the respective pretreatment protocol, HUVECs were left either untreated or incubated with TNF- α (10 ng/ml) for the respective times. To ensure an "intermittent" treatment with the respective anesthetic, media was changed during the washout periods (fig. 1). After this treatment, reverse-transcription polymerase chain reaction and fluorescence-activated cell sorting analysis of ICAM-1, VCAM-1, and E-selectin and infrared electrophoretic mobility shift assay were performed (fig. 1).

To rule out an influence of media change or different oxygen content in the gas mixtures, control experiments were performed. Fluorescence-activated cell sorting

Fig. 1. The experimental protocol. Anesthetics: 0.43 minimum alveolar concentration (MAC) xenon, 0.43 MAC nitrous oxide, 100 ng/ml morphine, 0.43 MAC isoflurane. Con = control cells; EMSA = electrophoretic mobility shift assay; FACS = fluorescence-activated cell sorting; PCR = reverse-transcription polymerase chain reaction; TNF = 10 ng/ml tumor necrosis factor α .



analysis on ICAM-1, VCAM-1, and E-selectin expression revealed that the media did not change it by itself and the concentration of 25% (present in the xenon and nitrous oxide mixture) or 80% oxygen (present in the isoflurane mixture) had no effect on TNF- α -induced adhesion molecule expression (data not shown).

Flow Cytometric Analysis of Cell Adhesion Molecules

Four hours after treatment, surface expression of adhesion molecules was measured by flow cytometry using fluorescein isothiocyanate-labeled antibodies against ICAM-1, VCAM-1, and E-selectin (Biosource and Calbiochem, Nivelles, Belgium). Briefly, cells were washed with ice-cold PBS three times and trypsinized. After detaching the cells and transferring them into fluorescence-activated cell sorting tubes, the digestion was stopped by addition of medium 199 containing 10% fetal calf serum. After centrifugation (218g, 4°C, 15 min), the cell pellet was washed with ice-cold PBS three times. Cells were incubated with 1% bovine serum albumin for 30 min at room temperature. After 30 min of incubation with the respective antibody at 4°C, the cells were again washed with PBS and resuspended in a volume of 300 μ l PBS for flow cytometric analysis. For the adjustment of instrument settings, control cells were prepared by performing the procedure as described above but by omitting the addition of the antibody. At least three different sets of experiments with cells from different isolations were performed in triplicate.

RNA Isolation and Reverse-transcription Polymerase Chain Reaction

RNA was prepared using an RNeasy[®] RNA isolation Mini kit (Qiagen, Hilden, Germany). Reverse-transcription polymerase chain reaction was performed using a One-step reverse-transcription polymerase chain reaction kit from Qiagen. Reverse-transcription polymerase chain reaction experiments were performed with primers for respective target enzyme ICAM-1 (sense: 5'-TAT-GGC-AAC-GAC-TCC-TTC-T-3', antisense: 5'-CAT-TCA-GCG-TCA-CCT-TGG-3') VCAM-1 (sense: 5'-ATG-ACA-TGC-TTG-

AGC-CAG-G-3', antisense: 5'-GTG-TCT-CCT-TCT-TTG-ACA-CT-3'), and E-selectin (sense: 5'-CTC-TGA-CAG-AAG-AAG-CCA-A-3', antisense: 5'-ACT-TGA-GTC-CAG-TGA-AGC-CA-3') and were standardized on glyceraldehyde 3-phosphate dehydrogenase (sense: 5'-TCA-CTC-AAG-ATT-GTC-AGC-AA-3', antisense: 5'-AGA-TCC-ACG-ACG-GAC-ACA-TT-3'). The conditions were as follows: CAMs: 30 min, 50°C (94°C for 1 min, 55°C for 1 min, 72°C for 1 min), 30 cycles; glyceraldehyde 3-phosphate dehydrogenase: 30 min, 50°C (93°C for 24 s, 55°C for 30 s, 73°C for 1 min), 30 cycles, adapted from previously described conditions.¹⁴ Polymerase chain reaction was followed by gel electrophoresis, ethidium bromide staining, and densitometric analysis using a Kodak Image station[®] (Eastman Kodak Comp., Rochester, NY).

Electrophoretic Mobility Shift Assay of Transcription Factor with Infrared Technology

Human umbilical vein endothelial cells were cultured in six-well plates until confluence. After the pretreatment protocol (fig. 1), they were left either untreated or treated with TNF- α (10 ng/ml) for 30 min. Nuclear and cytosolic extracts were prepared as described previously.¹⁵ Briefly, HUVECs were washed with PBS, scraped, and resuspended in 1,000 μ l hypotonic buffer. Cells were allowed to swell on ice for 15 min. Nonidet P-40 (10%, 100 μ l) was then added, followed by 10 s of vigorous vortexing and centrifugation at 12,000g for 30 s. The supernatant (containing the cytosolic protein) was removed. The nuclear pellet was extracted with 175 μ l hypertonic buffer by shaking at 4°C for 15 min. The extract was centrifuged at 12,000g, and the supernatant was frozen at -80°C. Protein concentrations were determined by the method of Lowry *et al.*¹⁶

A 22-mer double-stranded oligonucleotide probe containing a consensus binding-sequence for NF- κ B (5'-AGT TGA GGG GAC TTT CCC AGG C-3'), which is 5' end-labeled with an IRDye 700 (Licor Bioscience, Bad Homburg, Germany), was incubated with equal amounts of nuclear protein (5 μ g) for 20 min at room temperature in the dark in the binding buffer containing 100 mM Tris-HCl, 500 mM potassium chloride, 10

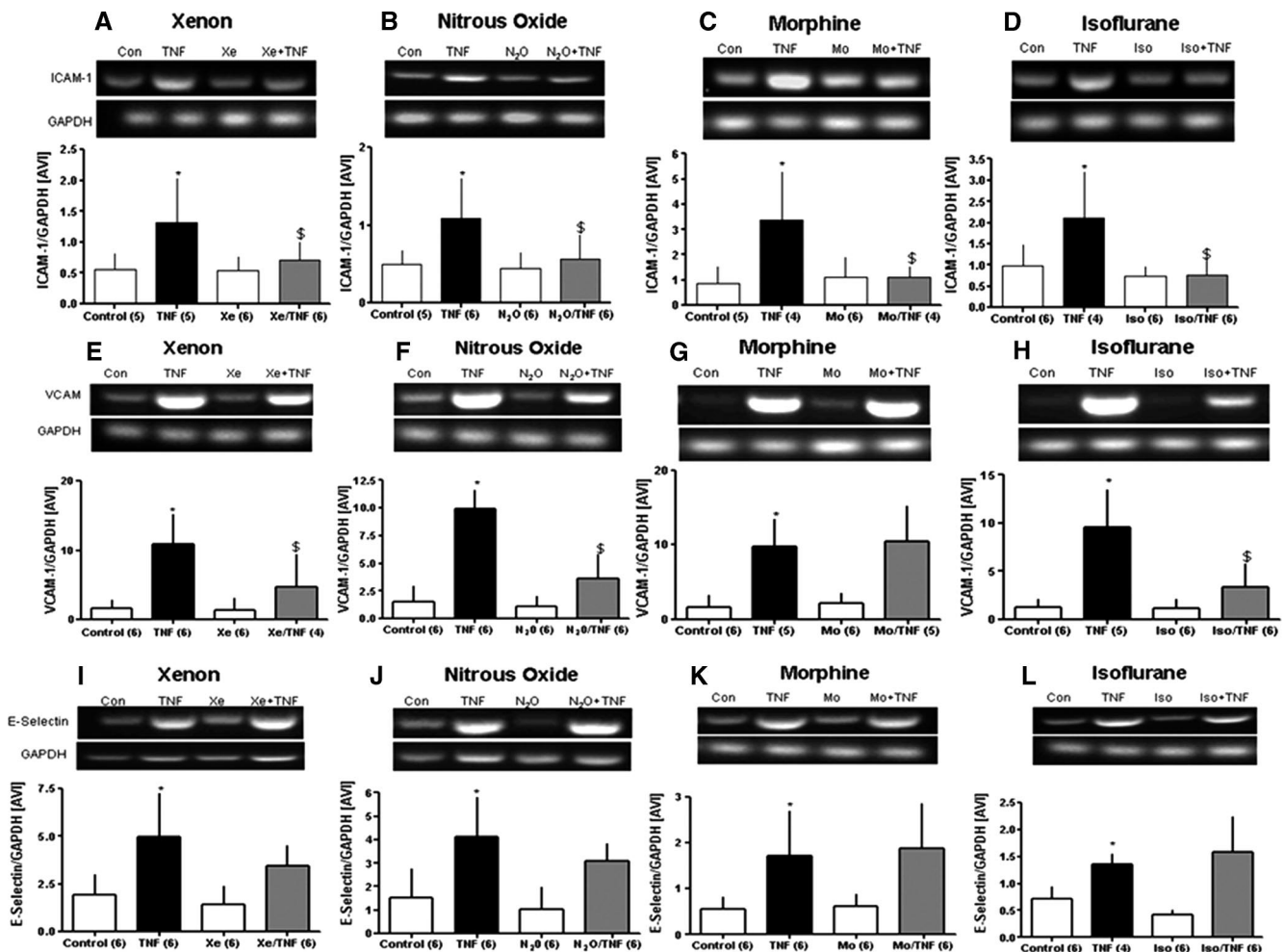


Fig. 2. Differential regulation of tumor necrosis factor α (TNF- α)-induced messenger RNA (mRNA) expression of cell adhesion molecules (CAMs) by anesthetics. mRNA expression of intracellular adhesion molecule 1 (ICAM-1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (A–D), vascular cell adhesion molecule 1 (VCAM-1) and GAPDH (E–H), and E-selectin and GAPDH (I–L) was determined by reverse-transcription polymerase chain reaction followed by gel electrophoresis and ethidium bromide staining. Human umbilical vein endothelial cells were cultured either in medium alone (Con) or in medium containing TNF- α (10 ng/mL, 1 h) with or without intermittent pretreatment of the cells with various anesthetics (xenon [Xe], nitrous oxide [N_2O], morphine [Mo], isoflurane [Iso]). Figures show representative gels out of three independent experiments performed from different cell preparations. Graphs show densitometric evaluation of signal intensities normalized to GAPDH (average light intensity [AVI]). Data are mean \pm SD. Values in parentheses show number of measurements. * $P < 0.05$, statistically significant versus TNF- α group. \$ $P < 0.05$, statistically significant versus control group.

mm dithiothreitol, and freshly added 25 mM dithiothreitol in 2.5% Tween-20. After addition of 1 μ L 10 \times orange loading dye, nucleoprotein-oligonucleotide complexes were resolved by electrophoresis (6% non-denaturing polyacrylamide gel, 80 V), and bands were visualized and quantified using the Odyssey IR Imager[®] (Licor Biosciences).

Statistical Analysis

Data are expressed as mean \pm SD. Group comparisons were analyzed by one-way analysis of variance (Graph Pad Prism version 4.00; GraphPad Software, Inc., San Diego, CA) followed by Bonferroni *post hoc* test for pairwise comparisons. Values with $P < 0.05$ were considered statistically significant versus the control group or the TNF- α group, respectively.

Results

Differential Regulation of TNF- α -induced mRNA Expression of CAMs

As demonstrated in figure 2, all four agents significantly decreased the TNF- α -induced messenger RNA (mRNA) expression of ICAM-1 (xenon [A]: 0.7 ± 0.3 , nitrous oxide [B]: 0.6 ± 0.3 , morphine [C]: 1.1 ± 0.4 , isoflurane [D]: 0.8 ± 0.3 vs. TNF- α : 1.3 ± 0.7 [A], 1.1 ± 0.5 [B], 3.4 ± 1.8 [C], 2.1 ± 1.1 [D] arbitrary units of average light intensity [AVI], respectively; $P < 0.05$). It is remarkable that the effect of intermittent pretreatment with the four substances nearly led to control levels of ICAM-1 (figs. 2A–D).

The expression of VCAM-1 was decreased by all inhalational anesthetics (xenon [E]: 4.7 ± 4.6 , nitrous oxide

[F]: 3.5 ± 2.2 , isoflurane [H]: 3.3 ± 2.4 vs. TNF: 10.8 ± 4.3 [E], 9.9 ± 1.7 [F], 9.6 ± 3.9 [H] AVI, respectively; $P < 0.05$) but not by the opioid morphine (morphine [G]: 10.5 ± 4.7 vs. TNF- α [G]: 9.7 ± 3.6 AVI), as demonstrated in figures 2E-H. In comparison with the effect of anesthetics on ICAM-1 expression, the effect on VCAM-1 was lower and control levels were not achieved (figs. 2E-H). E-selectin was not influenced by any of the four agents (figs. 2I-L). In comparison with morphine and isoflurane, the pretreatment with the anesthetic gases xenon and nitrous oxide showed a slight tendency to reduced TNF- α -induced E-selectin expression (figs. 2I and J).

Effects on TNF- α -induced CAM Protein Expression

To determine whether the observed decreased mRNA expression in fact leads to a reduced expression of adhesion molecule protein, fluorescence-activated cell sorting analysis of the three adhesion molecules was performed (fig. 3). Overall, the results for the expression of CAM protein were in accord with the results obtained from the polymerase chain reaction. All four agents reduced TNF- α -induced ICAM-1 expression (xenon [A]: 52.8 ± 2.7 , nitrous oxide [B]: 35.3 ± 12.0 , morphine [C]: 47.9 ± 22.5 , isoflurane [D]: 33.4 ± 4.5 vs. TNF: 67.5 ± 1.8 [A], 57.7 ± 19.2 [B], 89.6 ± 62.6 [C], 45.0 ± 18.9 [D] arbitrary units of mean fluorescence intensity, respectively; $P < 0.05$; figs. 3A-D). Also for protein expression, we found that the inhalational anesthetics suppressed TNF- α -induced VCAM-1 expression (xenon [E]: 31.9 ± 5.1 , nitrous oxide [F]: 40.3 ± 3.5 , isoflurane [H]: 76.4 ± 5.5 vs. TNF: 60.9 ± 28.0 [E], 60.3 ± 6.6 [F], 92.6 ± 9.2 [H], respectively; $P < 0.05$; figures 3E-H), whereas morphine had no effect (morphine [G]: 27.8 ± 2.5 vs. TNF- α [G]: 30.5 ± 5.9). The TNF- α -induced expression of E-selectin was not significantly influenced by the four agents (figs. 3I-L). In the case of E-selectin protein expression, morphine pretreatment lead to a slightly reduced TNF- α -induced expression in comparison with the other agents.

Prevention of TNF- α -induced Increase in NF- κ B Transcriptional Activity

We used nonradioactive infrared electrophoretic mobility shift assay to investigate the effect on NF- κ B, the transcription factor that is primary responsible for the induction of CAM. As clearly demonstrated in figure 4, all four agents abolished the TNF- α -induced increase in NF- κ B transcriptional activity (xenon [A]: 2.0 ± 1.6 , nitrous oxide [B]: 3.5 ± 2.2 , morphine [C]: 2.5 ± 1.5 , isoflurane [D]: 2.8 ± 0.7 vs. TNF- α : 6.6 ± 2.7 [A], 6.7 ± 1.4 [B], 5.7 ± 2.5 [C], 4.8 ± 0.9 [D] AVI, respectively; $P < 0.05$). The intermittent pretreatment led to a significant reduction reaching control levels after application of all substances (fig. 4).

Discussion

It is suggested that prevention of the cytokine-induced increase in adhesion molecule expression may be at least in part responsible for the reduction of cellular damage that might, for example, occur after ischemia-reperfusion of different organs. Knowing that some anesthetics might protect against ischemia-reperfusion of the heart, whereas others do not,¹⁷ we aimed to clarify whether different inhalational anesthetics and the opioid morphine might differentially influence cytokine-induced CAM expression in the *in vitro* model of HUVECs.

The main findings of the current study are that (1) intermittent pretreatment with all four agents directly effects the mRNA and protein expression of ICAM-1 in human endothelial cells; (2) that the inhalational anesthetics xenon, nitrous oxide, and isoflurane also suppress TNF- α -induced VCAM-1 expression; (3) that none of the agents had an effect on E-selectin; and (4) that all four agents prevent TNF- α -induced transcriptional activation of NF- κ B. These findings clearly suggest a differential regulation of TNF- α -induced CAM expression by anesthetics and morphine in HUVECs.

We already know from pharmacologic preconditioning of the heart that most volatile anesthetics,⁴ the chemically inert gas xenon,⁵ and morphine¹⁸ can mimic ischemic preconditioning and thereby protect the myocardium against cellular damage. In contrast, intravenous anesthetics such as ketamine can block ischemic preconditioning.¹⁹ Others, such as propofol, have no effect.²⁰ Because it is known that the two inhalational gases xenon and nitrous oxide provide their anesthetic and neuroprotective effects *via* similar pathways,²¹⁻²³ it is interesting that regarding effects on the heart, nitrous oxide showed no cardioprotective effect compared with xenon.¹⁷

The first studies addressing the effects of volatile anesthetics on the adhesion of polymorphonuclear neutrophil to the coronary endothelium came from Kowalski *et al.*⁷ They showed in the isolated guinea pig heart that continuous treatment with 1-2 MAC halothane, isoflurane, or sevoflurane suppressed the adhesion of polymorphonuclear neutrophils after myocardial ischemia.⁷ This effect is mediated at least in part *via* a decreased expression of the integrin CD11b, which is expressed on polymorphonuclear neutrophils.⁸ The same group transferred their observations to the model of HUVECs and demonstrated that halothane, isoflurane, and sevoflurane abolished the adhesion of polymorphonuclear neutrophils to HUVECs after stimulation with hydrogen peroxide.⁹ These studies were the first suggesting that *volatile* anesthetics might influence the expression of CAM on neutrophils targeting different adhesion molecules.

In the current study, we could not find an effect of the volatile anesthetic isoflurane on TNF- α -induced E-selectin expression. Our data are in contrast to the study of

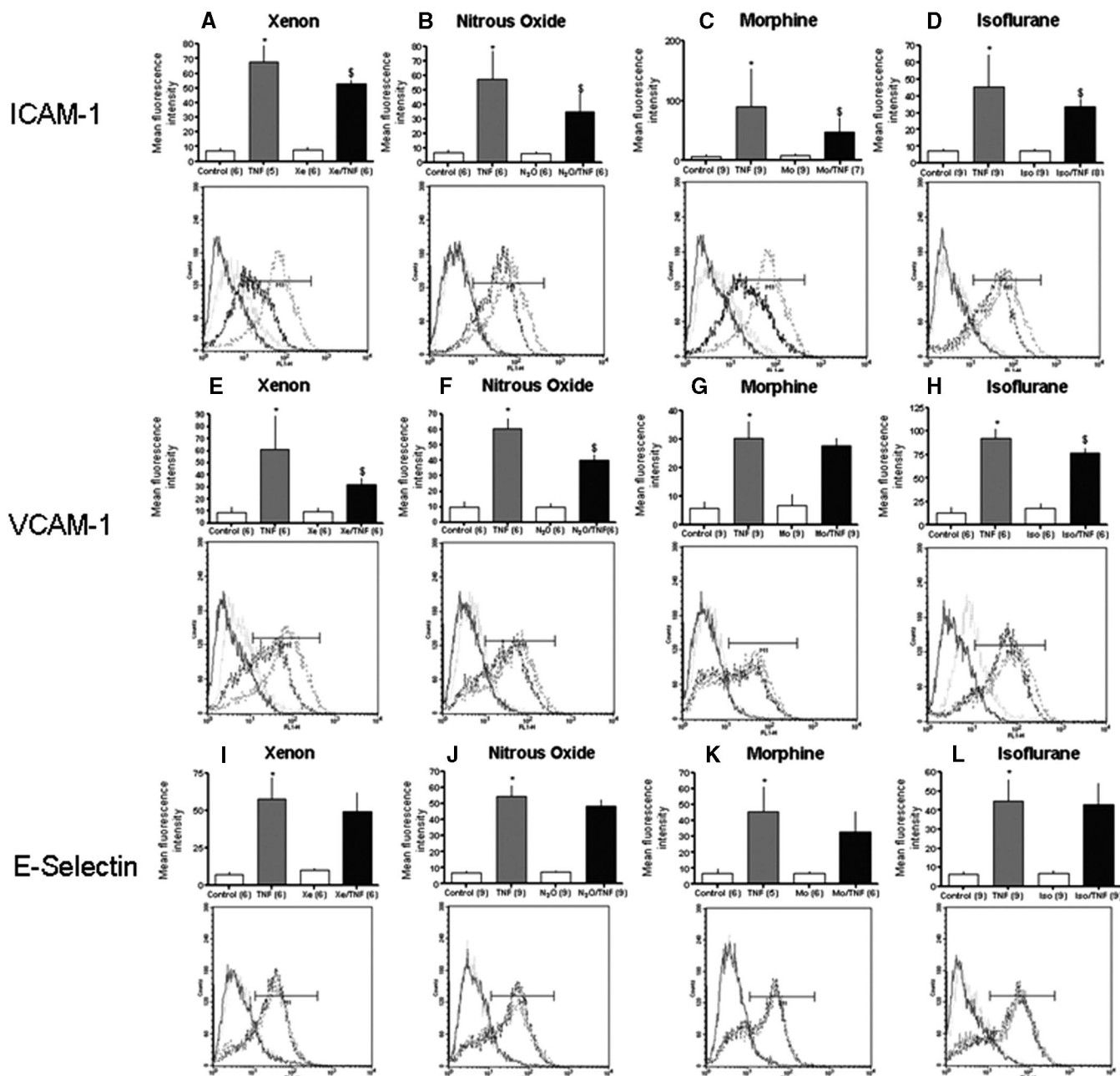


Fig. 3. Effects of the four anesthetics on tumor necrosis factor α (TNF- α)-induced cell adhesion molecule (CAM) protein expression. Expression of intracellular adhesion molecule 1 (ICAM-1; *A–D*), vascular cell adhesion molecule 1 (VCAM-1; *E–H*), and E-selectin (*I–L*) was detected by the corresponding fluorescein isothiocyanate-labeled antibodies in untreated, TNF- α -treated, and TNF- α + respective anesthetic-pretreated cells (xenon [Xe], nitrous oxide [N_2O], morphine [Mo], isoflurane [Iso]). Flow cytometric analysis was performed, and results are shown as histogram overlays (lower panels: gray line = control; light gray line = TNF- α alone; dashed light gray line = anesthetic alone; black line = TNF- α + anesthetic) showing numbers of cells versus fluorescence intensity at 520 nm. Upper panels show the quantification of two to three different experiments, each performed at least in triplicate. Data are mean \pm SD. Values in parentheses show number of measurements. * $P < 0.05$, statistically significant versus control group. \$ $P < 0.05$, statistically significant versus TNF- α group.

Biao *et al.*,¹² which showed that a 60-min pretreatment of HUVECs with 1 MAC of the volatile anesthetic desflurane suppressed the TNF- α -induced expression of all three adhesion molecules (ICAM-1, VCAM-1, and E-selectin) on a transcriptional level. These differences might be at least in part explained by the different treatment protocols; we used 3*5 min with intermittent washout

periods compared with 60 min continuous administration with desflurane in their study. Moreover, we used only half of the concentration (0.43 MAC) of the volatile anesthetic isoflurane. However, we chose this subanesthetic dose and the intermittent treatment protocol in accordance with findings from our *in vivo* studies, where these concentrations of xenon or isoflurane ap-

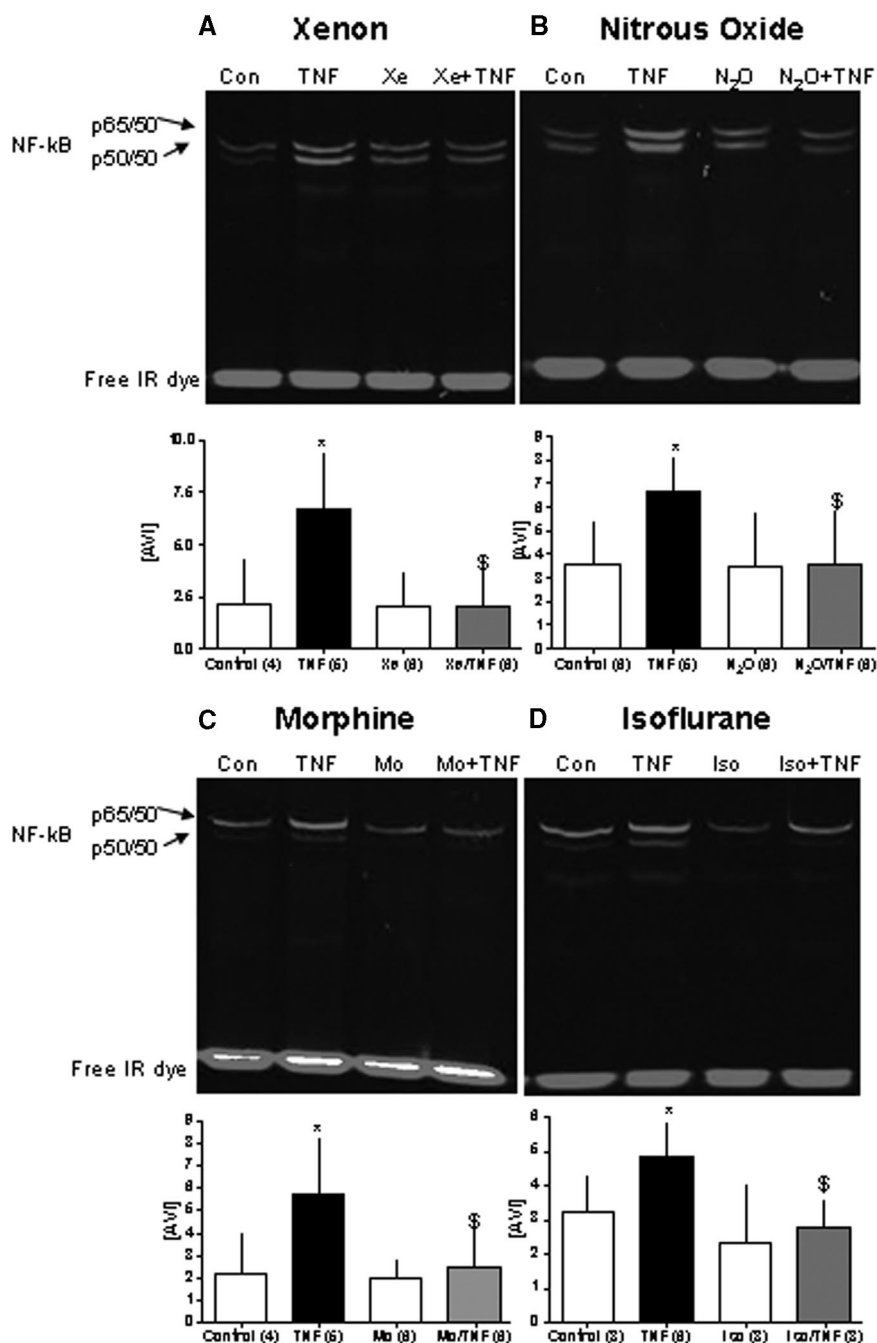


Fig. 4. Prevention of tumor necrosis factor α (TNF- α)-induced increase in nuclear factor κ B (NF- κ B) transcriptional activity after intermittent pretreatment with anesthetics. Human umbilical vein endothelial cells were cultured in either medium alone (Con) or in medium containing TNF- α (10 ng/ml) with or without intermittent pretreatment of the cells with the various anesthetics. Xenon (Xe; A), nitrous oxide (N₂O; B), morphine (Mo; C), isoflurane (Iso; D) (upper panel). NF- κ B binding activity was assessed by infrared (IR) electrophoretic mobility shift assay. NF- κ B is present in the cytoplasm as a heterodimer of p50 and p65 subunits. Data represent one representative out of three independent experiments from different cell preparations with similar results. Data are mean \pm SD (average light intensity [AVI]). Values in parentheses show number of measurements. * $P < 0.05$, statistically significant versus control group. \$ $P < 0.05$, statistically significant versus TNF- α group.

plied with a similar protocol had significant protective effect against ischemia-reperfusion injury of the heart.⁵ In accord with our study, a recent study of Lucchinetti *et al.*²⁴ showed that administration of subanesthetic concentrations of sevoflurane, before and during forearm ischemia in humans, suppressed leukocyte activation and protected the endothelium against ischemia-reperfusion injury. In addition, lower doses of isoflurane increased protein kinase C- ϵ activation and decreased infarct size to a greater extent than higher doses.²⁵ Hisano *et al.*²⁶ showed that isoflurane and sevoflurane can suppress E-selectin-mediated adhesion of HL60 cells to cytokine activated HUVECs under static conditions. How-

ever, as in our study, they could not find changes in the expression of E-selectin.²⁶

There are no further studies investigating the influence of volatile anesthetics on CAM (ICAM-1 or VCAM-1) expression on the endothelium. However, apart from suppressing TNF- α -induced adhesion molecule expression, there is also evidence from the literature that the volatile anesthetics isoflurane and halothane protect endothelial cells against cytokine- and hydrogen peroxide-induced apoptosis,²⁷ which plays also a pivotal role during endothelial cell damage in different pathophysiologic situations.

Some studies focused on selectins, which are known to mainly mediate the initial rolling of leukocytes along

the vessel, whereby ICAM-1 and VCAM-1 mediate the firm adhesion and diapedesis of leukocytes, together with the integrins expressed on the surface of the leukocyte.²⁸ A study from de Rossi *et al.*²⁹ focusing on a different group of CAM adhesion molecules expressed on neutrophils showed that isoflurane inhibits the activation of L-selectin, CD11a, and CD11b on human whole blood neutrophils. These data suggest that isoflurane mediates its protective effect in part *via* suppression of L-selectin, CD11a, and CD11b expression.

The latter group also investigated effects of the noble gas xenon on adhesion molecule expression on neutrophils *in vitro*. They found that xenon reduced the surface expression of P-selectin glycoprotein ligand 1 and L-selectin after stimulation with *N*-formyl-methionyl-leucyl-phenylalanine or phorbol-12-myristate-13-acetate.¹³ However, xenon did not affect the expression of the integrins CD11a and CD11b.¹³

Another study investigating the effects of xenon on adhesion molecule expression did not show an influence of xenon on the expression of different adhesion molecules such as L-selectin, CD18, and CD11b in an isolated cardiopulmonary bypass system.³⁰ This is in contrast to our results and the results of de Rossi *et al.*¹³ However, the cited studies did not investigate adhesion molecule expression on the endothelium, and therefore, these studies can only be compared to a limited extent with our results.

In the current study, both xenon and isoflurane significantly inhibited TNF- α -induced NF- κ B activity. In contrast, de Rossi *et al.*³¹ found that only isoflurane reduced NF- κ B activity after lipopolysaccharide stimulation in isolated monocytes, and that xenon, at a lower dose than that used by us, increased NF- κ B activity. This difference might result from different experimental settings and stimuli in both studies.

The second anesthetic gas we investigated was nitrous oxide. Because previous studies in the heart *in vivo* showed that nitrous oxide is, so far, the only inhalational anesthetic gas that has no protective effect on the myocardium,¹⁷ the results of the current study were surprising. In endothelial cells, nitrous oxide had similar effects like the noble gas xenon. It significantly abolished TNF- α -induced ICAM-1 and VCAM-1 expression as well as the increased transcriptional activation of NF- κ B. So far, this is the first study investigating an effect of nitrous oxide on HUVEC adhesion molecule expression; therefore, no comparison with the current literature is possible.

In contrast to the two inhalational gases, the intravenous analgesic morphine had no effect on TNF- α -induced VCAM-1 or E-selectin but suppressed ICAM-1 expression. In accord with our results, Wang *et al.* showed that morphine preconditioning can significantly attenuate ICAM-1 levels in human blood³² and also in rats *in vivo*.³³

Regarding the regulation of cytokine-induced NF- κ B transcriptional activity by morphine, Welters *et al.*³⁴

showed that morphine inhibits lipopolysaccharide-induced NF- κ B activation in human blood neutrophils and monocytes. Some controversy exists about the effects of morphine action on basal NF- κ B transcriptional activity. In rat hearts *in vivo*,³⁵ morphine increased NF- κ B activity, whereas in human T-lymphocytes, it had no effect on NF- κ B.³⁶

Regarding limitations of our study, it should be mentioned that we worked in an *in vitro* cell damage model of venous endothelial cells, which can only partly reflect aspects of a pathophysiologic situation. From our data, we cannot conclude whether the observed beneficial effects can also be transferred to arterial endothelial cells. Moreover, from our data obtained from an *in vitro* primary cell culture setting, it is not possible to transfer the beneficial effects of the anesthetics and morphine to the pathophysiologic state of ischemia-reperfusion injury.

Our results suggest that the suppression of TNF- α -induced adhesion molecule expression is differentially regulated by pretreatment with different anesthetics and the analgesic morphine. Moreover, the inhibition of TNF- α -induced NF- κ B activity, which was similar for all agents, does not necessarily lead to a suppressed expression of all three adhesion molecules.

In contrast to the existing literature on beneficial effects of anesthetics on cytokine-activated endothelial cells, we used for the first time a variety of anesthetics coming from completely different substance classes. Therefore, the results of our study provide novel aspects regarding effects of anesthetics on TNF- α -induced adhesion molecule expression on HUVECs.

These data may contribute to the understanding of the underlying mechanism of anesthetic- and morphine-induced prevention of endothelial cell damage.

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