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Sevoflurane Inhibits Unstimulated and Agonist-induced Platelet Antigen Expression and Platelet Function in Whole Blood In Vitro

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Background: **Previous studies have reported conflicting results about the effect of sevoflurane on platelet aggregation. To clarify this point, we investigated the effects of sevoflurane on platelet antigen expression and function *in vitro*.**

Methods: **Human whole blood was incubated for 1 h with 0.5 and 1 minimum alveolar concentration sevoflurane, 21% O_2, and 5% CO_2. A control sample was kept at the same conditions without sevoflurane. After stimulation with adenosine diphosphate or thrombin receptor agonist peptide 6, samples were stained with fluorochrome conjugated antibodies, and the expression of platelet glycoproteins GPIIb/IIIa, GPIb, and P-selectin, as well as activated GPIIb/IIIa, were measured with two-color flow cytometry. In addition, platelet function was assessed by means of thromboelastography and using the platelet function analyzer 100.**

Results: **Already in subanesthetic concentrations, sevoflurane inhibits unstimulated and agonist-induced GPIIb/IIIa surface expression and activated GPIIb/IIIa expression on platelets in whole blood. The agonist-induced redistribution of GPIb into the open canalicular system was also impaired by sevoflurane, whereas no effect on P-selectin expression in activated platelets could be found. Sevoflurane significantly reduced the maximum thromboelastographic amplitude. Furthermore, platelet function analyzer 100 closure times were significantly prolonged.**

Conclusion: **The results show that sevoflurane significantly impairs platelet antigen expression *in vitro*. It is especially the inhibition of GPIIb/IIIa expression and activation that impairs bleeding time as reflected in thromboelastographic measurements and platelet function analyzer 100 closure times. The exact inhibitory mechanism remains unclear.**

SINCE Ueda[1] demonstrated that halothane inhibits platelet aggregation in 1971, various studies have investigated the effects of volatile anesthetics on platelet aggregation. Sevoflurane in particular has recently been the subject of several investigations. In 1996, Hirakata *et al.*[2] reported that sevoflurane had strong effects on secondary platelet aggregation, probably through inhibition of thromboxane A_2 formation,[3] whereas Honemann *et al.*[4] found no influence on thromboxane A_2 signaling. The results of Hirakata *et al.*[2,3] were supported by a study of Dogan *et al.*[5], which also showed impaired platelet aggregation after sevoflurane anesthesia. More recently, Nozuchi *et al.*[6] have demonstrated that sevoflurane does not inhibit platelet aggregation induced by thrombin. However, the results remain contradictory, and the direct effects on platelet surface antigens still need to be studied.

Glycoprotein receptors within the platelet membrane are of particular interest in this regard. They are essential for platelet adhesion and platelet-mediated primary and secondary aggregation. Acquired or hereditary defects, as well as reduced expression of these glycoproteins, could result in platelet malfunction and impaired hemostasis.[7,8] Therefore, changes in platelet aggregation induced by sevoflurane could be reflected here.

Among the most important glycoproteins are the GPIIb/IIIa complex, inducing platelet aggregation *via* fibrinogen binding; the GPIb as a receptor of the von Willebrand factor; and the α-granule membrane protein P-selectin, mediating platelet endothel and leukocyte interactions.

To gain further insight into the mechanisms involved in the inhibition of platelet aggregation induced by sevoflurane, we investigated the influence of sevoflurane on the expression of different platelet surface glycoproteins in whole blood by means of flow cytometry. The expression of the platelet glycoproteins GPIIb/IIIa and GPIb and P-selectin were detected with fluorochrome-conjugated antibodies. The activated GPIIb/IIIa complex was examined using PAC-1, an antibody which recognizes the conformationally changed fibrinogen binding site. Platelet-related hemostasis was furthermore assessed with thromboelastography and the platelet function analyzer 100 (PFA).

Methods

Flow cytometry allows measurement of the specific characteristics of a large number of single cells. Before analysis, cells are labeled with fluorescence-conjugated antibodies. Thereafter, the flow cytometer detects the emitted fluorescence and light-scattering properties of each cell.

Antibodies and Reagents

The following were purchased from BD Pharmingen (San Jose, CA): anti-CD41a-phycoerythrin (PE; clone, HIP8), a monoclonal antibody–recognizing platelet GPIIb/IIIa complex independent of activation; anti-CD42b-PE (clone, HIP1), a monoclonal antibody for the

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subunit of GPIb; anti-CD62P-fluorescein-isothiocyanate (FITC; clone, AK-4), a monoclonal antibody directed against P-selectin expressed on platelet surface; and IgM-FITC (clone, G155-228) and IgG_1-FITC (clone, MOPC-21), antibodies for nonspecific binding. PAC-1-FITC (Becton-Dickinson, San Jose, CA) recognizes a neoepitope on the GPIIb/IIIa complex after undergoing the activation-induced conformational change. Dulbecco's phosphate-buffered saline without Ca^{2+} and Mg^{2+}, bovine serum albumin, adenosine diphosphate (ADP), and paraformaldehyde were obtained from Sigma Chemicals (St. Louis, MO). Thrombin receptor agonist peptide 6 (TRAP-6) was purchased from Bachem (Heidelberg, Germany).

Blood Collection and Incubation

After obtaining approval from the local ethics committee (Aachen, Germany) and informed written consent, blood samples were taken from healthy volunteers who had not received any medication for at least 2 weeks. Venous blood was collected without tourniquet from a cubital vein using a 21-gauge butterfly. The first 3 ml of blood was used to perform a hemogram and were then discarded. The next samples were drawn into polypropylene tubes containing sodium citrate (Sarstedt, Nuermbrecht, Germany). Nine parts of blood were anticoagulated with one part 3.8% trisodium citrate. All blood samples were immediately diluted to 1:1 with 37°C prewarmed Dulbecco's phosphate-buffered saline and were placed in polypropylene tissue culture dishes (Sarstedt).

One diluted blood sample was processed immediately to obtain baseline values. A second sample served as control and was incubated for 1 h in an incubator containing an atmosphere of 21% O_2 and 5% CO_2 at 37°C. The third sample was incubated with 1 minimum alveolar concentration (MAC) sevoflurane (2 vol%) or 0.5 MAC sevoflurane (1 vol%) also for 1 h. For the incubation, we used a recently developed chamber, which allows the delivery of volatile anesthetics at low gas flow rates in an atmosphere of 21% O_2 and 5% CO_2 at 37°C. Sevoflurane (Abbott, Wiesbaden, Germany) was delivered as a volatile–air mixture using an anesthetic machine (Titus; Draeger, Luebeck, Germany). Carbon dioxide was administered into the chamber from an external gas connection. After equilibration of the atmosphere inside the chamber, the fresh gas flow was kept at 0.5 l/min during the experiments. Oxygen, carbon dioxide, and sevoflurane concentrations and the temperature in the chamber were monitored continuously using a Datex AS/3 anesthesia monitor, including a multigas analyzer (Datex Ohmeda, Helsinki, Finland).

Sample Preparation and Flow Cytometric Analysis

To achieve comparable preconditions for staining with saturating antibody concentrations, the platelet count was adjusted in all samples to approximately 20×10^9/l by dilution with Dulbecco's phosphate-buffered saline containing 1% bovine serum albumin. Samples were then divided and stimulated with ADP (1 μM final concentration) or TRAP-6 (6 μM final concentration). After 5 min, 40 μl of either unstimulated or stimulated samples was added to polypropylene tubes containing saturating concentrations of fluorochrome-conjugated antibodies and antibodies for nonspecific binding. All aliquots were allowed to stain for 15 min at room temperature in the dark. The reaction was stopped with 1.5 ml cold Dulbecco's phosphate-buffered saline containing 1% bovine serum albumin and 1% paraformaldehyde. The cells were stored up to 30 min at 4°C in the dark until flow cytometric measurements were performed.

Flow cytometric two-color analyses were performed on a FACSCalibur flow cytometer and were analyzed using CellQuest 3.1 software (Becton Dickinson). Before each measurement, the flow cytometer was calibrated with fluorescence microbeads (Calibrite Beads; Becton Dickinson).

Platelets were identified by forward and side scatter and PE staining. For each sample, the data of 10,000 single platelets were collected. For further analysis, the platelets were gated in a side scatter *versus* fluorescence 2 (PE) dot plot. The mean FITC and PE fluorescence intensities of the gated platelet populations were calculated from fluorescence histograms. The percentage of platelets positive for PAC-1 was determined with a PAC-1-FITC *versus* CD42b-PE dot plot. The percentage of platelets positive for CD62P was measured in a CD62P *versus* CD41a-PE dot plot. Results are expressed as percentage of platelets positive for a marker and the mean fluorescence intensity (MFI) of the marker, reflecting the numbers of epitopes expressed on a single platelet. MFI and percentage of positive cells were calculated after subtraction of nonspecific isotype-specific antibody binding.[8]

Thromboelastography

Thromboelastography and PFA measurements were also performed on baseline, control, and sevoflurane samples. Thromboelastography was performed with the rotation thromboelastograph (ro-TEG®; Nobis Labordiagnostica, Endingen, Germany). For each thromboelastography, 300 l citrated blood was pipetted into the prewarmed (37C) cuvette of a rotation thromboelastograph, and coagulation was induced by adding 20 l $CaCl_2$. Analyzed parameters included coagulation or time from sample placement until initial fibrin formation (R time), clot formation or time taken for a fixed degree of viscoelasticity to be reached by the forming clot (K time), maximum amplitude, or maximum clot firmness (reflection of the absolute strength of the fibrin clot).

Table 1. Unstimulated and Agonist-induced Platelet Antigen Expression after Exposure to 1 MAC Sevoflurane

	Baseline	Control (60 min Incubation)	1 MAC Sevoflurane (60 min Incubation)
CD41a [MFI]	1,150 (995–1,330)	1,180 (1,009–1,380)	1,267 (1,071–1,562)
CD41a (1 μM ADP) [MFI]	2,157 (1,804–2,666)	2,068 (1,711–2,383)	1,272 (1,106–1,594)*
CD41a (6 μM TRAP-6) [MFI]	2,343 (1,899–2,753)	2,297 (1,753–2,719)	1,277 (1,075–1,600)*
PAC-1 [MFI]	9.9 (1.7–20.3)	9.4 (2.3–19.1)	6.2 (2–12.2)
PAC-1 (1 μM ADP) [MFI]	137.3 (82.4–187.7)	135.3 (86.5–193.3)	14.3 (2–33.9)*
PAC-1 (6 μM TRAP-6) [MFI]	178.1 (118.6–253.2)	179.1 (128.1–276.2)	10.3 (2.7–32.8)*
PAC-1 [% pos. platelets]	3.6 (0.5–9.5)	3 (0.7–9.8)	0.6 (0.3–1.4)*
PAC-1 (1 μM ADP) [% pos. platelets]	91.8 (81.6–98.2)	91.8 (78.5–97.2)	11.7 (0.6–44.3)*
PAC-1 (6 μM TRAP-6) [% pos. platelets]	91.1 (82.3–98.1)	92.5 (87–97.9)	6.2 (0.2–21.5)*
CD42b [MFI]	1,416 (1,075–1,746)	1,452 (1,251–1,741)	1,497 (1,148–1,950)
CD42b (1 μM ADP) [MFI]	1,083 (928–1,279)	1,105 (928–1,247)	1,422 (1,144–1,790)*
CD42b (6 μM TRAP-6) [MFI]	816.7 (654–958)	793.9 (635–991.4)	1,388.3 (1,100–1,688)*
CD62P [MFI]	6.6 (4.6–8.9)	5.7 (4.4–8)	7.4 (5.7–8.8)*
CD62P (1 μM ADP) [MFI]	22.2 (11.9–46.6)	22.7 (11.3–42.7)	22 (9.4–29.8)
CD62P (6 μM TRAP-6) [MFI]	61.6 (39.5–81.7)	68.3 (52.1–81)	63.7 (48.6–78.8)
CD62P [% pos. platelets]	2.3 (2–2.9)	2.3 (1.2–3.1)	4.4 (3.2–5.5)*
CD62P (1 μM ADP) [% pos. platelets]	7 (4.5–9.1)	8.5 (3.9–15.6)	17.7 (6.6–31.1)*
CD62P (6 μM TRAP-6) [% pos. platelets]	66.3 (43.2–84.2)	76.8 (67.1–84.3)	64.4 (43.3–86.8)

Data are expressed as mean, minimum, and maximum values.

* $P < 0.05$ *versus* control and baseline.

MFI = mean fluorescence intensity.

PFA Measurements

Platelet function analyzer measurements were performed on a PFA-100 system (Dade, Miami, FL). This system assesses platelet function in citrated whole blood by monitoring the blood flow through an aperture cut in a membrane coated with collagen and epinephrine or ADP. The time required for the occlusion of the aperture (closure time) has been reported to be indicative of the platelet function. The maximum value for closure time is 300 s, and values greater than 300 s are reported as nonclosure. Thromboelastography and PFA measurements were performed only with 0.5 MAC sevoflurane.

Statistical Analysis

To provide a better overview of the range of individual glycoprotein expression, data are given as mean, minimum, and maximum values. Because the Kolmogorow-Smirnow test revealed a normal distribution of the data, we used analysis of variance followed by the Bonferroni multiple comparison test to compare sevoflurane, baseline, and control samples. A P value of less than 0.05 was considered significant.

Results

Time-dependent Effects on Platelet Activation

To discover the effects of incubation time on platelet activation, we compared the results of the control samples with the baseline samples. After 60 min of incubation, none of the measured parameters showed a significant difference to baseline (see also tables 1–3).

Effect of Sevoflurane on GPIIb/IIIa Expression and PAC-1 Binding

Although sevoflurane had no significant effect on GPIIb/IIIa complex expression in unstimulated platelets, stimulation with ADP and TRAP-6 did not increase the number of GPIIb/IIIa epitopes expressed on the surface of those platelets incubated with sevoflurane (1 and 0.5 MAC), whereas GPIIb/IIIa epitopes almost doubled in the baseline and control samples.

The monoclonal antibody PAC-1 was used to identify the activated GPIIb/IIIa complex. After incubation with sevoflurane (0.5 and 1 MAC), PAC-1 binding of unstimulated as well as ADP- or TRAP-6–stimulated platelets was significantly lower compared with baseline and control samples. MFI and the number of cells positive for PAC-1 increased only slightly in platelets exposed to sevoflurane, whereas both values showed a 10-fold increase in baseline and control samples after activation. Incubation with 0.5 MAC sevoflurane inhibited PAC-1 binding less than did incubation with 1 MAC sevoflurane. Results are summarized in tables 1 and 2.

Effects of Sevoflurane on GPIb Expression

Exposure to 1 and 0.5 MAC sevoflurane did not change surface expression of GPIb in unstimulated platelets. Interestingly, activation with ADP and TRAP-6 of platelets incubated with sevoflurane did not result in a decrease in surface-expressed GPIb, whereas both activators induced a significant decrease in surface GPIb in baseline and control platelets (tables 1 and 2).

Effects of Sevoflurane on P-selectin Expression

Incubation with sevoflurane (0.5 and 1 MAC) resulted in a significant increase in CD62P-MFI and positive cells

Table 2. Unstimulated and Agonist-induced Platelet Antigen Expression after Exposure to 0.5 MAC Sevoflurane

	Baseline	Control (60 min Incubation)	0.5 MAC Sevoflurane (60 min Incubation)
CD41a [MFI]	1,220 (1,069–1,435)	1,159 (1,048–1,381)	1,213 (949–1,364)
CD41a (1 μM ADP) [MFI]	2,245 (1,958–2,597)	2,076 (1,724–2,429)	1,301 (973–1,493)*
CD41a (6 μM TRAP-6) [MFI]	2,493 (2,166–2,864)	2,308 (1,907–2,616)	1,216 (898–1,431)*
PAC-1 [MFI]	11 (2.6–25)	11.1 (3.1–21.4)	7.1 (2.1–13)*
PAC-1 (1 μM ADP) [MFI]	146.6 (83.9–189.4)	130 (93.7–173.3)	23 (8.5–50.7)*
PAC-1 (6 μM TRAP-6) [MFI]	168.6 (112.1–275)	158.9 (87–301.6)	14.7 (4.3–29.3)*
PAC-1 [% pos. platelets]	7.2 (1.3–17.1)	5.1 (1.1–11.4)	2.2 (0.3–6.8)*
PAC-1 (1 μM ADP) [% pos. platelets]	93.7 (82.4–97.6)	93.6 (86.7–97.5)	25.5 (3–59.9)*
PAC-1 (6 μM TRAP-6) [% pos. platelets]	93.4 (83.6–98.6)	91.1 (80.5–98.5)	16.2 (1.72–38.5)*
CD42b [MFI]	1,391 (1,260–1,616)	1,470 (1,170–1,644)	1,423 (1,040–1,663)
CD42b (1 μM ADP) [MFI]	1,038 (800–1,215)	1,135 (841–1,435)	1,363 (997–1,620)*
CD42b (6 μM TRAP-6) [MFI]	810 (574–1,193)	790 (660–1,067)	1,310 (1,016–1,550)*
CD62P [MFI]	5.8 (4.4–7.6)	6 (3.4–11.1)	7.9 (4.3–14.6)*
CD62P (1 μM ADP) [MFI]	20.3 (7.7–37.2)	20.5 (9.3–31.6)	23.8 (15.3–36.1)
CD62P (6 μM TRAP-6) [MFI]	66.56 (44.8–94.2)	67.2 (38.3–105.6)	60.1 (36.6–86.3)
CD62P [% pos. platelets]	3 (2.1–3.8)	2.9 (2–3.9)	4.4 (2.5–6.3)*
CD62P (1 μM ADP) [% pos. platelets]	7.9 (0.59–19)	8.4 (3.5–16.5)	26.3 (9.8–57.5)*
CD62P (6 μM TRAP-6) [% pos. platelets]	70.8 (49.1–85.5)	70.3 (50.1–92.5)	62.2 (37.2–90.9)

Data are expressed as mean, minimum, and maximum values.

* $P < 0.05$ *versus* control and baseline.

MFI = mean fluorescence intensity.

in unstimulated platelets. Stimulation with ADP increased the number of positive cells for P-selectin but not the MFI in platelets exposed to sevoflurane. Activation with TRAP-6 did not result in significant differences in surface expression of P-selectin in any of the three groups, although overall P-selectin expression was higher compared with unstimulated and ADP activated platelets (tables 1 and 2).

Thromboelastographic and PFA Measurements

In comparison with baseline and control samples, the samples incubated with sevoflurane (0.5 MAC) showed a significantly increased R time and a significantly reduced maximum amplitude. Clot formation time could not be measured because the clot never reached the degree of viscoelasticity required for its determination. PFA closure times of the baseline and control samples for both cartridges remained within normal values, whereas closure times of the blood exposed to sevoflurane were greater than 300 s analog to nonclosure. Because the results obtained with 0.5 MAC sevoflurane were already highly significant, we did not repeat the thromboelastographic and PFA measurements with 1 MAC sevoflurane (table 3).

Discussion

In the current study, we have been able to show that sevoflurane significantly altered platelet glycoprotein expression and platelet function, even in subanesthetic concentrations *in vitro*. Already in unstimulated blood, sevoflurane reduced basal PAC-1 binding and the percentage of PAC-1–positive cells. Stimulation of sevoflurane-incubated platelets with ADP or TRAP-6 did not result in an increase of surface-expressed GPIIb/IIIa, nor did the amount of PAC-1 binding on a single cell and the number of PAC-1–positive cells increase considerably. At 0.5 MAC sevoflurane, PAC-1 binding and the percentage of positive cells for PAC-1 were greater in comparison

Table 3. Thromboelastography and PFA Measurements

	Baseline	Control (60 min. incubation)	0.5 MAC sevoflurane (60 min incubation)
Thromboelastography			
R time (min)	10.7 (7–14.4)	11.3 (9.8–14.6)	17.7 (16.1–20.6)*
K time (min)	6.2 (5.4–6.9)	6.3 (4.6–10.6)	†
Maximum amplitude (mm)	53 (49–55)	51 (50–53)	8 (6–11)*
PFA measurements			
Collagen/ADP (s)	98.6 (97–100)	101.3 (92–116)	> 300*
Collagen/Epinephrine (s)	143.6 (132–162)	139.3 (132–150)	> 300*

Data are expressed as mean, minimum, and maximum value.

* $P < 0.05$ *versus* control and baseline. † Clot formation time could not be measured because the clot never reached the degree of viscoelasticity required for its determination.

with 1 MAC, suggesting a possible dose-dependent inhibition of receptor activation.

It is well-established that platelet adhesion is mediated *via* glycoprotein GPIb receptors through interaction with the von Willebrand factor and that further physiologic activation of platelets *via* intracellular signaling pathways leads not only to an increased expression of the GPIIb/IIIa receptor complex, but also to a conformational change and exposure of the fibrinogen binding site. Subsequent fibrinogen bridging allows firm attachment of adjacent platelets. This process is a prerequisite for platelet aggregation and thrombus formation. Therefore, an inhibition of the GPIIb/IIIa complex results in a prolonged bleeding time.[9,10] Because PAC-1 only binds to activated GPIIb/IIIa epitopes, the reduced binding on platelets exposed to sevoflurane *in vitro* could reflect a serious incapacity to generate the fibrinogen binding site and could thus induce platelet aggregation.

These results were confirmed by the prolonged bleeding time in PFA measurements.[11,12] Thromboelastography also revealed an incapacity to produce a sufficient clot firmness in sevoflurane-incubated blood. Furthermore, the thromboelastographic patterns resembled the patterns produced by abciximab-modified thromboelastography where the GPIIb/IIIa receptor is selectively blocked by a monoclonal antibody fragment (c7E3 Fab; ReoPro; Lilly, Bad Homburg, Germany), and the resulting maximum amplitude is a function of the fibrinogen concentration.[13,14]

Considering the fact that an activated GPIIb/IIIa receptor complex is a prerequisite for primary and secondary platelet aggregation, our findings do not correspond with the studies of Hirakata *et al.* and Nozuchi *et al.*[6] Hirakata *et al.*[2,3] found that sevoflurane inhibited secondary platelet aggregation induced by ADP and epinephrine, and Nozuchi *et al.*[6] reported that sevoflurane did not inhibit aggregation induced by thrombin. In our study, after incubation with sevoflurane, neither a weak agonist, such as ADP, nor a strong agonist, such as TRAP-6, was able to recruit and activate a sufficient number of GPIIb/IIIa epitopes on the platelet surface required for adequate fibrinogen binding. Therefore, not only secondary but also primary aggregation is impaired by sevoflurane. The different study results may be partly explained by the different methods used. In contrast to the cited studies, we analyzed platelets in whole blood instead of platelet suspensions. We also used sevoflurane as a gas, not as a liquid, and allowed platelets to incubate for 1 h.

Interestingly, not only was fibrinogen binding of platelets impaired by sevoflurane, but the often-described activation-induced redistribution of GPIb from the surface to the internal membranes of the open canalicular system also failed to occur.[15–18] This leads to the conclusion that perhaps more than one inhibitory mechanism is involved. Our results stand in contrast to the results of Froehlich *et al.*,[19] who observed a redistribution. Maybe the different incubation times and different concentrations of ADP and TRAP-6 led to the divergent findings.

Nevertheless, α-granule secretion on activation seems to be unaffected by sevoflurane as determined by binding of a monoclonal antibody to P-selectin. P-selectin is located in the membranes of α granules and becomes externalized on the platelet surface after platelet activation and granule secretion.[15,20] In unstimulated platelets, P-selectin surface expression was even higher in platelets exposed to sevoflurane.

The mechanism by which sevoflurane suppresses platelet response to various stimuli remains unclear. The hypothesis of Hirakata *et al.*[2] that sevoflurane inhibits thromboxane A_2 formation by suppressing cyclooxygenase activity cannot explain the effects of sevoflurane on primary aggregation by inhibiting GPIIb/IIIa expression and activation. The findings of Hirakata *et al.*[2] can possibly be explained by the fact that GPIIb/IIIa mediates the formation of thromboxane A_2, and GPIIb/IIIa receptor blockade impairs the formation of this secondary feedback agonist.[21]

A direct inhibitory effect of sevoflurane on the GPIIb/IIIa receptor, as well as an inhibition of intracellular signaling pathways, might be possible, whereas a direct effect on ADP and thrombin surface receptors seems to be improbable because both agonists interact with specific receptor types, including the purinase-activated receptors PAR1 and PAR4 for thrombin and the purinergic receptors P2Y1 and $P2T_{AC}$ for ADP. Although the signaling pathways that deliver messages from these receptors to the GPIIb/IIIa complex have not been completely characterized, it seems likely that a link exists from the G-protein–coupled agonist receptors to the GPIIb/IIIa receptor complex, including phospholipase Cβ, inositol phosphates, and protein kinase C. Therefore, not only a direct inhibitory effect of sevoflurane on the GPIIb/IIIa receptor but also a possible interference with parts of the signaling pathway is imaginable.[22,23] Kohro and Yamakage[24] investigated the effect of halothane on platelet function and proposed a decrease in intracellular free Ca^{2+} and production of inositol 1,4,5 -triphosphate as the possible inhibitory mechanism. A similar mechanism is imaginable for the effects of sevoflurane on platelets. However, further studies are necessary to confirm these speculations.

In summary, sevoflurane inhibits agonist-induced GPIIb/IIIa activation and surface expression on platelets in whole blood already in subanesthetic concentrations *in vitro*. The agonist-induced redistribution of GPIb into the open canalicular system was also impaired by sevoflurane, whereas no effect on P-selectin expression in activated platelets as an indicator of activation dependent α degranulation could be found. Although Hirakata *et al.*[3] observed an impaired platelet aggregation in pa-

tients anesthetized with sevoflurane, and Nathan *et al.*[25] noticed a higher blood loss in patients undergoing gynecologic ambulatory anesthesia with sevoflurane, it must be further evaluated whether our *in vitro* findings have clinical implications.

References

1. Ueda I: The effects of volatile general anesthetics on adenosine diphosphate-induced platelet aggregation. ANESTHESIOLOGY 1971; 34:405–8
2. Hirakata H, Ushikubi F, Toda H, Nakamura K, Sai S, Urabe N, Hatano Y, Narumiya S, Mori K: Sevoflurane inhibits human platelet aggregation and thromboxane A2 formation, possibly by suppression of cyclooxygenase activity. ANESTHESIOLOGY 1996; 85:1447–53
3. Hirakata H, Nakamura K, Sai S, Okuda H, Hatano Y, Urabe N, Mori K: Platelet aggregation is impaired during anaesthesia with sevoflurane but not with isoflurane. Can J Anaesth 1997; 44:1157–61
4. Honemann CW, Nietgen GW, Podranski T, Chan CK, Durieux ME: Influence of volatile anesthetics on thromboxane A2 signaling. ANESTHESIOLOGY 1998; 88: 440–51
5. Dogan IV, Ovali E, Eti Z, Yayci A, Gogus FY: The in vitro effects of isoflurane, sevoflurane, and propofol on platelet aggregation. Anesth Analg 1999; 88:432–6
6. Nozuchi S, Mizobe T, Aoki H, Hiramatsu N, Kageyama K, Amaya F, Uemura K, Fujimiya T: Sevoflurane does not inhibit human platelet aggregation induced by thrombin. ANESTHESIOLOGY 2000; 92:164–70
7. Weiss HJ, Turitto VT, Baumgartner HR: Platelet adhesion and thrombus formation on subendothelium in platelets deficient in glycoproteins IIb-IIIa, Ib, and storage granules. Blood 1986; 67:322–30
8. Schmitz G, Rothe G, Ruf A, Barlage S, Tschope D, Clemetson KJ, Goodall AH, Michelson AD, Nurden AT, Shankey TV: European Working Group on Clinical Cell Analysis: Consensus protocol for the flow cytometric characterisation of platelet function. Thromb Haemost 1998; 79:885–96
9. Clemetson KJ, Clemetson JM: Platelet GPIb-V-IX complex: Structure, function, physiology, and pathology. Semin Thromb Hemost 1995; 21:130–6
10. Jennings LK, White MM: Expression of ligand-induced binding sites on glycoprotein IIb/IIIa complexes and the effect of various inhibitors. Am Heart J 1998; 135:S179–83
11. Kundu SK, Heilmann EJ, Sio R, Garcia C, Davidson RM, Ostgaard RA: Description of an in vitro platelet function analyzer: PFA-100. Semin Thromb Hemost 1995; 21(suppl 2):106–12
12. Cox D: Methods for monitoring platelet function. Am Heart J 1998; 135:S160–9
13. Kettner SC, Panzer OP, Kozek SA, Seibt FA, Stoiser B, Kofler J, Locker GJ, Zimpfer M: Use of abciximab-modified thrombelastography in patients undergoing cardiac surgery. Anesth Analg 1999; 89:580–4
14. Gottumukkala VN, Sharma SK, Philip J: Assessing platelet and fibrinogen contribution to clot strength using modified thromboelastography in pregnant women. Anesth Analg 1999; 89:1453–5
15. Escolar G, White JG: Changes in glycoprotein expression after platelet activation: Differences between in vitro and in vivo studies. Thromb Haemost 2000; 83:371–86
16. Hourdille P, Heilmann E, Combrie R, Winckler J, Clemetson KJ, Nurden AT: Thrombin induces a rapid redistribution of glycoprotein Ib-IX complexes within the membrane systems of activated human platelets. Blood 1990; 76: 1503–13
17. Michelson AD, Ellis PA, Barnard MR, Matic GB, Viles AF, Kestin AS: Downregulation of the platelet surface glycoprotein Ib-IX complex in whole blood stimulated by thrombin, adenosine diphosphate, or an in vivo wound. Blood 1991; 77:770–9
18. Michelson AD, Benoit SE, Kroll MH, Li JM, Rohrer MJ, Kestin AS, Barnard MR: The activation-induced decrease in the platelet surface expression of the glycoprotein Ib-IX complex is reversible. Blood 1994; 83:3562–73
19. Frohlich D, Rothe G, Schmitz G, Hansen E: Volatile anaesthetics induce changes in the expression of P-selectin and glycoprotein Ib on the surface of platelets in vitro. Eur J Anaesthesiol 1998; 15:641–8
20. Dore M: Platelet-leukocyte interactions. Am Heart J 1998; 135:S146–S51
21. Gawaz M, Ruf A, Pogatsa-Murray G, Dickfeld T, Rudiger S, Taubitz W, Fischer J, Muller I, Meier D, Patscheke H, Schomig A: Incomplete inhibition of platelet aggregation and glycoprotein IIb-IIIa receptor blockade by abciximab: Importance of internal pool of glycoprotein IIb-IIIa receptors. Thromb Haemost 2000; 83:915–22
22. Shattil SJ: Signaling through platelet integrin alpha IIb beta 3: Inside-out, outside-in, and sideways. Thromb Haemost 1999; 82:318–25
23. Daniel JL, Dangelmaier C, Jin J, Kim YB, Kunapuli SP: Role of intracellular signaling events in ADP-induced platelet aggregation. Thromb Haemost 1999; 82:1322–6
24. Kohro S, Yamakage M: Direct inhibitory mechanisms of halothane on human platelet aggregation. ANESTHESIOLOGY 1996; 85:96–106
25. Nathan N, Peyclit A, Lahrimi A, Feiss P: Comparison of sevoflurane and propofol for ambulatory anaesthesia in gynaecological surgery. Can J Anaesth 1998; 45:1148–50