ROLE OF NO AND ENDOTHELIN IN HEMOGLOBIN-INDUCED PULMONARY VASOCONSTRICTION

Axel Heller*, Max Ragaller*, Joachim Schmeck†, Heidi Flüth‡, Michael Müller*, Detlev-Michael Albrecht*, and Thea Koch*
*Department of Anesthesiology and Intensive Care Medicine, University Hospital Carl Gustav Carus D-01307 Dresden; and †Department of Anesthesiology and Operative Intensive Care Medicine, University Hospital, D-68167 Mannheim, Germany

ABSTRACT—The underlying mechanisms of hemoglobin (Hb)-induced vasoconstriction are not yet well understood. The aim of this study was to elucidate the influence of nitric oxide (NO) and endothelin (ET) on Hb-induced pulmonary vasoconstriction. Therefore, an autologous Hb preparation was administered into isolated rabbit lungs, in which pulmonary artery pressure (PAP) and weight gain was monitored. Either glyceroltrinitrate (GTN; 10⁻⁵ M; n = 6), L-arginine (10⁻² M; n = 6), L-NAME (10⁻⁴M; n = 6), ET₁ or ET₅ receptor antagonists (BQ₁₂₃, 10⁻⁶M, n = 6) or (BQ₇₈₈, 10⁻⁶ M, n = 6) were added to the perfusion fluid and NO and thromboxane A₂ levels were measured. Results: In the control group the Hb-stimulation resulted in a pressure response up to 25.1 ± 2.1 mmHg (p < .05), which was 136 ± 6% of the reference value. The PAP increase was significantly (p < .05) blunted after GTN (71 ± 5%), L-arginine (93 ± 6%) and BQ₇₈₈ (88 ± 7%). Pretreatment with L-NAME (139 ± 13%) or BQ₁₂₃ (115 ± 9%) did not show significant changes in PAP. Conclusion: The reduction of the Hb-induced pulmonary hypertension by NO-donors points toward the inactivation of NO by free hemoglobin. Likewise, ET₅-receptor mediated vasoconstrictive effects without changes in NO concentrations seem to play a pathogenetic role in the Hb-induced pulmonary vasoconstriction.

INTRODUCTION

Vascular tone in the pulmonary circulation is regulated by a variety of mediators. In the healthy organism, the pulmonary vascular resistance is controlled by the balance of vasoconstrictor and -dilator substances. Growing interest has been focused on the impact of the potent vasoconstrictor endothelin (ET) and the vasodilator nitric oxide (NO). These mediators are potentially involved in pulmonary vasoconstriction due to free hemoglobin (1, 2). After trauma, burn injury, and subarachnoid hemorrhage, free hemoglobin induces severe vasoconstriction associated with hyperperfusion in the microcirculation (3). Although a great number of in vivo and in vitro, studies have investigated the vasoactive properties of hemoglobin, the pathophysiological mechanisms associated with serious cardiovascular, humorl, and immunologic (4) side effects following hemoglobin administration have not been completely elucidated. The vasoconstrictor effects have been shown in the systemic (5) as well as in the coronary (6) and cerebral circulation (7). Particularly the development of hemoglobin solutions as oxygen carrying blood substitutes demanded for explanations of these observed side effects. Imbalances between constrictor and dilator mediators regulating vascular tone, namely an interference of hemoglobin with vasodilative NO, seems to be of significance. Since NO is supposed to play an important role as physiological vasodilator in conductance and resistance vessels, considerations on how NO may be inactivated in the circulation are given in recent studies (8). NO is synthesized from l-arginine by the action of nitric oxide synthase (NOS), a NADPH-dependent enzyme. NO induces via the activation of soluble guanylate cyclase release of cyclic guanosine monophosphate cGMP, thus resulting in relaxation of smooth muscle cells and vasodilation (8). The hemoglobin-induced vasoconstriction is discussed to be due to scavenging of NO on the one hand and due to interactions between NO and hemoglobin at the guanylate cyclase activation site on the other hand (9).

Another interesting aspect concerning the pathomechanisms of vasoconstriction seems to be the release of the extremely potent 21 amino acid peptide vasoconstrictor endothelin-1 (10). There is accumulating evidence that ET₁ may also account for the severe cerebral vasospasm associated with subarachnoid hemorrhage (11). Previous studies of our group indicated the involvement of PAF in pulmonary vasoconstriction due to hemoglobin, whereas diclofenac catalase and deferoxamine did not influence vascular tone in the lung (12). Since the underlying mechanisms of hemoglobin-induced vasoconstriction are not yet well understood, the aim of this study was to investigate the effects of a stroma-free autologous hemoglobin preparation on pulmonary vascular resistance and mediator release and to determine the pathogenetic role of NO and especially the involvement of ET₁ in Hb-induced vasoconstriction. For that purpose, experiments were performed in isolated perfused and ventilated rabbit lungs, using the NO-donors glyceroltrinitrate (GTN), l-arginine, the NO synthase inhibitor L-NAME on the one hand and the selective ET₁ (BQ₁₂₃) and ET₅ (BQ₇₈₈) receptor antagonists to analyze the mediating receptor subtypes of ET₁ on the other hand.
MATERIALS AND METHODS

This study was approved by the Animal Subject Protection Committee of the local government. The care and handling of animals were in accordance with the principles expressed in the Helsinki Declaration.

Isolated rabbit lung

The techniques of preparing and perfusing isolated rabbit lungs have been previously described in detail (12). Female chinchilla rabbits (Otocologus cuniculus) weighing 2,100 ± 196 g (mean ± SD) were anesthetized with ketamine (50 mg/kg, Ketanest®; Parke Davis, Germany) and xylazine (4 mg/kg Rompun®; Bayer, Germany) and anecocagulated with heparin-sodium 1,000 U/kg, injected in the ear vein. After placement of a tracheostomy tube, the rabbits were mechanically ventilated with room air by means of a respirator (Servo ventilator 900D, Siemens, Elema, Sweden). The thorax was opened via the diaphragm, and after a median sternotomy a catheter was inserted into the pulmonary artery. The lung organ preparation was isolated and suspended from a weight transducer (Hottinger, Baldwin Møtechne Type U1, Darmstadt) in a temperature-controlled (37°C) and humidified chamber. After the cannulaization procedure, the lungs were perfused with 200 mL Krebs-Henseleit buffer solution (55.5 g/L in the perfusion (KHBB) by a roller pump (Harvard Apparatus, Inc., 7566-10, Cole Palmer Instruments Co., Chicago, IL) at a constant volume inflow of 150 mL/min in a recirculating system. The lungs were ventilated with 4% CO2 in air (frequency 25 min−1, tidal volume 30 mL, PEEP 5–1 mbar) and, in order to avoid atelectasis formation, intermittently flushed by increasing the expiratory pressure up to 3 mbar for three inspirations. The pulmonary arterial (PAP) and airway pressures (AP) were continuously recorded via Statham strain gauge transducers. Due to a constant perfusion flow, alterations of perfusion pressure directly reflect alterations of pulmonary vascular resistance. Intermittently samples of perfusate were taken for measurements of pH, PO2, PC02, O2-saturation (blood gas analysis system 288, Ciba Corning, Germany), oncotic pressure (Onkomet BMT 921, Dr. Karl Thoma, 84081 Ulm, Germany) as well as for determination of thromboxane B2 and NO metabolites. Initially, the lungs were perfused with KHBB-solution, using low flow rates in the opened circulatory system to remove remaining blood from the vascular bed. The perfusion fluid was then exchanged for fresh buffer via two separate perfusion circuits two min after the beginning of the extracorporeal circulation and 15 min later, after the flow was increased to 150 mL/min. After another 30 min steady state period, these lungs had a constant mean PAP of 7.8–9.1 mmHg (zero-referenced at the hilum). The only lungs selected for the study were those that showed a homogenous white appearance with no signs of hemoastasis or edema formation, and which were completely isogravimetric during the steady state period. In pilot experiments, the perfusion with KHBB has been documented to maintain integrity of the microcirculation for more than 5 h in our model, which was assessed by measurements of PAP and weight gain, by biochemical analysis (LDH, AA-nitratobilites, histamine) as well as by ultrastructural studies. During the observation period, neither significant alterations in LDH, eicosanoids and histamine release nor structural abnormalities (e.g., destruction of endothelial or epithelial cells) were found.

Experimental protocol

Thirty-six lung preparations were randomly assigned to six groups. Following a 30 min equilibration period, the first perfusate sample was drawn for measurements of baseline values. Thereafter, autologous SPH (Hb = 90–130 g/L) was applied repetitively, as a bolus of 10 mL yielding in a final concentration of 23–25 mmol/L, yielding in final concentrations of Na+ 138 mmol/L, K+ 4.5 mmol/L, Mg2+ 1.33 mmol/L, Cl− 135 mmol/L, Ca2+ 2.38 mmol/L, glucose 12 mmol/L, HCO3− 12 mmol/L. The osmolality was approximately 330 mosm/kg (Micro-Osmometer, Rocheing Møtechne, Berlin). The pH of the buffer solution was adjusted to 7.4 with 1 M NaHCO3. Effects due to endotoxin contamination of the plasma-free perfusate can be excluded in our model, as assessed in previous experiments. No hemodynamic reactions, thromboxane generation, or histamine release were observed following endotoxin addition to the perfusate in the absence of plasma complement components.

Stroma-free hemoglobin solution

Rabbit hemoglobin was prepared from freshly drawn and heparinized autologous rabbit blood. After centrifugation at 1,500 g for 10 min, the red blood cells were concentrated after removal of plasma and buffy coat, resuspended, and again centrifuged and washed three times with sterile physiological saline. Subsequently, the red cells were lysed by addition of sterile distilled water and treatment with liquid nitrogen for 1 min. Stromal and other solid fragments were removed by centrifugation (2,000 g for 15 min) and filtration through sterile gauze. The supernatant was diluted with saline to achieve hemoglobin concentrations between 90–130 g/L (388 blood gas analysis system, Ciba Corning, Germany). 10 mL of the prepared lysate was injected into the arterial line as described in the experimental protocol. The obtained lysate had the following specifications: hemoglobin: 90 ± 20 g/L, methemoglobin: 5 ± 2%, CO Hb: 2 ± 1%, pH: 7.38 ± 0.12, osmolality: 290 ± 12 mosm/kg, colloidal oncotic pressure: 30 ± 4 mmHg, Na+: 143 ± 8 mmol/L, K+: 4.1 ± 0.7 mmol/L, endotoxin: <2 pg/mL (LAL test).
The endothelin-receptor antagonists BQ23, and BQ788 were obtained from Alexis (Laufingen, Switzerland). Glycerolintrinitrate (Ampo-Trinitron) was purchased from Merck (Darmstadt, Germany), l-arginine and l-NAME from Sigma Chemicals (St. Louis, MO) and diclofenac (Voltaren) from Ciba-Geigy (Wehr, Germany).

**Statistical analysis and data presentation**

Data are presented as means ± standard error of means (SEM). Differences between groups were tested by one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls multiple comparison procedure. Significance was accepted at p < .05. During the first SFH reaction, PAP was considered as 100% in each lung for comparisons of further changes in PAP due to inhibitors and SFH application. Thus, the following values express changes from the first stimulation 2 and 7 min after onset of SFH perfusion.

**RESULTS**

Baseline values of PAP 7.8–9.1, and airway pressure between 4 and 5.5 mmHg were similar in all groups and in agreement with previous studies reported by our group (12,13). Hemoglobin administration induced an acute pressure increase up to 18.9 ± 1.2 mmHg within 2 min after administration, which declined to 13.7 ± 9 mmHg at the end of SFH perfusion. During another 23 min equilibration period (time point 30 min), approximately baseline levels were achieved. The acute vasoconstrictive response was significantly (p < .05) enhanced after repetitive SFH injection in the controls (Fig. 1), resulting in pulmonary artery pressures of 25.1 ± 2.1 mmHg (2 min, 133 ± 8.1% of first SFH) and of 18.6 ± 1.2 (7 min, 136 ± 6.2% of first SFH). The sustained increase in pulmonary vascular resistance was interrupted after 7 min by washing out the SFH and exchanging the whole perfusion fluid for fresh buffer (CP). The pressure increase in the lungs pretreated with GTN was significantly reduced to 67.1 ± 8.2% (2 min, p < .05) and to 71.4 ± 5.1% (7 min, p < .01). After pretreatment with l-arginine 93.3 ± 5.5% (7 min, p < .05) and the ETB receptor antagonist BQ788 88.1 ± 6.5% (7 min, p < .05), the PAP increase was significantly attenuated compared with the corresponding control values. Fig. 1 represents the time course of PAP in control lungs. Mean values of pressure increase after the second SFH application, expressed as percentage of first PAP increase 7 min following SFH-injection in the differently treated groups, are shown in Fig. 2 (for absolute data see Table 1). The inhibition of NO-synthase by l-NAME failed to exert significant changes in the vascular resistance due to SFH. PAP increase did not differ from controls (140.9 ± 15.0% (2 min) and 138.7 ± 13.4% (7 min) of first SFH stimulation). The influence of ETB receptor antagonist on SFH-induced pressure response was examined by preadministration of BQ123, which did not prevent the increase in PAP 155.6 ± 18.1% (2 min) and 115.7 ± 8.7% (7 min). Lung edema in terms of weight increase monitored in our model did not occur in either group during the observation period. Mean maximal weight increases at the end of the experiment at time point 60 min ranged between 0.5 and 2.3 g from baseline.

The analysis of perfuse samples revealed a baseline value of circulating TXB2 of 2 ± 4 ng/mL and an immediate increase (p < .001) after the first SFH injection in all groups (mean 6.6 ± 7 ng/mL). Following the subsequent change of perfusion buffer TXA2 levels reached almost baseline and were comparable in all groups. Although preadministration of NO donors and BQ788 were able to attenuate the pressure reaction due to SFH, no significant inhibitory effect on TXA2 release could be found. Compared with the reference stimulations, the following percentages of TXA2 release were obtained, which did not reach statistical significance in between-group testing: controls 78.6 ± 19.1%, GTN 128.3 ± 12.5%, l-Arg 117.7 ± 26.7%, l-NAME 116 ± 38.7%, BQ123 112.6 ± 4%, BQ788 66.6 ± 14.5%. Onset of saturation, Pr2 and Pco2 did not significantly change throughout the observation period.

NOx levels as an indicator for NO-synthase activity were measured during each hemoglobin injection (Table 2). Baseline values of NOx in the perfusate were similar in all groups. Compared with the first SFH administration (reference value) the second SFH stimulation revealed a reduction of NOx levels in the control group (82.3 ± 15.4%), whereas pretreatment of the lungs with GTN 10⁻⁵ M significantly elevated nitrate and nitrite levels to 197.2 ± 10% (p < .05) (Fig. 3). Corresponding to these results the injection of the NO-synthase inhibitor l-NAME 10⁻⁴ M was followed by a reduced NOx release into the perfusate (23.8 ± 2.5%; p < .05). Although pretreatment with the NO-synthase substrate l-arginine and the ETB receptor antagonist BQ788 was able to suppress the pressure reaction due to hemoglobin, only a slight but no significant increase of
TABLE 1. Mean pulmonary artery pressure [mmHg]

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GTN</th>
<th>L-Arg</th>
<th>L-NAME</th>
<th>BQ123</th>
<th>BQ786</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.SFH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td>18.9 ± 1.2</td>
<td>17.4 ± 4.1</td>
<td>15.2 ± 1.1</td>
<td>15.2 ± 3.2</td>
<td>11.7 ± 1.6</td>
<td>19.5 ± 1.3</td>
</tr>
<tr>
<td>7 min</td>
<td>13.7 ± 0.9</td>
<td>15.1 ± 2.1</td>
<td>13.8 ± 2.0</td>
<td>13.9 ± 3.4</td>
<td>11.8 ± 2.0</td>
<td>14.9 ± 1.0</td>
</tr>
<tr>
<td>2.SFH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td>25.1 ± 2.1</td>
<td>11.7 ± 1.0*</td>
<td>15.2 ± 0.6</td>
<td>21.4 ± 3.2</td>
<td>18.2 ± 3.3</td>
<td>19.9 ± 2.2</td>
</tr>
<tr>
<td>7 min</td>
<td>18.6 ± 1.2</td>
<td>10.7 ± 0.5**</td>
<td>12.9 ± 0.7*</td>
<td>19.2 ± 2.6</td>
<td>13.6 ± 1.2</td>
<td>13.1 ± 0.9*</td>
</tr>
</tbody>
</table>

*p < .05; **p < .01 vs. control.

TABLE 2. Nitrate and nitrite (NOx) concentration in the perfusate [μmol/L]

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GTN</th>
<th>L-Arg</th>
<th>L-NAME</th>
<th>BQ123</th>
<th>BQ786</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.SFH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td>12.1 ± 1.2</td>
<td>13.9 ± 1.3</td>
<td>10.5 ± 1.5</td>
<td>12.4 ± 1.0</td>
<td>13.2 ± 2.4</td>
<td>11.5 ± 1.1</td>
</tr>
<tr>
<td>7 min</td>
<td>10 ± 2.2</td>
<td>27 ± 1.5*</td>
<td>11.1 ± 1.8</td>
<td>2.9 ± 1.0*</td>
<td>9.9 ± 0.9</td>
<td>11.8 ± 1.5</td>
</tr>
</tbody>
</table>

*p < .05 vs. control.

Fig. 3. Percentage of nitrate and nitrite (NOx) values in relation to the first hemoglobin application (reference value) 7 min after onset of hemoglobin challenge in all experimental groups (n = 6 each). *p < .05 vs. control.

NOx could be found compared with the control group (L-Arg 104.8 ± 4.8% and BQ786 101.8 ± 5.5%). Blockade of the ETa receptor with BQ232 revealed a NOx decrease comparable with controls (81.0 ± 16.6%).

DISCUSSION

Numerous experimental and clinical studies have provided evidence of vasocostrictive effects due to stroma-free hemoglobin. These effects have been observed in the coronary (15), cerebral (16), pulmonary (12), and systemic circulation (1). Despite intensive research efforts, the underlying pathomechanisms are not completely clarified.

Due to the extraordinary high affinity of free hemoglobin to the vasodilator NO, it is supposed to be bound and inactivated by hemoglobin (8, 17). Other investigators, however, observed hemoglobin induced vasocostriction independent of NO inactivation (18). In vitro studies demonstrated vasocostrictive effects of hemoglobin even in deendothelized vessels (19). Since the vascular endothelium is the site of NO production these findings point toward NO-independent effects. Beny et al. demonstrated endothelium-dependent and -independent contractions of coronary vessels that did not result from NO scavenging of hemoglobin. (20). The controversy described in the current literature does not allow a definite conclusion concerning the pathogenic role of NO as mediator of hemoglobin induced vasocostriction. Another interesting aspect in the discussion of potential pathomechanisms is the involvement of the extremely potent vasocostricitor endothelin (ET) (10). Studies in different species revealed an increase of ET, that might be responsible for cerebral vasospasm after subarachnoid hemorrhage (11) and increases in pulmonary artery pressure (21). While a hemoglobin-induced release of ET-I has been shown (22), controversial results have also been reported (23). Summarizing data from literature it seems likely that hemoglobin induces the release of ET-I.

In addition, the activation of the arachidonic acid cascade followed by the formation of vasocostrictive TXA2, PAF, and PAF or the generation of reactive oxygen species by autooxidation of the hemoglobin molecule (24) have been discussed to be involved in the hemoglobin induced vasocostriction.

The current study aims to elucidate this controversy concerning the SFH-induced effects in the lung vasculature. In previous studies, we investigated the impact of cyclooxygenase products, PAF, free iron and oxygen radicals on pulmonary vasocostriction and mediator release (12). The results pointed toward a crucial role of PAF but not of oxygen radicals or iron ions. Thromboxane A2, however, seemed to play a minor role in the course of SFH-induced vasocostriction. The present study investigates the effect of NO, its precursor L-arginine, and its synthesis inhibitor L-NAME as well as the effect of endothelin on hemoglobin-induced pulmonary vascular reaction. Special interest was focused on the question as to which endothelin receptor subtypes mediate vasocostrictive effects due to SFH application. Therefore, selectively acting ETa (BQ232) and ETb (BQ786) receptor antagonists were added to the perfusate prior to SFH administration to clarify the role of ETb as mediator of the observed pressure response. Experiments were performed in isolated rabbit lungs, which allow investigation of the basic pathomechanisms involved in such complex reactions and analysis of the multiple interactions between free hemoglobin, endothelium, and organ tissue. According to results of Barnard (21) and our own previously published data (12), experiments conducted in this study revealed a similar vasocostrictive potential of SFH even in small doses. Since hemoglobin preparations showed vasocostrictive properties independent from the degree of modification (25), the use of our hemoglobin preparation with the
described properties, e.g., the absence of LPS together with the reactivity to the administered drugs implicate that the vasoconstrictor effect cannot be attributed to insufficient purification but seems likely to be due to hemoglobin itself. Furthermore, the obtained results are in accordance with experiments performed with ultrapure hemoglobin preparations (26). The observations of the current study demonstrate a dependency of hemoglobin-induced pulmonary vasoconstriction on nitric oxide concentrations on the one hand but also on ET$_B$ receptor mediated reactions on the other hand. Administration of glycercrotiltrinitrate and L-arginine resulted in an increase of NO$_x$ and significantly reduced pressure increase in the pulmonary vasculature following hemoglobin administration. In pilot experiments, we observed that L-arginine (10$^{-2}$ M) did not alter vascular tone in our model when given solely. Pretreatment with Hb arginine, however, did reduce vascular tone, pointing toward substrate dependency of eNOS in states of an increased turnover, as evidenced in our study, during NO-scavenging by Hb. Inhibition of NO synthase by L-NAME, however, did suppress NO$_x$ generation but had interestingly minor influence on pulmonary artery pressure, which was supposed to increase. This finding supports the thesis that NO does not solely account for the vasoconstrictive properties of SFH (27, 28).

Moreover, a significant reduction of pulmonary artery pressure occurred due to inhibition of ET$_B$ receptors without changes in NO$_x$ levels, which were observed after ET$_B$ as well as after ET$_A$ receptor antagonism. Acute effects of ET$_1$ have been observed after various stimuli (13), and studies have been performed to identify an intracellular pool of ET$_1$. From current results, it can be postulated that ET$_1$ is not stored in intracellular vesicles (29). Some investigators, however, believe that the ET$_1$ precursor big-ET$_1$ may be stored. From this pool, ET$_1$ can quickly be generated by granulocyte-derived proteases (30).

Since endothelins stimulate numerous signal transduction mechanisms, including the intracellular release of Ca$^{2+}$ with subsequent activation of endothelial nitric oxide synthase, the regulation of both NO and ET$_1$ in the endothelium is closely related. Increasing nitric oxide decreases ET$_1$ production in bovine endothelial cells (31). Conversely, inhibition of NO synthesis augments pulmonary ET$_1$ release, independent of the status of oxygenation (32). While early investigators postulated vasoconstrictive properties of the ET$_A$ and dilatative effects of ET$_B$ receptor activation (33), recent physiologic studies indicate more diverse and complex functions. Activating either ET$_A$ or ET$_B$ receptors can contract smooth muscle (34). These findings suggested the existence of at least two different subtypes for the ET$_A$ (ET$_{A1}$ and ET$_{A2}$) and ET$_B$ (ET$_{B1}$ and ET$_{B2}$) receptors (35). The data of the current study as well as investigations describing ET$_B$ mediated vasoconstriction in the rabbit lung (33, 36) are in agreement with this hypothesis. While the reduction of the pressure response after ET$_B$ antagonism in the present study was not associated with reduced NO$_x$ levels, ET$_B$ mediated secondary activation of endothelial NO synthase (29) cannot account for the observed pressure reduction. Furthermore, no changes in TXA$_2$ release were detected in the isolated rabbit lung which previously have been reported to be ET$_B$ mediated (32). Thus, the ET$_B$ receptor contributes to HB-induced pulmonary vasoconstriction by actions that are different from NO or TXA$_2$ release and which cannot be further differentiated in the present experimental setting.

Summarizing, the results obtained from the isolated lung the reduction of vasoconstrictive effects by the application of NO donors point toward interactions between hemoglobin and endogenous produced nitric oxide. Furthermore, endothelin seems to contribute to the vasoconstrictive effects predominantly mediated via the ET$_1$ receptor. The postulated involvement of PAF in hemoglobin induced pulmonary vasoconstriction (12) remains unclear. Whether PAF directly influences smooth vessel musculature or interferes with the regulation of ET$_1$ and NO synthesis, which exert the vasoconstrictive effects, has not been differentiated. Regarding this problem, Wang and coworkers demonstrated in a recent study that NO attenuates PAF induced pulmonary hypertension (27). Based on current knowledge, application of SFH induces imbalances between tonus regulating vasoconstrictors and vasodilators, thus resulting in a vasoconstrictive overall effect. In view of therapeutic interventions, the pathophysiologic mechanisms of the observed vasoconstrictive effects due to stroma-free hemoglobin require further clarification.

ACKNOWLEDGMENTS

The authors thank K. Salomon, J. Schulte, A. Tapper, and M. Lehmer (Institute of Anesthesiology, University Hospital, Mannheim, Germany), and J. Christopher (Center of Medical Research, University Hospital, Mannheim, Germany) for excellent technical assistance. This study was supported by the Research Fund of the Faculty of Clinical Medicine Mannheim, University of Heidelberg.

REFERENCES