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Angaben zur Veröffentlichung / Publication details:

Heller, Axel R., Maximilian Ragaller, Joachim Schmeck, Heidi Flüth, Michael Patrick Müller, D. Michael Albrecht, and Thea Koch. 1998. "Role of NO and endothelin in hemoglobin-induced pulmonary vasoconstriction." *Shock* 10 (6): 401–6. https://doi.org/10.1097/00024382-199812000-00004.

ROLE OF NO AND ENDOTHELIN IN HEMOGLOBIN-INDUCED PULMONARY VASOCONSTRICTION

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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^{-4}M; n = 6), ET_A- or ET_B-receptor antagonists (BQ_{123}, 10^{-6}M, n = 6) or (BQ_{788}, 10^{-6} M, n = 6) were added to the perfusion fluid and NO_x and thromboxane A_2 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 BQ_{788} (88 ± 7%). Pretreatment with L-NAME (139 ± 13%) or BQ_{123} (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, ET_B-receptor mediated vasoconstrictive effects without changes in NO_x 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 subarachnoidal hemorrhage, free hemoglobin induces severe vasoconstriction associated with hypoperfusion in the microcirculation (3). Although a great number of *in vivo* and *in vitro*, studies have investigated the vasoactive properties of hemoglobin, the pathophysiologic mechanisms associated with serious cardiovascular, humoral, 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 ET_1 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 ET_1 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 ET_A-(BQ_{123}) and ET_B- (BQ_{788}) receptor antagonists to analyze the mediating receptor subtypes of ET_1 on the other hand.

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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 (*Orticolagus caniculus*) 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 anticoagulated 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 Meβtechnik Type U1, Darmstadt) in a temperature-controlled (37°C) and humidified chamber. After the cannulation procedure, the lungs were perfused with 200 mL Krebs-Henseleit hydroxy-ethyl-starch buffer solution (KHHB) by a roller pump (Masterflex® 7566-10, Cole Palmer Instruments Co., Chicago, IL) at a constant volume inflow of 150 mL min^{-1} in a recirculating system. The lungs were ventilated with 4% CO_2 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, Po_2, Pco_2, O_2-saturation (blood gas analysis system 288, Ciba Corning, Germany), oncotic pressure (Onkometer BMT 921, Dr. Karl Thomac GmbH, Germany) as well as for determination of thromboxane B_2 and NO_x concentrations. Initially the lungs were perfused with KHHB-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 hemostasis or edema formation, and which were completely isogravimetric during the steady state period. In pilot experiments, the perfusion with KHHB 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-metabolites, 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 SFH (Hb = 90-130 g/L) was applied repetitively, as a bolus of 10 mL yielding in a final concentration of .55 g/L in the perfusion fluid. Subsequently samples of perfusate were taken at 7 and 30 min post-SFH-injection to investigate mediator release and vascular reactions. Perfusion with SFH was maintained for 7 min. Subsequently, the perfusion fluid was completely exchanged for fresh KHHB-solution followed by a 20 min equilibration period in which PAP returned to a stable baseline level before the renewed SFH application. A second bolus of 10 mL SFH was injected and the same protocol as described for the first SFH application was performed. The first SFH administration without treatment was identical in all groups and served as reference value, whereas the second dose was given in the presence or absence of inhibitor substances. For comparison of groups, the first pressure reaction in the absence of inhibitors (first SFH application) was considered 100%. Six preparations in which SFH was given two times in absence of any inhibitor served as controls. The same protocol was carried out in experiments in which either the NO donors GTN (10^{-5} mol; n = 6) or L-arginine (10^{-2} mol; n = 6) or the NO synthase inhibitor L-NAME (10^{-4} mol; n = 6) was given into the perfusate 10 min prior to the second injection of SFH. In further experimental setups, the SFH induced vasoactive effects were examined in the presence of ET_A- (BQ_{123} 10^{-6} mol, n = 6) or ET_B-receptor antagonist (BQ_{788} 10^{-6} mol, n = 6). The doses of the intervention compounds were chosen according to previous publications (13) and preceding dose response studies in our model.

Measurement of TXB_2

Thromboxane B_2 (TXB_2) was assayed from 100 μL of recirculating KHHB as stable hydrolysis product of thromboxane A_2 by an enzyme immunoassay (TiterZyme® TXB_2 EIA, PerSeptive Diagnostics Inc., Framingham, MA). A goat anti-rabbit antibody has been precoated onto the well plate. A rabbit polyclonal antibody to TXB_2 is bound by the antibody on the coated well. Variable amounts of TXB_2 in the sample compete with a fixed amount of alkaline phosphatase-labeled TXB_2 for a limited number of sites on the rabbit anti-TXB_2 antibody. The higher the concentration of the compound in the sample, the less labeled compound is bound to the specific antibody. The unbound material is removed by washing. A colored product is formed inversely proportional to the amount of unlabeled TXB_2 bound. The absorbance at 405 nm is correlated to the concentration by a standard curve. Photometric measurement was performed with an AR 2001 photometer (Anthos Labtech Instruments, Krefeld, Germany). The cross-reactivity of TXB_2-antiserum with 2,3-Dinor-TXB_2 was 55.8%, 1.5% with 11-Dehydro TXB_2, 1% with PGB_2, and .5% with PGD_2. Other eicosanoids exert cross reactivity less than .1%.

Measurement of nitrite and nitrate (NO_x)

A commercially available colorimetric test kit was used for the determination of total NO_x concentration in a two step process (Cayman Chemicals, Ann Arbor, MI). In the first step, nitrate was converted into nitrite utilizing nitrate reductase. After that step, Griess reagent (14) was added, which converts nitrite into a deep purple azo compound. Photometric measurement (AR 2001 photometer) of the light absorption at 540 nm due to this azo chromophore determines the NO_2 concentration. Azide, ascorbic acid, dithiothreitol, and mercaptoethanol interfere with the color development in concentrations of 100 μM and phosphate >50 mM interferes with the conversion of nitrite to nitrate.

Materials

A cell- and plasma-free perfusion medium was used in the present study in order to avoid the complex interactions with different circulating cells, which may mask direct effects on vascular tone and mediator release. The perfusate consisted of a Krebs-Henseleit buffer solution with a colloid oncotic pressure between 23–25 mmHg, yielding in final concentrations of Na^+ 138 mmol/L; K^+ 4.5 mmol/L; Mg^{2+} 1.33 mmol/L; Cl^- 135 mmol/L; Ca^{2+} 2.38 mmol/L; glucose 12 mmol/L; HCO_3^- 12 mmol/L. The osmolality was approximately 330 mosm/kg (Mikro-Osmometer, Roebling Meβtechnik, Berlin). The pH of the buffer solution was adjusted to 7.4 with 1 M $NaHCO_3$. 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 (288 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: .3 ± .1%, pH: 7.38 ± .12, osmolality: 290 ± 12 mosm/kg, colloid oncotic pressure: 30 ± 4 mmHg, Na^{2+}: 143 ± 8 mmol/L, K^+: 4.1 ± .7 mmol/L, endotoxin: <2 pg/mL (LAL test).

The endothelin-receptor antagonists BQ_{123} and BQ_{788} were obtained from Alexis (Läuflingen, Switzerland). Glyceroltrinitrate (Aquo-Trinitrosan) 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 ET_B receptor antagonist BQ_{788} 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 ET_A- receptor antagonist on SFH-induced pressure response was examined by preadministration of BQ_{123}, 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 .5 and 2.3 g from baseline.

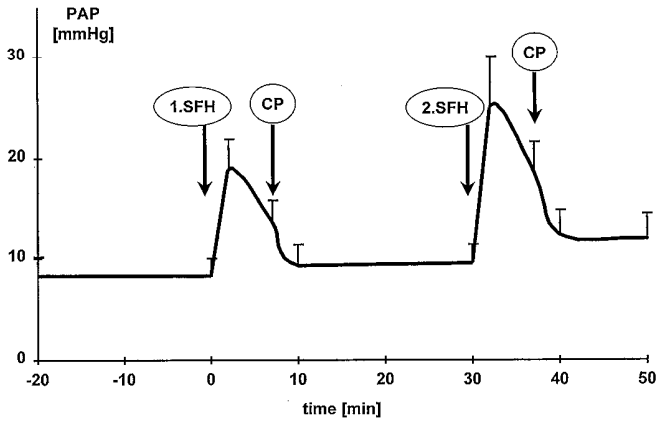

FIG. 1. **Time course of mean arterial pressure in control lungs (n = 6).** An acute pressure increase was observed after each administration of hemoglobin solution (SFH). Hemoglobin challenge was terminated by a complete change of perfusion buffer (CP).

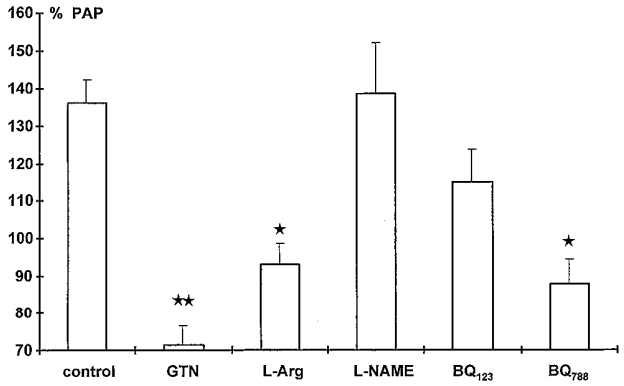

FIG. 2. **Percentage of pulmonary artery pressure in relation to the first hemoglobin application (reference value) 7 min after onset of hemoglobin challenge in all experimental groups (n = 6 each).** $^*p < .05$; $^{**}p < .01$ vs. control.

The analysis of perfusate samples revealed a baseline value of circulating TXB_2 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 TXA_2 levels reached almost baseline and were comparable in all groups. Although preadministration of NO donors and BQ_{788} were able to attenuate the pressure reaction due to SFH, no significant inhibitory effect on TXA_2 release could be found. Compared with the reference stimulations, the following percentages of TXA_2 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%, BQ_{123} 112.6 ± 4%, BQ_{788} 66.6 ± 14.5%. Oxygen saturation, Po_2 and Pco_2 did not significantly change throughout the observation period.

NO_x levels as an indicator for NO-synthase activity were measured during each hemoglobin injection (Table 2). Baseline values of NO_x in the perfusate were similar in all groups. Compared with the first SFH administration (reference value) the second SFH stimulation revealed a reduction of NO_x levels in the control group (82.3 ± 15.4%), whereas pretreatment of the lungs with GTN 10^{-5} M significantly elevated nitrite and nitrate 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 NO_x release into the perfusate (23.8 ± 2.5%; $p < .05$). Although pretreatment with the NO-synthase substrate L-arginine and the ET_B receptor antagonist BQ_{788} was able to suppress the pressure reaction due to hemoglobin, only a slight but not significant increase of

TABLE 1. **Mean pulmonary artery pressure [mmHg]**

	Control	GTN	L-Arg	L-NAME	BQ_{123}	BQ_{788}
1.SFH						
2 min	18.9 ± 1.2	17.4 ± 4	15.2 ± 1.1	15.2 ± 3.2	11.7 ± 1.8	19.5 ± 1.3
7 min	13.7 ± .9	15 ± 2.1	13.8 ± 2	13.9 ± 3.4	11.8 ± 2	14.9 ± 1
2.SFH						
2 min	25.1 ± 2.1	11.7 ± 1*	15.2 ± .6	21.4 ± 3.2	18.2 ± 3.3	19.9 ± 2.2
7 min	18.6 ± 1.2	10.7 ± .5**	12.9 ± .7*	19.2 ± 2.6	13.6 ± 1.2	13.1 ± .9*

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

TABLE 2. **Nitrite and nitrate (NO_x) concentration in the perfusate [μmol/L]**

	Control	GTN	L-Arg	L-NAME	BQ_{123}	BQ_{788}
1.SFH	12.1 ± 1.2	13.9 ± 1.3	10.5 ± 1.5	12.4 ± 1	13.2 ± 2.4	11.5 ± 1.1
2.SFH	10 ± 2.2	27 ± 1.5*	11.1 ± 1.8	2.9 ± .1*	9.9 ± .9	11.8 ± 1.5

*$p < .05$ vs. control.

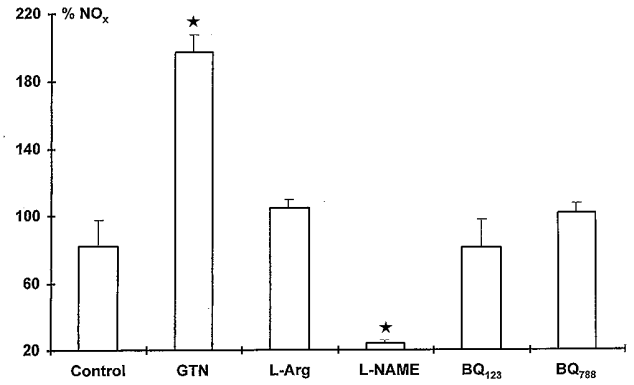

FIG. 3. **Percentage of nitrite and nitrate (NO_x) values in relation to the first hemoglobin application (reference value) 7 min after onset of hemoglobin challenge in all experimental groups (n = 6 each).** *$p < .05$ vs. control.

NO_x could be found compared with the control group (L-Arg 104.8 ± 4.8% and BQ_{788} 101.8 ± 5.5%). Blockade of the ET_A receptor with BQ_{123} revealed a NO_x decrease comparable with controls (81.0 ± 16.6%).

DISCUSSION

Numerous experimental and clinical studies have provided evidence of vasoconstrictive 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 vasoconstriction independent of NO inactivation (18). *In vitro* studies demonstrated vasoconstrictive 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 vasoconstriction. Another interesting aspect in the discussion of potential pathomechanisms is the involvement of the extremely potent vasoconstrictor endothelin (ET_1) (10). Studies in different species revealed an increase of ET_1 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-1 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_1.

In addition, the activation of the arachidonic acid cascade followed by the formation of vasoconstrictive TXA_2, PGF_2, 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 vasoconstriction.

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 ions and oxygen radicals on pulmonary vasoconstriction and mediator release (12). The results pointed toward a crucial role of PAF but not of oxygen radicals or iron ions. Thromboxane A_2, however, seemed to play a minor role in the course of SFH-induced vasoconstriction. 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 vasoconstrictive effects due to SFH application. Therefore, selectively acting ET_A (BQ_{123}) and ET_B (BQ_{788}) receptor antagonists were added to the perfusate prior to SFH administration to clarify the role of ET_1 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 vasoconstrictive potential of SFH even in small doses. Since hemoglobin preparations showed vasoconstrictive 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 reagibility 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 glyceroltrinitrate 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-scavenge 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 dilative 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_B 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. Christophel (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.

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