Efficiency of Aerosolized Nitric Oxide Donor Drugs to Achieve Sustained Pulmonary Vasodilation

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ABSTRACT
Inhalation of nitric oxide (NO) causes selective pulmonary vasodilation, but demands continuous supply of the gaseous agent. We investigated the suitability of aerosolization of NO-donor drugs for achieving sustained reduction of pulmonary vascular tone. In buffer-perfused rabbit lungs, stable pulmonary hypertension was achieved by continuous infusion of the thromboxane-analogue U46619. The NO-donor drugs molsidomine, 3-morpholinosydnone-imine (SIN-1), sodium nitroprusside (SNP) and glyceryl-trinitrate reduced the pulmonary hypertension in a dose-dependent fashion, whether admixed to the perfusate or inhaled as alveolar-accessible aerosol particles (aerosolization time 3–6 min), with an efficiency ranking of SNP > SIN-1 > molsidomine and glyceryl-trinitrate. Notably, nearly identical dose-response curves were obtained when corresponding molar quantities of the most potent agents, SNP and SIN-1, were applied either via transbronchial or via intra-vascular routes, with respect to rapidity of onset, extent (pressure reduction to near baseline) and duration (>90 min) of vasorelaxation. Appearance of sydnonimines in the perfusate after aerosolization and reduction of SIN-1 efficacy when nebulized in non-recirculatingly perfused lungs demonstrated substantial entry of this prodrug into the vascular space after alveolar deposition. In contrast, undiminished vasodilatory efficiency of aerosolized SNP under conditions of non-recirculating perfusion suggested predominant efficacy via local NO release for this agent. We conclude that short aerosolization maneuvers of NO-donor drugs are suitable to achieve dose-dependent, extensive and sustained vasodilation in the pulmonary circulation, thus offering a new therapeutic approach in pulmonary hypertension.

Pulmonary arterial hypertension is an important feature of acute and chronic lung disease and may occur secondary to cardiac disorders. Vasodilator therapy is hampered by lack of specificity, as it may cause both pulmonary and systemic vasodilation with potentially dangerous arterial hypotension. This feature was observed for a variety of vasorelaxant drugs, e.g., prostacyclin (Barst et al., 1994, Pepke-Zaba et al., 1991, Rossaint et al., 1993), urapidil (Adnot et al., 1987), calcium channel blockers (Rich, 1995) and adenosine (Morgan et al., 1991), as well as the NO-donor drugs GTN or SNP (Prielipp et al., 1988, Vanderford et al., 1991). Furthermore, i.v. or p.o. application of all these agents may worsen arterial hypoxemia due to their lack of intrapulmonary selectivity: their vasodilatory effect is not restricted to well-ventilated lung areas, but also includes poorly aerated or non-ventilated lung regions, thereby increasing perfusion of these regions and thus ventilation-perfusion mismatch and shunt-flow.

In view of these drawbacks of vasodilator therapy in pulmonary hypertension, substantial progress was made by the finding that inhalation of the gaseous vasorelaxant agent NO is capable to effect pulmonary vasodilation in the absence of any substantial systemic vascular effect (Frostell et al., 1991, Pepke-Zaba et al., 1991). Due to its extreme short half-life and instantaneous inactivation by hemoglobin binding upon entry into the intravascular space, the vasodilatory property of the inhaled NO is restricted to vessels in the near vicinity of the alveolar compartment (Rimar and Gillis, 1993). In addition, this new approach is endowed with the capability to improve arterial oxygenation, as the access of the vasodilatory agent is largely restricted to well-ventilated lung regions, thereby redistributing blood flow to these areas and improving ventilation-perfusion matching. In clinical studies, such a profile of selective pulmonary vasodilation was confirmed in acute lung failure with accompanying moderate pulmonary hypertension (Pierobe et al., 1995, Rossaint et al., 1993). In addition, NO inhalation was shown to decrease the pulmonary vascular resistance in patients with chronic and partly excessive pulmonary hypertension due to different

ABBREVIATIONS: GTN, glyceryl trinitrate; MOL, molsidomine; N-ethoxy carbonyl-3-4-morpholinosydnone imine; NO, nitric oxide; NO_x, sum of nitrite + nitrate + peroxynitrite + NO; PAP, pulmonary arterial pressure; ppb, parts per billion; SIN-1, 3-morpholinosydnone imine; SIN-1C, 3-morpholinoiminoacetonitrile; SNP, sodium nitroprusside.
underlying diseases, such as persistent pulmonary hypertension of the newborn, primary pulmonary hypertension of the adult, end-stage pulmonary fibrosis, chronic obstructive pulmonary disease and mitral valve disease (Adnot et al., 1993, Channick et al., 1994, Girard et al., 1992, Kinsella et al., 1992, Moinard et al., 1993, Pepke-Zaba et al., 1991, Roberts et al., 1992).

These findings suggest that, in addition to the effects of vascular remodeling, persistent vasoconstriction substantially contributes to the augmentation of pulmonary vascular resistance in chronic disease, giving credit to further attempts to establish continuous, selective pulmonary vasodilator therapy. Long-term infusion of drugs with short halflives such as prostacyclin may reduce pulmonary hypertension but is laborious, prone to i.v.-line complications and lacks selectivity for the pulmonary circulation (Barst et al., 1994). Use of gaseous NO demands continuous inhalation of this agent, as the pulmonary hypertension resumes within a few minutes of ceasing NO inhalation both under experimental and clinical conditions (Frostell et al., 1991, Rossaint et al., 1993). Against this background, it was of interest to find that selective pulmonary vasodilation may also be achieved by using aerosol technology for alveolar deposition of a vasodilatory drug such as prostacyclin, an agent with nonselective vasorelaxant properties when applied i.v. (Walmrath et al., 1993, 1995, 1996). We now followed this line of reasoning and investigated, in an established experimental model of pulmonary hypertension in isolated perfused lungs (Rimar and Gillis, 1993), whether aerosolization might also be used for the alveolar deposition of different NO-donor drugs, aiming to achieve prolonged pulmonary vasodilation by ongoing local NO release from these prodrugs. In particular, the following questions were posed: 1) Is this route of application as efficient as the intravascular administration of the NO-donors with respect to rapidity and extent of pulmonary vasodilation, and can sustained vasorelaxation of the lung vessels be achieved by a short maneuver of drug nebulization? 2) Can the bioactivity of the different NO-donors be monitored by continuous measurement of NO exhalation and/or the release of NO/NO-decomposition products into the vascular space? 3) Are the NO-donors retained by the alveolar epithelial barrier, or does spillover of the prodrugs into the vascular space contribute to the vasodilatory effects?

Materials and Methods

Reagents. SNP [sodium nitrosoylpentacyanoferrate(III), Niprus] was obtained from Schwarz Pharma (Monheim, Germany). MOL and SIN-1 were kindly provided by Cassella (Frankfurt, Germany). U46619 (stable thromboxane analogue) was obtained from Paesel-Lorei (Frankfurt, Germany). GTN in aqueous solution (Aqu-O-Trin-troasan) and all other chemicals were purchased from Merck (Darmstadt, Germany).

Lung model. The model of perfused rabbit lungs has previously been described in detail (overview in Seeger et al., 1994). Briefly, rabbits of either sex weighing 2.6 to 2.9 kg were anaesthetized with heparin and deeply anesthetized with a mixture of ketamine and xylazine (50 and 16 mg/kg, respectively). Tracheostomy was performed, and the animals were ventilated with room air, using a Harvard respirator (tidal volume, 30 ml; frequency, 30/min; positive endexpiratory pressure, 1 cmH2O). After mid-ster nal thoracotomy, catheters were placed into the pulmonary artery and the left atrium, and perfusion with Krebs-Henseleit buffer was started. The buffer contained 120 mM NaCl, 4.3 mM KCl, 1.1 mM KHPO4, 24 mM NaHCO3, 2.4 mM CaCl2 and 1.3 mM MgPO4, as well as 2.4 g/liter glucose. In parallel with the onset of artificial perfusion, gas supply was changed to a mixture of 16% O2, 5% CO2 and 79% N2. After extensive rinsing of the lung vasculature, the lungs were recirculatingly perfused with a pulsatile flow of 100 ml/min (total volume 150 ml); left atrial pressure was set at 2 mmHg (referred to the hilum). Lungs were suspended from a force transducer, and the whole system was equilibrated at 37°C. Lungs included in the study 1) had a homogeneous white appearance with no signs of hemostasis, edema or atelectasis; 2) had PAP and ventilation pressures in the normal range and 3) were isogravimetric during an initial steady state period of at least 30 min.

Detection of NO. NO release into both the alveolar and intra vascular compartment was on-line monitored as described in a previous study (Spriestersbach et al., 1995). Briefly, an aliquot of the mixed expired gas (160 ml/min) was continuously sampled at the ventilator exhaust valve and transferred to a chemiluminescence NO-analyzer (UPK 3100; UPK, Butzbach, Germany). The detection limit of NO in gas was 1 ppb (parts per billion, v/v). Daily calibration was performed with certified gases (NO in oxygen-free nitrogen; Messer Griesheim, Herborn, Germany). For monitoring of buffer fluid NO and NO metabolites (NO, nitrate, nitrite and peroxy nitrite, all summarized as NO2), a small aliquot of the lung effluent (600 μl/min) was continuously transferred into a reaction vessel containing 80 ml of 0.1 M vanadium (III) chloride in 2 M HCl at 98°C. This reagent quantitatively reduces the NO decomposition products back to NO. Arising NO was carried by oxygen-free nitrogen continuously flushed through the device (160 ml/min), which after passage of a liquid trap and an acidic vapor trap entered a second chemiluminescence detector. Calibration was performed with buffer fluids containing known concentrations of nitrite and nitrate.

Aerosol delivery. Aqueous solutions of either NO-donor drug were placed in a jet nebulizer (Pari, Starnberg, Germany), driven at a pressure of 1.5 bar with the same gas mixture as used for lung ventilation. The mass median aerodynamic diameter of the particles generated by this nebulizer was 2.5 μm with a geometric S.D. of 2.1. Aerosols were delivered to the inspiration loop of the ventilator by use of a bag-in-box system; the nebulized amount of fluid was 18 μl/min. Lung deposition of these aerosols was determined in separate experiments by a recently described laserphotometric technique (Schmekl et al., 1996). It was found that, independent of the drug admixed to the solution used for nebulization, the present mode of aerosolization and lung ventilation resulted in a deposition fraction of 36 ± 4%. In all experiments with drug aerosol application, this deposition rate was taken into consideration when delivering the present doses of the different agents to the bronchoalveolar space.

Detection of molsidomine and metabolites. Perfusate measurements of molsidomine, SIN-1 as the main metabolite of the prodrug molsidomine, and the decomposition product SIN-1C were kindly performed by W. Gärnert (Cassella Frankfurt, Germany) according to established high-performance liquid chromatography techniques (Bevan and Modha, 1981, Dell and Chamberlain, 1978).

Experimental protocol. After an initial steady state period of 30 min, continuous infusion of the stable thromboxane A2-mimetic U46619 was started. The dosage was varied to achieve a sustained increase in PAP to 2- to 3-fold baseline levels; the mean dose was 16 ± 10 ng × kg-1 × min-1 as averaged over all experiments. A stable PAP plateau was achieved after ~40 min, and control experiments (data not given in detail) showed that ongoing infusion of U46619, this level of pulmonary hypertension was maintained for at least 120 min with variations in PAP of less than 2 mmHg. For assessing the efficacy of the NO-donor drugs MOL, SIN-1, SNP and GTN, these agents were administered after maintenance of the elevated PAP plateau for at least 10 min, during which the U46619 infusion was continued. The drugs were either bolus-injected into the recirculating buffer fluid, using total doses of 0.015, 0.15 and 1.5
μmoles (perfusate volume 150 ml), or the same absolute quantities were aerosolized for delivery to the lungs via inhalation. The time required for aerosol application ranged between 3 and 6 min for molsidomine, SIN-1 and SNP, independent of the dosage employed due to use of different concentrations in the nebulizer. For delivery of 1.5 μmoles GTN, however, the aerosolization required 60 min due to the relatively low concentration of this drug in aqueous solution (1 mg/ml as compared to 5 mg/ml in ethanol-containing preparations).

Separate experiments were performed to assess the intravascular recovery of sydnonimines (1.5 μmoles of molsidomine or 0.15 μmoles of SIN-1, both delivered as aerosol or admixed to the buffer fluid). In these experiments, repetitive sampling of the pulmonary venous effluent was undertaken for high-performance liquid chromatography analysis as described above. For further differentiation between “local” and “systemic” (recirculation of the drug or an active metabolite after transition from the alveolar into the vascular compartment) effects of aerosolized NO-donors, SNP (0.015 and 0.15 μmoles) and SIN-1 (1.5 μmoles) were nebulized in lungs which were nonrecirculatingly perfused by continuously discarding the complete venous effluent and replacing it by fresh buffer fluid.

**Data analysis.** All values were expressed as mean ± S.E.M. For comparison of means, one-way analysis of variance was performed. P values of less than 0.05 were considered to represent a significant difference. For analysis of intravascular NOx data, the increase per time was considered. All statistical procedures were performed with the SPSS for MS Windows analysis system.

**Results**

**Base-line conditions.** Under baseline conditions, the pulmonary artery pressure ranged between 6 and 10 mmHg in all experiments. In response to U46619 infusion, rapid vasoconstrictor response with plateauing of PAP values between 17 and 23 mmHg occurred throughout (fig. 1). Progressive intravascular NOx accumulation was observed as previously described (Spriestersbach et al., 1995), at a rate of 10 to 40 nmol × 1⁻¹ × min⁻¹. In parallel, continuous exhalation of NO was noted, ranging between 50 and 110 ppb in the ex-
pired air. U46619 infusion did not affect the kinetics of either NOx release or NO exhalation.

**Intravascular administration of NO-donor drugs.** After intravascular administration, all NO-donors reduced the U46619-elicited pulmonary hypertension in a dose-dependent fashion (figs. 1–4). When compared on a molar basis, SNP was the most effective drug with a sustained reduction of PAP to near baseline levels even at the “medium” dose of 0.15 μmoles, which was accompanied by a small augmentation of NO exhalation (P < .05), whereas no acceleration of the perfusate NOx accumulation was evident at this dosage. The use of a 10-fold higher dose (1.5 μmoles) of SNP did not further enhance the PAP-lowering capacity, but the intravascular accumulation of large quantities of NOx was observed (P < .001), whereas there was only a marginal further increase in NO exhalation (P < .05). SIN-1 was slightly less effective than SNP; it similarly reversed the pulmonary hypertension to near baseline levels with rapid onset of action at the highest dose of 1.5 μmoles. The intravascular application of this drug was accompanied by a solely marginal (P > .05) increase in NO exhalation, whereas a significant (P < .01) acceleration of the perfusate NOx accumulation was noted only at the highest dosage used. Intravascular GTN reduced PAP dose-dependently with rapid onset of action, but the maximum extent of pressure reduction was less than that noted for SIN-1 and SNP, even at the maximum dose of 1.5 μmoles, moreover a rapid loss of efficacy was also noted. In contrast, short peaks of very large amounts of NO were detected in the exhaled air (P < .01 for 0.15 μmoles; P < .001 for 1.5 μmoles), and this drug effected the highest concentrations of NOx in the recirculating buffer fluid of all agents (P < .02 for 0.15 μmoles; P < .001 for 1.5 μmoles; respectively). Intravascular molsidomine displayed vasorelaxant efficacy only at the maximum dose of 1.5 μmoles. Delayed-onset but sustained PAP-reduction was then noted, and a significant airspace or intravascular release of NO or its decomposition products was not detected.

![Graph](https://example.com/graph.png)

**Fig. 2.** Dose-dependent effects of aerosolized versus intravascular molsidomine on pulmonary hypertension, nitric oxide (NO) exhalation and intravascular accumulation of (peroxy-)nitrite/nitrate (NOx). U46619 was continuously infused to increase pulmonary artery pressure (PAP) as indicated. The molsidomine application is indicated by an arrow and time was set at zero; the total quantities of the drug for either route of application are given. Mean ± S.E.M. of n = 5 independent experiments each; error bars are missing when falling into symbol.
**Aerosol administration of NO-donor drugs.** Using the transbronchial route for drug application, SNP was again found to be the most potent vasodilator of all NO-donor drugs investigated. Comparable to the intravascular administration of this agent, reduction of the pulmonary hypertension to near baseline levels was achieved with the “medium” dose of 0.15 μmoles of nebulized SNP, accompanied by an increase in NO exhalation (P < .05) in the absence of a significant effect on perfusate NOx accumulation. The kinetics of pressure reduction were, however, slightly retarded as compared to the perfusate admixture of this drug. Again, the use of a 10-fold higher dose did not further enhance the PAP-lowering capacity, but the intravascular and the airspace release of NO were markedly increased (P < .01 each). Aerosolized SIN-1 reduced the elevated PAP with time course and dose-dependency comparable to intravascular administration of this drug, but relatively more exhalation of NO (P < .01 for 0.15 μmoles) and somewhat less increase in intravascular NO degradation products (P > .05) were detected. The vasorelaxant properties of aerosolized molsidomine surpassed those noted upon intravascular administration of this drug: even the dose of 0.15 μmoles provoked some reduction in PAP values, and sustained efficacy was noted upon nebulization of 1.5 μmoles molsidomine. The delay in onset of action was similar to the perfusate application of this agent. A moderate release of NOx/NO into both the intravascular and alveolar compartment was noted upon use of the highest molsidomine dose (P < .05 each). Aerosolized GTN exerted only a marginal effect of the U46619-elicited pulmonary hypertension at the highest dose of 1.5 μmoles.

**Sydnonimine recovery.** When 0.15 μmoles SIN-1 were administered via the intravascular route, 66% was recovered in the intravascular space after 5 min (fig. 5). After aerosolization of the same dose, 25.2% of the deposited SIN-1 was detectable within the intravascular compartment after this time period. Subsequently, perfusate SIN-1 concentrations
Glyceryl Trinitrate

### Aerosol
- □ 1.5 μmoles
- ▲ 0.15
- ▼ 0.015
- ■ 1.5 μmoles

### Intravascular
- ○

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**Fig. 4.** Dose-dependent effects of aerosolized versus intravascular glyceryl trinitrate (GTN) on pulmonary hypertension, nitric oxide (NO) exhalation and intravascular accumulation of (peroxy-)nitrite/nitrate (NOx). U46619 was continuously infused to increase pulmonary artery pressure (PAP) as indicated. The GTN application is indicated by an arrow and time was set at zero; the total quantities of the drug for either route of application are given. Mean ± S.E.M. of n = 5 independent experiments each; error bars are missing when falling into symbol.

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decreased progressively, paralleled by a corresponding increase in the metabolite SIN-1C. Molsidomine recovery in the perfusate was 80% when assessed 5 min after intravascular administration of this drug, and 48% of the aerosolized dose appeared within the intravascular space after this time span (fig. 6). After both routes of administration, perfusate molsidomine concentrations decreased slowly, paralleled by a moderate increase in the primary metabolite SIN-1 and the successive metabolite SIN-1C.

**Lung inflation pressure.** Neither U46619-infusion nor intravascular or transbronchial application of NO-donor drugs exerted any change in the inflation pressures during constant-volume ventilation.

### Discussion

In the model of U46619-elicited pulmonary hypertension, which is operative largely via precapillary vasoconstriction in perfused rabbit lungs (Lindeborgh *et al.*, 1995, Rimar and Gillis, 1995), short aerosolization maneuvers of different NO-donor drugs turned out to be very effective for achieving pulmonary vasodilation. SNP and SIN-1 were found to be the most potent agents, effecting sustained reduction of pulmonary artery pressure to near base-line levels when applied by nebulization. Interestingly, these prodrugs displayed nearly smaller and only transient pressure decrease under nonrecirculating conditions.

**Efficacy of SNP and SIN-1 aerosols under nonrecirculating conditions.** Separate control experiments ascertained the provocation of a constant vasoconstrictor response by continuous infusion of U46619 also under nonrecirculating conditions. Under these circumstances, aerosolized sodium nitroprusside decreased the elevated PAP with virtually identical kinetics and extent as observed for the standard protocol with recirculation of the buffer fluid (fig. 7). Aerosolized SIN-1, in contrast, induced a significantly far
Fig. 5. Intravascular recovery of 3-morpholinosydnone imine (SIN-1). A total of 0.15 μmoles of SIN-1 was either aerosolized or administered i.v. at time zero, and SIN-1 as well as its decomposition product 3-morpholininoacetanilide (SIN-1C) were quantified in samples of pulmonary venous effluent. Mean ± S.E.M. of three independent experiments each are given.

identical dose-effect relationships with respect to onset, extent and duration of pulmonary vasodilation whether administered via the inhalative route or infused into the pulmonary artery of the isolated lungs. Molsidomine, possessing overall less vasodilatory potency in this model, was even slightly more effective when nebulized as related to intravascular administration, and GTN was the least effective agent for both routes of application in the perfused lungs.

All agents presently used are prodrugs, with NO being the essential product that ultimately promotes the pharmacological action, i.e., the pulmonary vasodilation as assessed in our study. However, the conditions for the liberation of NO and the “yield” of this active radical in competition with other directly released nitrous compounds markedly differ between the various drugs used, and this is partly reflected by the appearance of NO in the exhaled air and NOx in the recirculating medium. The pharmacokinetics of NO in buffer-perfused rabbit lungs were previously analyzed in detail (Spriestersbach et al., 1995). In essence, it was found that NO appearing at the gas-fluid interface of the lung largely escapes into the gaseous alveolar compartment due to its very low buffer-gas partition coefficient. When decomposing to products such as nitrite, nitrate or peroxynitrite—which occurs most likely, the more remote the site of NO generation is from the gaseous compartment—the substance is kept in aqueous solution including the buffer medium. The perfusate NOx comprises both nitrite and nitrate as well as peroxynitrite, whereas only minute quantities of the volatile NO itself remain dissolved in the buffer medium when this reaches the acidic vanadium (III) column employed for detection. NOx may thus represent decomposition products of previously generated NO, but may also represent nitrous agents directly liberated from the prodrug, as occurring extensively in the case of GTN (see below). Keeping these facts in mind, the following features of NO liberation from the different prodrugs appear to support the present findings:

Glyceryl trinitrate. Both enzymatic and nonenzymatic bioactivation pathways are established for the NO liberation from organic nitrate esters such as GTN (Feelisch and Kelm, 1991, Feelisch and Noack, 1987, Noack and Feelisch, 1991). The former include denitration by cytosolic GSH-S-transferase, producing mainly inorganic nitrite, and reduction to thionitrite esters, which form NO via nitrosothiol intermediates. The latter is operative in the presence of various thiol-containing compounds. The relative contribution of enzymatic vs. nonenzymatic pathways depends on the local enzymatic outfit and the availability of thiol compounds and is not established for the capillary endothelial and the alveolar epithelial surface of the lung. Overall, the generation of nitrite must be assumed to far exceed the formation of NO; a ratio of 14:1 was, e.g., shown in the presence of cysteine in vitro (Noack and Feelisch, 1991). This fact explains the very high levels of NOx measured on intravascular administration of GTN, which sharply contrast to the relatively low vasodilatory efficacy of this agent. In addition, the decomposition of intravascular GTN obviously occurred very rapidly in the lung, as evident from the fast initial kinetics of NOx appearance and the short peak of NO exhalation. A presently unexplained finding is the fact that the peak of NO exhalation in response to intravascular GTN was the highest among all agents in spite of the low vasodilatory efficacy of this drug; it may be speculated that this represents generation of NO very close to the gaseous surface but remote from the smooth muscle cells of the precapillary resistance vessels constricted by U46619. The aerosol application of GTN was hampered by the fact that only low concentrations of this agent in aqueous solution are available, demanding long nebulization times in contrast to all other drugs presently used, which partly explains the very slow onset of action on inhaled GTN. Higher concentrations of GTN are available in ethanol-containing solutions, but the aerosolization of ethanol as vehicle was avoided, as this agent itself may exert pharmacological effects including endogenous NO liberation upon nebulization into the bronchoalveolar compartment (Greenberg et al., 1993). In addition, the conditions of enzymatic or nonenzymatic bioactivation of GTN at the alveolar surface are not yet established, but the current data suggest rather low bioconversion of GTN in this compartment.

Molsidomine and SIN-1. Molsidomine has to be cleaved enzymatically to generate the direct NO-liberating prodrug SIN-1, an event hitherto described only for hepatic passage (Feelisch et al., 1989, Meinertz et al., 1985, Noack and Feelisch, 1989). Our data of vasodilation exerted by molsidomine in the isolated perfused lungs, in companion with NO/NOx generation and the appearance of the active metabolite SIN-1 in the intravascular space, unequivocally demonstrate that enzymatic activity capable of molsidomine cleavage to SIN-1 resides also in lung parenchyma, at least in rabbit species. SIN-1 possesses the capability of spontaneous NO liberation, independent of thiol-compounds and accompanied by the appearance of the SIN-1C metabolite; moreover in vitro studies showed that all sydnonimines generate NO at a nearly equimolar rate at nitrite/nitrate, i.e., with a relatively high yield (Feelisch et al., 1989). As anticipated from these pharmacokinetic data, molsidomine exerted moderate (on a molar basis) but very sustained pulmonary vasodilation, both upon intravascular and alveolar deposition: the time course of PAP reduction in response to 1.5 μmoles molsidomine, and the fact that within 1 hr of application of this agent far less than 50% was converted to the SIN-1 metabolite, both suggest that the duration of the vasodilatory action of this agent will
Intravascular Recovery of Molsidomine

![Graph showing intravascular recovery of molsidomine.](image)

**Fig. 6.** Intravascular recovery of molsidomine. A total of 1.5 μmoles of molsidomine was either aerosolized or intravascularly administered at time zero, and molsidomine as well as the metabolites 3-morpholinosydnone imine (SIN-1) and 3-morpholinoiminoacetonitrile (SIN-1C) were quantified in samples of pulmonary venous effluent (see different scaling of the metabolite concentrations). Means ± S.E.M. of three independent experiments each are given.

far exceed the present 90-min observation period. Not surprisingly, direct application of SIN-1 was more effective than molsidomine with respect to rapidity and extent of pulmonary vasodilation, accompanied by more rapid appearance of SIN-1C signalling decomposition with NO release. Virtually identical efficiency was observed for intravascular and alveolar deposition of this agent. In addition, when using the highest dose of 1.5 μmoles SIN-1, nearly maximum vasodilatory capacity was still observed after 90 min, demonstrating a sustained mode of action also for this drug. Despite their pronounced vasodilatory efficacy, both molsidomine and SIN-1 provoked only moderate NO exhalation and perfusate NO\textsubscript{x} accumulation. In comparison to the GTN data one might speculate that the sydnonimine-derived NO was much more “efficiently” used for pharmacological action at the site of the vessel smooth muscles, with less escape into the alveolar and intravascular compartment, possibly due to differences in the cell entry of the prodrug, but no basic pharmacological data are presently available to substantiate such a speculation.

We cannot exclude that—in addition to nitric oxide liberation—the stable metabolite SIN-1C may contribute to the vasodilatory effect of SIN-1. However, the kinetics of SIN-1 decomposition and SIN-1C accumulation in our experiments in relation to the time course of the PAP-reduction (fig. 3 and 5) suggest a predominant role of SIN-1 derived NO. A significant impact of SIN-1C seems unlikely, as extensive and maximum vasodilatation occurs within the first minutes after SIN-1 application, when SIN-1C concentrations are low; moreover, pulmonary hypertension resumes in parallel to SIN-1 decay and despite of SIN-1C increase.

**Sodium nitroprusside.** The in vivo-breakdown of SNP is probably initiated by the contact between SNP and sulphydryl-groups bound on cell membranes, resulting in the formation of an unstable nitroprusside radical, which then dissociates into cyanide and nitric oxide (Gerber and Nies, 1992, Ivankovich et al., 1978, Schulz, 1984). The dose-response curves of this agent nearly corresponded to those of SIN-1 with respect to rapidity of onset of PAP decrease, maximum vasodilatory effect and duration of vasorelaxation, with slightly higher efficiency of SNP. Again, aerosol administration of SNP effected nearly the same profile of action as the intravascular administration of this NO-donor, with somewhat less initial kinetics of vasorelaxation. With respect to airspace NO and perfusate NO\textsubscript{x} appearance, SNP took an “intermediate” position between GTN and the sydnonimines: dose-dependent release into either compartment was noted, with some predominance of NO exhalation upon aerosolization and NO\textsubscript{x} accumulation upon buffer admixture of SNP. The total quantities clearly surpassed those in response to SIN-1 in spite of a virtually identical vasodilatory efficacy.

Overall, the first question raised by this study, the suitability of aerosolization of NO-donor drugs, packaged into particle sizes with access to the alveolar space for achieving prolonged vasodilation of the pulmonary circulation, may be answered affirmatively and without reservation. Even the prodrug molsidomine, requiring preceding enzymatic cleavage, is effective upon nebulization, although with much less potency as compared to SNP and SIN-1. In contrast to the sustained vasodilation induced by these agents, reduction of pulmonary artery pressure by inhalation of gaseous nitric oxide ceases within <30s upon stop of supply even in blood-free-perfused lungs (data not given).

The second question, concerning the reliability of NO exhalation (or perfusate NO\textsubscript{x} accumulation) to monitor bioconversion and pharmacological activity of the NO-donors, can only be answered affirmatively with much reservation. Within one pharmacological agent, there was a clear link between dosage, vasodilatory property and NO/NO\textsubscript{x} release. However, identical bioactivities (PAP decrease) of the different drugs, as, e.g., exerted by SNP and SIN-1, were accompanied by different levels of NO/NO\textsubscript{x}, and most striking was the discrepancy between the high levels of NO/NO\textsubscript{x} appearance in the gaseous and intravascular compartment and the low pulmonary vasodilatory efficacy of GTN. This finding suggests hitherto unknown differences in the partitioning of the various compounds into the tissue compartments from either the perfusate or aerosolization domain. In addition, NO binding to various target molecules may be operative as the underlying event of such a “loose” coupling between intragran NO liberation and relaxation of the vascular smooth muscle cells. In addition, these observations give rise to a
showed substantial entry of the prodrug into the vascular compartment after inhalative delivery. The latter is even more evident for the nebulization of molsidomine, with maximum perfusate levels of this agent approaching 60% of those measured after direct buffer admixture. Concerning the sydnonimines, the inhalative route of application thus apparently effects pulmonary vasodilation and delivers substantial quantities of the active prodrug to the systemic circulation. The latter feature will, of course, cause loss of selectivity for the pulmonary circulation, the extent of which has to be ruled out in intact animal experiments with direct comparison of pulmonary and systemic vasodilatory effects upon aerosolization and intravenous infusion of these agents. In contrast, SNP displayed virtual identical vasodilatory efficacy whether used under recirculating or non-recirculating conditions. Although no direct measurements of SNP entry into the vascular space were performed, these findings suggest predominant or even exclusive effects of NO continuously liberated from a prodrug that was retained in the alveolar (or interstitial) lung compartment. This is further supported by pilot experiments demonstrating that aerosolized SNP was also effective in blood perfused rabbit lungs, in which rapid inactivation of intravascular NO by hemoglobin occurs. The permeability characteristics of the alveolar epithelial barrier for different types of drugs remain to be elucidated, and presently only speculations can be undertaken on the basis of available physiological data (Boucher, 1994a and b; Saumon and Basset, 1993). Due to the huge surface of the single alveolar epithelial type I cells, i.e., the relative scarcity of interepithelial junctions, and the tightness of these junctions, the entry of any hydrophilic agent into the interstitial space is largely restricted, and it may remain deposited on the epithelial barrier for hours. In contrast, any agent that possesses the capability of transmembrane and (thus) transcellular passage must be anticipated to reach the interstitial and subsequently the intravascular compartment rapidly due to the extreme “flatness” of the alveolar epithelial layer. It is tempting to speculate that these features may be the basis the pharmacokinetic differences between the hydrophilic SNP and the amphiphilic sydnonimines. Further studies are clearly necessary to substantiate this assumption.

In conclusion, short aerosolization maneuvers of NO-donor drugs turned out to be an efficient approach for achieving dose-dependent, extensive and sustained vasodilation of the lung vasculature in an experimental model of pulmonary hypertension. NO exhalation may be used to monitor the bioactivities of the different NO-donors, but the levels of NO liberation into the gaseous (and intravascular) space in relation to the vasorelaxant property substantially differ among the various agents. The selectivity of the vasodilatory effect for the pulmonary circulation will largely depend on the pharmacokinetic fate of the prodrug used, whether it remains deposited on the alveolar epithelial barrier after aerosol delivery or rapidly transits this layer with intravascular entry; such differential features may even be used for different therapeutic goals. Overall, aerosolization of NO-donors may offer a new therapeutic concept for the treatment of chronic pulmonary hypertension.

References
