

Intratracheal instillation of a novel NO/nucleophile adduct selectively reduces pulmonary hypertension

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Brilli, Richard J., Brian Krafte-Jacobs, Daniel J. Smith, Dominick Roselle, Daniel Passerini, Amos Vromen, Lori Moore, Csaba Szabó, and Andrew L. Salzman. Intratracheal instillation of a novel NO/nucleophile adduct selectively reduces pulmonary hypertension. *J. Appl. Physiol.* 83(6): 1968–1975, 1997.—We examined the pulmonary and systemic hemodynamic effects of administering soluble nitric oxide (NO) donor compounds (NO/nucleophile adducts, i.e., NONOates) directly into the trachea of animals with experimentally induced pulmonary hypertension. Steady-state pulmonary hypertension was created by using the thromboxane agonist U-46619. Yorkshire pigs were randomly assigned to one of four groups: *group 1*, intratracheal saline (control; $n = 8$); *group 2*, intratracheal sodium nitroprusside ($n = 6$); *group 3*, intratracheal ethylputreanine NONOate ($n = 6$); and *group 4*, intratracheal 2-(dimethylamino)-ethylputreanine NONOate (DMAEP/NO; $n = 6$). Pulmonary and systemic hemodynamics were monitored after drug instillation. *Group 4* had significant reductions in pulmonary vascular resistance index (PVRI) at all time points compared with steady state and compared with *group 1* ($P < 0.05$), whereas systemic vascular resistance index did not change. The mean change in mean pulmonary arterial pressure in *group 4* was $-33.1 \pm 1.2\%$ compared with $+6.4 \pm 1.3\%$ in *group 1* ($P < 0.001$), and the mean change in mean arterial pressure was $-9.3 \pm 0.7\%$ compared with a control value of $-0.9 \pm 0.5\%$ ($P < 0.05$). *Groups 2* and *3* had significant decreases in both PVRI and systemic vascular resistance index compared with steady state and with *group 1*. In conclusion, intratracheal instillation of a polar-charged tertiary amine NONOate DMAEP/NO results in the selective reduction of PVRI. Intermittent intratracheal instillation of selective NONOates may be an alternative to continuously inhaled NO in the treatment of pulmonary hypertension.

nitric oxide donor; pulmonary hypertension

ACQUIRED PULMONARY HYPERTENSION is a common finding in many diseases, including sepsis, meconium aspiration, acute respiratory distress syndrome, and congenital heart disease associated with pulmonary overcirculation; it is also found in patients after cardiopulmonary bypass (17, 19, 39, 49). Intravenous therapy with vasodilating agents such as nitroglycerin, nitroprusside, prostaglandin E₁, milrinone, and amrinone is intended to reduce pulmonary hypertension. Because these medications lack selectivity for the pulmonary vascular bed and exacerbate ventilation-perfusion matching by overriding the hypoxic pulmonary vasoconstriction response, their use is associated with unwanted side effects, including systemic hypotension and hypoxemia (34). An effective, easily administered

pulmonary vasodilator that does not induce systemic hypotension or hypoxemia would be of great clinical importance.

The free radical nitric oxide (NO) is a potent vasodilator and when inhaled as a gas, selectively dilates the pulmonary vascular bed (24, 30). Although the exact mechanism by which inhaled NO dilates the pulmonary vessels is unknown, it is presumed that NO is distributed to distal ventilated alveolar segments where it passes readily, due to its great lipophilicity, through the epithelium into the cytosol of the arteriolar vascular smooth muscle. It then activates guanylyl cyclase, inducing the production of guanosine 3',5'-cyclic monophosphate, which in turn causes a decrease in intracellular calcium in vascular smooth muscle cells, leading to vascular relaxation (33, 42). NO that diffuses into the vascular lumen is inactivated by its interaction with hemoglobin to form methemoglobin. In this manner, it is presumed that NO is a selective pulmonary vasodilator, since it is rapidly metabolized in the systemic circulation (8, 28, 37, 38). Multiple clinical studies have shown that inhaled NO is a selective pulmonary vasodilator and is useful in the treatment of diseases associated with pulmonary vascular hypertension (1, 2, 5, 16, 21, 40, 41, 48).

Because of its extremely short half-life, inhaled NO must be delivered continuously, and withdrawal of therapy has been associated with rebound hypoxemia (11). In addition, delivery of inhaled NO requires specialized delivery equipment and environmental monitoring systems to avoid exposure of health-care workers to NO and NO₂, a toxic NO by-product (3, 15, 43, 47). The delivery of an aqueous, slow-release form of NO to the pulmonary vascular bed would offer several advantages compared with NO gas delivery, including a simplified delivery system, the ability to provide intermittent therapy, and decreased environmental release of NO or NO₂.

Compounds formed by reacting NO with various nucleophiles, also known as NO/nucleophile adducts (NONOates), have vasorelaxant properties and appear to induce vasodilation via spontaneous nonenzymatic release of NO (6, 25, 26). NONOates contain the following generic chemical structure: X[N(O)NO]⁻. These compounds are stable as solids, highly soluble in aqueous media, and release NO at predictable rates (22). Their vasorelaxant properties correlate with their first-order rates of spontaneous release of NO in simple aqueous buffers (6). Systemic intravenous administration of NONOates during induced pulmonary hyperten-

sion produces a dose-dependent decrease of both pulmonary arterial and systemic arterial pressure (22, 46). It has been suggested that, for intravenous use, alteration of the carrier nucleophile of a NONOate may allow targeting of specific vascular beds (46).

Because direct continuous inhalation of NO selectively targets the pulmonary vasculature, we hypothesized that direct tracheal instillation of a NONOate with limited mucosal permeability would permit selective pulmonary vasodilation. In addition, alteration of the polarity and charge of the carrier nucleophile could limit the transmucosal flux of the nucleophile without limiting the transmucosal diffusion of the released NO (7). To test this hypothesis, we investigated the hemodynamic effects of direct tracheal instillation of the charged nucleophile adduct 2-(dimethylamino)-ethylputreanine/NO (DMAEP/NO, a tertiary amine with a cationic-charged end group) in a porcine model of pulmonary hypertension and compared these effects with those of other intratracheally administered nitrovasodilators, sodium nitroprusside (SNP, a commonly used NO donor) and ethylputreanine NO (EP/NO, an ester NONOate with an uncharged end group) (Fig. 1).

MATERIALS AND METHODS

Animal Instrumentation and Experimental Procedures

This study was approved by the Animal Care and Use Committee of the Children's Hospital Research Foundation. The care and handling of the animals were performed in accordance with National Institutes of Health guidelines. Twenty-six male immature random-bred Yorkshire pigs (~6–10 wk old, 10–15.5 kg body mass) were fasted on the day before surgery. Sedation was achieved with intramuscular ketamine hydrochloride (20 mg/kg) and atropine sulfate (0.05 mg/kg). Endotracheal intubation was performed under 2% isoflurane anesthesia, and mechanical ventilation was instituted with a Ventimeter ventilator (Air Shields, Hatboro, PA). General anesthesia was maintained with 0.5–0.6% isoflurane, 70% nitrous oxide, and balance oxygen. The respiratory rate and tidal volume were adjusted to maintain the arterial P_{CO_2} at 40 ± 5 Torr (5.3 kPa). Heating pads and blankets were used to maintain a normal core body temperature of $39.0 \pm 0.4^\circ\text{C}$.

A right femoral cut down was performed for insertion of a double-lumen 4-Fr catheter in the femoral vein for fluid and drug administration, and a 5-Fr single-lumen catheter was placed in the femoral artery for blood pressure monitoring and arterial blood-gas sampling (Cook, Bloomington, IN).

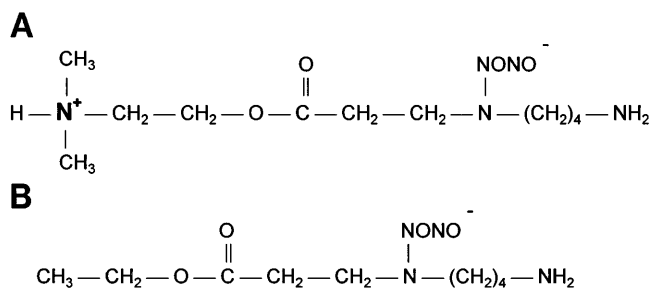


Fig. 1. Chemical structure of nitric oxide (NO)/nucleophile adducts (NONOates). A: 2-(dimethylamino)-ethylputreanine/NO (DMAEP/NO); N^+ , cationic-charged end group of this NONOate. B: ethylputreanine/NO (EP/NO).

A midsternotomy was performed, and the pericardium was incised. A 16-mm ultrasonic cardiac output flow probe (Transonic Systems, Ithaca, NY) was positioned around the pulmonary artery. The probe was connected to a previously calibrated blood flowmeter (model T206, Transonic Systems). Intracardiac catheters (3-Fr \times 24 in., model 50010, DLP, Grand Rapids, MI) were inserted in the right atrium, left atrium, and pulmonary artery for pressure measurement and were secured in place with a purse-string suture. A search for a patent ductus arteriosus was undertaken, and, if present, the ductus was ligated. The pericardial space was filled with warmed saline and the sternum approximated with towel clips. During the surgical procedure, animals received either D_5 Ringer lactate or Ringer lactate solution, as appropriate, at a rate of $20 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ to maintain normal serum glucose concentration. After closure of the sternotomy, the rate of intravenous infusion was reduced to $5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. A 6-Fr feeding tube was placed through a T connector attached to the proximal end of the tracheal tube, with the tip of the feeding tube positioned 1 cm distal to the end of the tracheal tube and above the carina. After the surgical procedure, animals were allowed to stabilize for 30 min.

Hemodynamic and Blood-Gas Measurements

Mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), left atrial pressure, and right atrial pressure were determined with the use of calibrated transducers (Cobe Cardiovascular, Arvada, CO) driving an amplifier monitor (Horizon 2000, Mennen Medical, Clarence NY) with digital readout. Oxygen saturation and heart rate were recorded with a pulse oximeter (Ohmeda 5250 RGM Louisville, CO). Cardiac output was recorded from the digital output of the flowmeter connected to the flow probe positioned around the pulmonary artery. Gas tensions and methemoglobin levels in arterial blood were determined by using a blood-gas analyzer (model 278, Ciba-Corning Diagnostic, Madfield, MA) and corrected for core temperature.

Cardiac index (CI) was calculated as cardiac output-to-animal weight (in kg). Pulmonary vascular resistance index (PVRI) was calculated as $79.9 \times (\text{MPAP} - \text{left atrial pressure})/\text{CI}$ ($\text{dyn} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{kg}$), and systemic vascular resistance index (SVRI) as $79.9 \times (\text{MAP} - \text{right atrial pressure})/\text{CI}$ ($\text{dyn} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{kg}$).

Drug Preparation

Thromboxane agonist preparation. A stock solution of 9,11-dideoxy-11 α ,9 α -epoxymethano-prostaglandin $F_{2\alpha}$ (U-46619; Sigma Chemical, St. Louis, MO) was suspended in 95% ethanol and stored at -20°C . A fresh working solution was prepared in 0.9% saline to achieve a concentration of 100 $\mu\text{g}/\text{ml}$.

NONOate preparation. The chemical preparation of these NONOates has not been previously reported and is outlined below.

The monoester 2-(dimethylamino)-ethylputreanine was synthesized by dissolving 2.0 g (14 mmol) of 2-(dimethylamino)-ethylacrylate (Aldrich Chemical, Milwaukee, WI) into 50 ml of dry tetrahydrofuran (THF). This solution was added dropwise to 5 M excess of 1,4-diaminobutane (70 mmol, 6.6 g) (Aldrich Chemical) in 50 ml of THF at room temperature over 8 h with vigorous stirring. After the reaction continued for an additional 8 h, the THF was removed by rotary evaporation, leaving a yellow oily product. The oil was washed with five separate 25-ml portions of anhydrous diethyl ether to remove excess diaminobutane. The resulting 2.6 g of soft waxy 2-(dimethylamino)-ethylputreanine was dried in a vacuum oven at room temperature overnight.

DMAEP/NO was prepared by dissolving 500 mg (2.16 mmol) of 2-(dimethylamino)-ethylputreanine in 80 ml of THF in a high-pressure glass bottle (Ace Glass, Vineland, NJ). The mixture was degassed and exposed to 70 pounds/in.² (psi) of NO for 48 h while being vigorously stirred. The pressure was then released, and the liquid layer was discarded. The NONOate, which accumulated as a yellow wax on the walls of the reaction flask, was washed with ether and dried under vacuum. The reaction yielded 485 mg of product. The ultraviolet (UV) spectrum of the NONOate (0.1 mol/l sodium hydroxide) revealed a λ_{\max} 250 nm and extinction coefficient (ϵ) 6.4 mM⁻¹ cm⁻¹; ¹H nuclear magnetic resonance [heavy sodium hydroxide (D₂O/OD), pH 8] δ 1.54 (m, 4H), 2.26 (s, 6H), 2.42 (t, 2H), 2.60 (t, 2H), 2.84 (m, 4H), 3.17 (t, 2H), 3.73 (t, 2H) (where m is multiplet, s is singlet, and t is triplet). The rate of release of NO was determined by rapidly mixing 5 mg of the NONOate in 50 ml of pH 7.4 buffer at 25°C and by measuring the absorbance at 250 nm every 10 min for 6 h. Subsequent readings were taken at appropriate intervals after 20–25 h. A first-order plot ($r^2 = 0.996$) yielded a half-life of 135 min.

EP was synthesized by dissolving 2 g (20 mmol) of ethylacrylate (Aldrich Chemical) into 50 ml of ethanol. This solution was added dropwise to 5 M excess of 1,4-diaminobutane (100 mmol, 8.8 g) (Aldrich Chemical) in 50 ml of ethanol at room temperature over 8 h with vigorous stirring. After the reaction continued for an additional 8 h, the ethanol was removed by rotary evaporation to leave a colorless oily product. The oil was washed with five separate 25-ml portions of anhydrous diethyl ether to remove excess diaminobutane. The resulting 4.3 g of soft waxy EP was dried in a vacuum oven at room temperature overnight.

EP/NO was prepared by dissolving 500 mg (4.99 mmol) of EP in 80 ml of distilled THF in a high-pressure glass bottle (Ace Glass). The mixture was degassed and exposed to 70 psi of NO for 48 h while being vigorously stirred. The pressure was then released, and the liquid layer was discarded. The NONOate, which accumulated as a sticky wax on the walls of the reaction bottle, was washed with ether and dried under vacuum. The reaction yielded 432 mg of product. The UV spectrum of the NONOate (0.1 mol/l sodium hydroxide) revealed a λ_{\max} 250 nm and ϵ 2.44 mM⁻¹ cm⁻¹; ¹H nuclear magnetic resonance (D₂O/OD; pH 8) δ 1.15 (t, 3H), 1.6 (m, 4H), 2.26 (t, 2H), 2.42 (t, 2H), 2.6 (t, 2H), 2.85 (m, 2H), 3.16 (q, 2H, where q is quadruplet). The rate of release of NO was determined by rapidly mixing 5 mg of the NONOate in 50 ml of pH 7.4 buffer at 25°C and by measuring the absorbance at 250 nm every 10 min for 6 h. Subsequent readings were taken at appropriate intervals after 20–25 h. A first-order plot ($r^2 = 0.997$) yielded a half-life of 143 min.

Immediately before instillation into the trachea, the NONOates were dissolved in 10 ml of saline. SNP (10 mg) was diluted in 10 ml of 5% dextrose in water to achieve a final concentration of 1 mg/ml and shielded from light.

Pulmonary Hypertension Protocol

Following surgical preparation, baseline hemodynamic and arterial blood-gas measurements were obtained. U-46619 was then infused into the femoral vein continuously at 0.5–2.0 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ to increase PVRI two- to threefold above baseline measurements. The U-46619 infusion was continued throughout the entire experiment. PVRI data were obtained every 5 min until 15 min of stable increased PVRI measurements occurred. This steady-state pulmonary hypertension (SS) represented the mean of the three previous sequential PVRI measurements. The percent changes in pulmonary vascular resistance in response to the administration of the study drugs was calculated relative to this SS

measurement. Throughout the study period, mechanical ventilatory support was adjusted to maintain arterial pH between 7.35 and 7.45, arterial PCO₂ between 35 (4.7 kPa) and 45 Torr (6 kPa), and arterial PO₂ between 75 (10 kPa) and 150 Torr (20 kPa).

Drug Administration

Animals were randomly assigned to one of four groups: *group 1* animals received intratracheal saline (control) ($n = 8$); *group 2* animals received intratracheal SNP ($n = 6$); *group 3* animals received intratracheal EP/NO ($n = 6$); and *group 4* animals received intratracheal DMAEP/NO ($n = 6$). Equimolar NO dosing was used for EP/NO and DMAEP/NO; however, delivery of an equimolar dose of SNP resulted in profound systemic hypotension and death for the animals. Pilot experiments with SNP revealed that administration of 15% of the equimolar NONOate dose resulted in maximal vasodilation without hemodynamic compromise; therefore, this dose of SNP was used. All study drugs were instilled over a 2- to 3-min period directly into the trachea via the 6-Fr feeding tube. Hemodynamic data were continuously monitored and recorded at 5- to 10-min intervals over 1 h after the administration of the study drug. Arterial blood-gas measurements were obtained every 15 min.

At the conclusion of the study, all animals were euthanized with pentobarbital sodium. A postmortem search for intracardiac communication was conducted, and samples of lung tissue were obtained for histological examination.

Statistics

Data were analyzed by using the SigmaStat Statistics Package (Jandel, San Rafael, CA). Multiple comparisons between groups were performed by two-way analysis of variance for repeated measures with Bonferroni post hoc correction. Univariate analysis of variance for repeated measures was used to analyze within-group differences. Time-matched between-group differences and contrasts with baseline values within groups were analyzed by using the Student-Newman-Keuls test. Normally distributed continuous data were analyzed by the unpaired two-tailed Student's *t*-test. Significance was noted for $P < 0.05$. Data are expressed as means \pm SE.

RESULTS

All animals survived the experimental protocol. Steady-state pulmonary hypertension was achieved after infusion of U-46619: 1) MPAP increased in all groups by 13.5 ± 0.8 Torr (1.8 kPa); 2) the mean percent increase in PVRI among all groups was $199.4 \pm 8.6\%$; 3) MAP increased in all groups by 4.2 ± 0.7 Torr (0.6 kPa); 4) SVRI increased in all groups by $15.9 \pm 2.4\%$; 5) CI decreased by $9.6 \pm 2.3\%$ among all groups. Left and right atrial pressures were unchanged.

The administration of DMAEP/NO (*group 4*), caused a significant decrease in PVRI at all time points relative to SS and in comparison with the control group (*group 1*) (Fig. 2). SVRI did not change significantly. MPAP decreased significantly at each time point relative to SS and in comparison with *group 1* (Fig. 3). The mean percent change in MPAP over time was $-33.1 \pm 1.2\%$ compared with $+6.4 \pm 1.3\%$ for *group 1* ($P < 0.001$). A small but significant decrease in MAP was observed at 10, 15, 20, and 25 min relative to SS and in comparison with *group 1* (Fig. 4). The mean change in

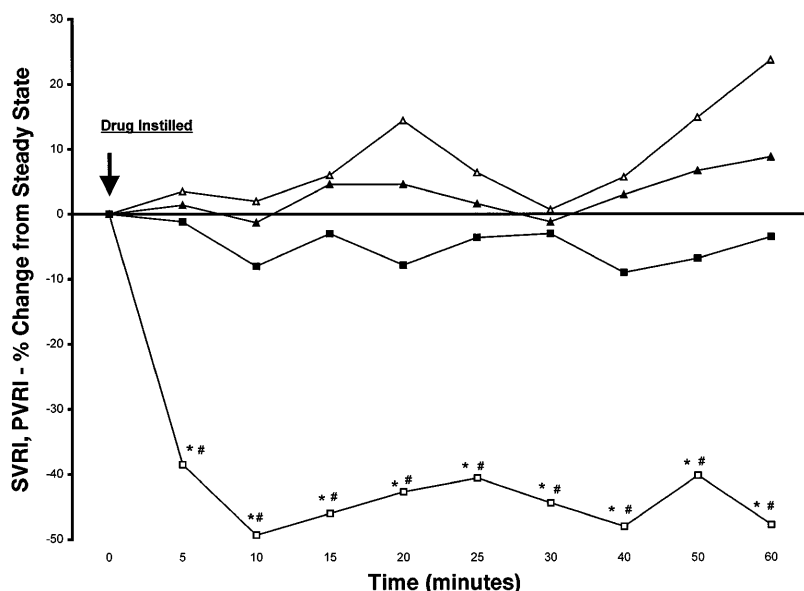


Fig. 2. Intratracheal instillation of a charged polar tertiary amine NONOate: effect on pulmonary vascular resistance index (PVRI) and systemic vascular resistance index (SVRI). Data are means \pm SE. Group 1: $n = 8$; control-PVRI (Δ); control-SVRI (\blacktriangle). Group 4: $n = 6$; DMAEP/NO-PVRI (\square); DMAEP/NO-SVRI (\blacksquare). * $P < 0.05$ in comparison with steady-state pulmonary hypertension, by one-way repeated-measures analysis of variance (ANOVA). # $P < 0.05$ in comparison with control group, by two-way repeated-measures ANOVA.

MAP over time in group 4 was $-9.3 \pm 0.7\%$ compared with $-0.9 \pm 0.5\%$ ($P < 0.05$) in group 1. CI did not change over time after administration of DMAEP/NO. These data demonstrate that tracheal instillation of a charged tertiary amine NONOate selectively lowers PVRI without a significant change in SVRI.

The administration of EP/NO (group 3) caused a significant decrease in PVRI at time points 0–30 min relative to SS and in comparison with the group 1 animals (Fig. 5). The SVRI decreased significantly from SS throughout the 60-min study period and in comparison with group 1 at 15 and 60 min (Fig. 5). MPAP decreased significantly relative to SS and in comparison with group 1 at time points 5–40 min ($P < 0.05$) (Fig. 3). The mean percent change in MPAP over time was $-17.5 \pm 1.9\%$ compared with $+6.4 \pm 1.3\%$ for group 1 ($P < 0.001$). The MAP decreased significantly relative to SS at time points 15–60 min ($P < 0.05$) and was significantly different than group 1 at time 15 min ($P < 0.05$) (Fig. 4). The mean decrease in MAP over time was $7.6 \pm 0.7\%$ compared with group 1 at $-0.9 \pm 0.5\%$ ($P < 0.001$). CI did not change significantly during

the experimental time period. These data demonstrate that tracheal instillation of an uncharged NONOate lowers both PVRI and SVRI.

The administration of SNP (group 2) caused a significant decrease in PVRI at 15 min compared with group 1 and at times 10, 15, 20, 25, and 40 min relative to SS ($P < 0.05$). SVRI was significantly decreased throughout most of the 60-min study relative to SS and group 1 ($P < 0.05$). MPAP decreased significantly compared with group 1 at 5–30 min ($P < 0.05$) (Fig. 3). The mean decrease in MPAP over time was $19.0 \pm 3.1\%$ and was significantly different compared with group 1 ($P < 0.001$). MAP decreased significantly throughout the study relative to SS and was significantly less than in group 1 at 5–20 min ($P < 0.05$) (Fig. 4). The mean decrease in MAP over time was $11.5 \pm 1.2\%$ compared with group 1 of $-0.9 \pm 0.5\%$ ($P < 0.001$). These data demonstrate that tracheal instillation of a non-NONOate NO donor lowers PVRI, SVRI, MPAP, and MAP.

Histological examination of the lung tissue obtained at the conclusion of the experimental protocol revealed no differences in pulmonary parenchymal architecture

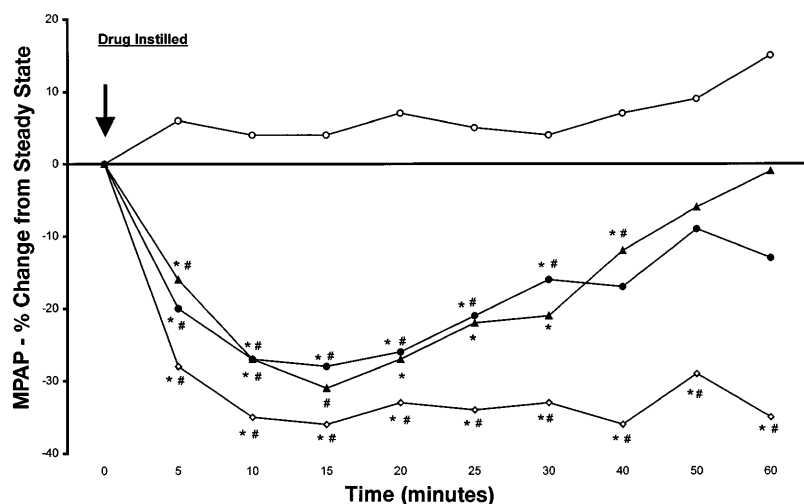
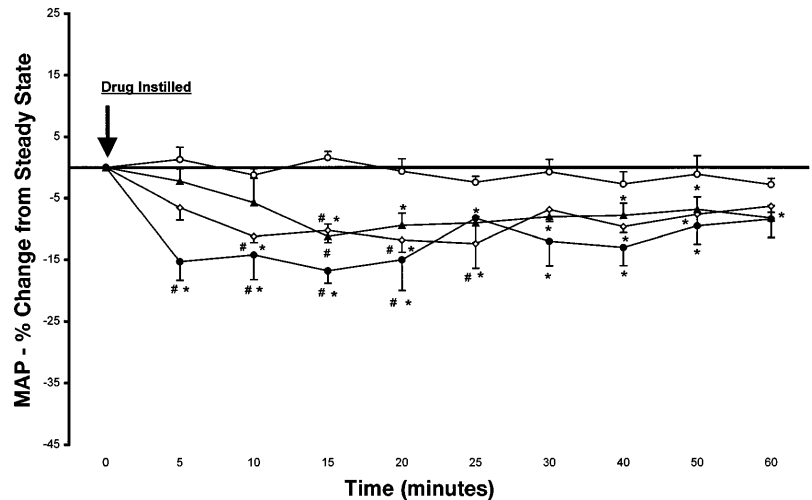


Fig. 3. Effect of intratracheal instillation of NO donor compounds on mean pulmonary arterial pressure (MPAP). Data are means \pm SE. Group 1: $n = 8$; control (\circ). Group 2: $n = 6$; sodium nitroprusside (SNP; \bullet). Group 3: $n = 6$; EP/NO (\blacktriangle). Group 4: $n = 6$; DMAEP/NO (\diamond). * $P < 0.05$ in comparison with steady-state pulmonary hypertension, by one-way repeated-measures ANOVA. # $P < 0.05$ in comparison with control group, by two-way repeated-measures ANOVA.

Fig. 4. Effect of intratracheal instillation of NO donor compounds on mean arterial pressure (MAP). Data are means \pm SE. Group 1: $n = 8$; control (\circ). Group 2: $n = 6$; SNP (\bullet). Group 3: $n = 6$; EP/NO (\blacktriangle). Group 4: $n = 6$; DMAEP/NO (\diamond). * $P < 0.05$ in comparison with steady-state pulmonary hypertension, by one-way repeated-measures ANOVA. # $P < 0.05$ in comparison with control group, by two-way repeated-measures ANOVA.



between the study groups. Methemoglobin levels remained $<0.4\%$ in all animals tested.

DISCUSSION

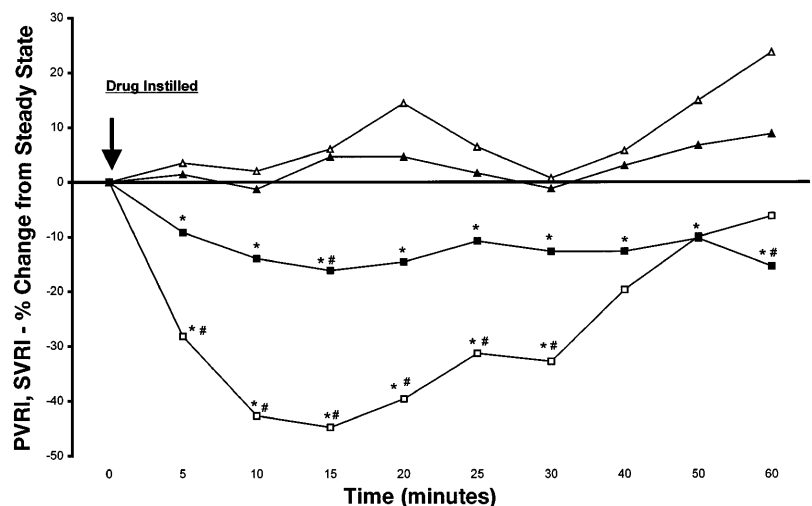
NONOates are compounds that spontaneously release NO, have vasorelaxant properties, and when administered intravenously can cause systemic and pulmonary vasodilation with concomitant hypotension (6, 46). Previous reports have suggested that these vasorelaxant properties may be related to the extent of NO release and to the structure of the carrier nucleophile (22, 25, 26). In an attempt to produce selective pulmonary vasodilation, we examined the effects of direct tracheal instillation of two chemically distinct, putreanine-based NONOates (EP/NO and DMAEP/NO) and compared them with a commonly used non-NONOate nitrovasodilator (SNP).

This is the first report describing the pulmonary and systemic hemodynamic effects of direct tracheal instillation of NONOates in aqueous form. Our results demonstrate that rapid intratracheal instillation of this class of compounds can significantly affect the pulmonary vasculature. Moreover, instillation of DMAEP/NO resulted in a selective and sustained de-

crease in the pulmonary hypertension induced by U-46619, without a clinically significant effect on systemic vascular resistance or systemic arterial pressure. Compared with the other NO donors tested, the effect of DMAEP/NO on pulmonary hypertension was both more selective and had a longer duration of action. The hemodynamic activity of all NO donors tested persisted well beyond the instillation period. This prolonged duration of action may obviate the need for continuous NO inhalation and allow for the intermittent administration of NO donors.

At the outset of this study, we postulated that selective pulmonary vasodilation might occur after delivery into the trachea of an NO donor with minimal mucosal permeability. Polar-charged compounds have limited transmucosal flux (7, 31, 32, 45). A NONOate with a polar-charged nucleophile structure might have limited mucosal transit, whereas the NO spontaneously released would easily diffuse into the local pulmonary vascular bed. The NO would then produce pulmonary vasodilation and become inactivated as it combined with hemoglobin (36, 37). We chose a choline analog ester of putreanine as the nucleophile for use in producing a NONOate because it is a naturally occurring

Fig. 5. Intratracheal instillation of EP/NO: effect on PVRI and SVRI. Data are means \pm SE. Group 1: $n = 8$; control-PVRI (\triangle); control-SVRI (\circ). Group 3: $n = 6$; EP/NO-PVRI (\blacklozenge); EP/NO-SVRI (\blacksquare). * $P < 0.05$ in comparison with steady-state pulmonary hypertension, by one-way repeated-measures ANOVA. # $P < 0.05$ in comparison with control group, by two-way repeated-measures ANOVA.



polyamine. Although little is known about the catabolism and elimination of polyamines, putrescine, as a breakdown product of spermidine, is a highly charged polyamine produced in the cytoplasm of all nucleated cells, which, when combined with a choline analog, forms the charged tertiary amine NONOate DMAEP/NO (4). This report demonstrates the selective pulmonary vasodilating effects of the charged tertiary amine NONOate, DMAEP/NO; however, additional work is necessary to examine the role of the NO carrier nucleophile in producing this pulmonary vasodilation, compared with other nonpolar, noncharged compounds.

Although both NONOates tested significantly reduced U-46619-induced pulmonary hypertension, a statistically significant decrease in MAP occurred at some time points after administration. This decrease was small, <10%. Further pharmacological study will be necessary to minimize this effect on systemic arterial blood pressure. For DMAEP/NO, there was no statistically significant effect on SVRI or CI; however, both parameters decreased below SS values. This decrease in SVRI and CI may have contributed to the reduction in MAP observed with DMAEP/NO. The significant decrease in SVRI noted after EP/NO administration may have been compensated by increased CI, resulting in a less pronounced decrease in MAP. SNP is commonly utilized as an intravenous vasodilator; however, this is the first demonstration of the sustained hemodynamic effects after intratracheal administration of this agent. Not unexpectedly, SNP administration resulted in significant and comparable decreases in both SVRI, PVRI, and MAP.

We are aware of only one other study testing the tracheal administration of NONOates. Hampl et al. (13) examined the efficacy of nebulized diethylenetriamine/NO (DETA/NO) in a rat model of chronic pulmonary hypertension. In their study, DETA/NO, a primary amine with a charged cationic end group, exhibited selective pulmonary vasodilation when aerosolized intratracheally. In contrast, the present report demonstrates that intratracheal administration of a NONOate with a charged cationic end group (DMAEP/NO) is a selective pulmonary vasodilator compared with a NONOate with an uncharged end group (EP/NO). These data support the hypothesis that the presence of a cationic end-group charge on the carrier nucleophile is important in conferring pulmonary vascular selectivity to the NONOate. We speculate that this pulmonary vascular selectivity is secondary to reduced respiratory epithelial permeability of the charged carrier nucleophile. This concept awaits further testing in an *in vitro* model. Further comparisons between our study and that of Hampl are limited because the models are different (acute vs. chronic, swine vs. rat, and liquid instillation vs. aerosol).

Multiple reports have described the selective pulmonary vasodilating effect of inhaled NO (8, 20, 28, 38, 50). Unfortunately, the delivery of NO by inhalation remains complex (14, 15, 47, 50). Because the vasorelaxant properties of inhaled NO are not sustained, continuous therapy is necessary (10, 18, 23, 40). Effective

administration requires specialized delivery equipment, including NO gas-containing tanks, a closed ventilator circuit, and NO and NO₂ monitoring devices to minimize health-care worker's exposure to these two gases (9, 12, 27). NO₂ is produced in the ventilator circuit as a by-product of NO and O₂ (29). It is yet unclear what effects this NO₂ may have on the lung; however, prolonged exposure to NO₂ results in decreased antioxidant activity, lipid peroxidation, and alveolar permeability in animals and humans (12, 35, 44). This report demonstrates that intratracheally delivered aqueous NONOates can have similar vasorelaxant properties compared with inhaled NO, without many of the aforementioned delivery problems. Gas-containing tanks and NO/NO₂ monitoring devices are not necessary with the use of NONOates as described in this report. Environmental exposure issues are minimized, and intermittent therapy by direct liquid instillation is possible. DMAEP/NO, with a duration of action of at least 1 h, is a promising agent, although further study is necessary to evaluate this and other NONOates with varying durations of action.

Some limitations to our study should be mentioned. The study was of short duration and did not address optimal dosing; therefore, duration of action and toxicity to the lung must be addressed in future investigations. Although this study demonstrates reduction in pulmonary hypertension, the effect of these compounds on oxygenation was not examined. The efficacy of these compounds in other forms of lung disease with pulmonary hypertension must also be investigated. Further work to elucidate the intrapulmonary distribution of these compounds is underway in our laboratory.

In summary, this report demonstrates that the intermittent intratracheal delivery of a charged polar tertiary amine NONOate selectively dilates the pulmonary vasculature with minimal systemic hemodynamic effects. NONOates delivered intermittently with minimal equipment and with potentially reduced risk to patients and health-care workers may be an alternative to continuously inhaled NO. This preliminary study demonstrates promise for the use of intratracheally delivered aqueous NONOates and is evidence that NONOates may have important therapeutic roles in the future management of pulmonary hypertension.

We thank Jennifer Giles for her work in the surgical preparation of the animals, Alvin Denenberg for help in preparing the pharmacological agents, and Dr. Edgar Ballard for his histological evaluation of the lung specimens.

This work was presented in part at the 26th Educational and Scientific Symposium of the Society of Critical Care Medicine, San Diego, CA, February 7, 1997.

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Received 10 March 1997; accepted in final form 31 July 1997.

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