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European Journal of Pharmacology 430 (2001) 311–315

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Short communication

Comparison of responses to novel nitric oxide donors in the feline pulmonary vascular bed

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Received 13 July 2001; received in revised form 7 August 2001; accepted 8 August 2001

Abstract

Pulmonary vascular responses to the novel diazeniumdiolate nitric oxide (NO) donors diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt, were investigated and compared in the intact-chest cat. Under conditions of controlled blood flow, when tone in the pulmonary vascular bed had been raised to a high steady level, intralobar injections of diethylamine/NO (0.3–10 μg), diethylenetriamine/NO (10–30 μg), spermine/NO (10–30 μg), sulfite/NO (10–30 μg), and angeli's salt (10–30 μg) caused dose-related decreases in lobar arterial pressure without changing left atrial pressure. In terms of relative vasodilator activity in the pulmonary vascular bed, the dose of the compounds that decreased lobar arterial pressure 4 mm Hg (ED_4 mm Hg) was significantly lower for diethylamine/NO compared to *S*-nitroso-*N*-acetylpenicillamine which was significantly less than diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt. The half-life of the vasodilator responses, as measured by 50% response recovery time, to diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt was similar for doses with similar magnitudes of vasodilation, while the half-life to *S*-nitroso-*N*-acetylpenicillamine was significantly less than the diazeniumdiolate NO donors. The present data demonstrate that the diazeniumdiolate NO donors diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt have potent but relatively short-lasting vasodilator activity in the pulmonary vascular bed of the cat. © 2001 Published by Elsevier Science B.V.

Keywords: Cat; Circulation; Diazeniumdiolate; Diethylamine/NO; Diethylenetriamine/NO; Spermine/NO; Sulfite/NO; Angeli's salt

1. Introduction

Endothelium-derived relaxing factor, actively known to be nitric oxide (NO) or a nitrosothiol derivative of nitric oxide, has been found to play an integral role in regulating a wide range of organ systems (Furchgott and Zawadski, 1980; Ignarro et al., 1981; Patel et al., 1999). A gaseous nitrogen monoxide radical, NO, serves as a second messenger in the central and peripheral nervous systems, regulation of blood pressure, platelet aggregation, cell proliferation, and modulation of myocardial contractility (Kelly et al., 1996; Kerwin et al., 1995; Marin and Rodriguez-Martinez, 1997). Many disease states, such as hypertension, angina, myocardial infarction, erectile dysfunction, and congestive heart failure, have been linked to alterations in NO (Bivalacqua et al., 2000; Marin and Rodriguez-Martinez, 1997). Administration of substances that release NO have been shown to be potent vasodilators and are mediated through the activation of soluble guanylyl cyclase and production of cyclic 3′, 5′-guanosine monophosphate (cGMP) leading to vasorelaxation (De Witt et al., 1994; Ignarro and Kadowitz, 1985; Lincoln, 1989). Nitroglycerin and sodium nitroprusside are examples of substances that release NO and induce relaxation of smooth muscle by increasing cGMP (Ignarro et al., 1991). Recently, a new class of NO donors has been shown to have vasodilator activity similar to that of nitroglycerin and sodium nitroprusside (Maragos et al., 1991). Diazeniumdiolates are NO/nucleophile adducts containing the structure $X[N(O)NO]^-$, where X represents a nucleophile residue and have been reported to generate bioactive NO without redox activation at predictable rates within a physiological pH in vitro (Maragos et al., 1991). These NO donors are thought to directly activate soluble guanylyl

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0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)01289-4

cyclase without requiring tissue activation by spontaneously releasing NO. However, the vasodilator activity of diazeniumdiolate NO donors and the time-course of the vasodilator response in vivo have not been fully investigated. Therefore, the present study was undertaken to compare the responses of the diazeniumdiolate NO donors, diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt, in the pulmonary vascular bed of the cat under conditions of controlled blood flow when tone in the bed had been raised to a high steady level.

2. Methods

Adult cats of either sex weighing 2.5–3.7 kg were sedated with ketamine hydrochloride (10–15 mg/kg i.m.) and were anesthetized with pentobarbital sodium (30 mg/kg i.v.). The animals were restrained in the supine position on a fluoroscopic table, and supplemental doses of anesthetic were administered as needed to maintain a uniform level of anesthesia. The trachea was intubated with a cuffed pediatric endotracheal tube, and the animals spontaneously breathed room air enriched with 95% O_2/5% CO_2. Systemic arterial (aortic) pressure was measured from a catheter inserted into the aorta from the femoral artery, and intravenous injections were made into a catheter positioned in the inferior vena cava from a femoral vein. For perfusion of the left lower lung lobe, a triple-lumen, 28 cm, 6F balloon perfusion catheter was passed under fluoroscopic guidance from an external jugular vein into the artery to the left lower lung lobe. After the animals had been heparinized (1000 U/kg i.v.), the lobar artery was vascularly isolated by distension of the balloon cuff on the perfusion catheter. The lobe was perfused with a perfusion pump (model 1210, Harvard Instruments) by way of the catheter lumen beyond the cuff with blood withdrawn from a femoral artery. Lobar arterial pressure was measured from a second catheter port 5 mm beyond the cuff on the perfusion catheter. The perfusion rate was adjusted so that lobar arterial perfusion pressure approximated mean pressure in the main pulmonary artery and was not changed thereafter. The flow rate ranged from 31 to 42 ml/min, and left atrial pressure was measured with a radiopaque 6F double-lumen catheter or a 6F radiopaque polyethylene catheter passed transseptally into the left atrium. Mean vascular pressures, measured with Spectromed DTX Plus transducers zeroed at the right atrial level, were recorded on a Grass model 7 recorder after characteristic waveforms had been confirmed. These procedures have been described previously (De Witt et al., 1994).

Diethylamine/nitric oxide, diethylenetriamine/nitric oxide, spermine/nitric oxide, sulfite/nitric oxide, and angeli's salt were synthesized as previously described (Keefer et al., 1996; Maragos et al., 1991; Saavedra et al., 1996). Their molecular weights are 155, 163, 280, 176, and 140 g/mol, respectively. Each agent was dissolved in 10 mM NaOH; this alkaline stock solution was then diluted with phosphate buffered saline (PBS; pH 7.4), and the dosing

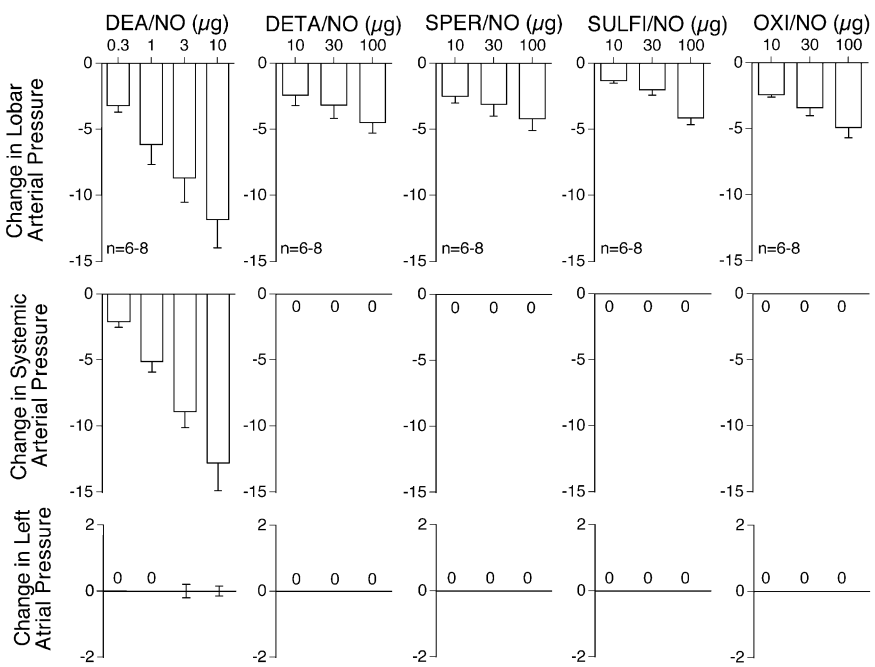

Fig. 1. Bar graph comparing changes in lobar arterial pressure, systemic arterial pressure and left atrial pressure in response to diethylamine/NO (DEA/NO), diethylenetriamine/NO (DETA/NO), spermine/NO (SPER/NO), sulfite/NO (SULFI/NO), and angeli's salt (OXI/NO). The compounds were injected into the perfused lobar artery when baseline pressure in the perfused lobar artery was raised to a high steady value (32–38 mm Hg) with an infusion of U-46619; *n* indicates number of experiments. Changes in left atrial pressure ranged from −0.2 to 0 mm Hg. All pressures measured in mm Hg.

solution was administered immediately after preparation into the perfused lobar artery. The solvents for these drugs had no significant effect on baseline vascular pressure or on responses to the vasoactive agents. *S*-nitroso-*N*-acetylpenicillamine was dissolved in normal saline. All solutions were stored in a freezer in amber bottles. The NO donor solutions were prepared fresh for every experiment and all other working solutions were prepared on a frequent basis and kept on crushed ice during an experiment. Diethylamine, diethylenetriamine, spermine, and ammonium sulfite in the absence of an attached NO moiety were also investigated. All agents were injected into the perfused lobar artery in fixed small volumes, and injections of the compounds were randomized. The thromboxane A_2 mimic, U-46619 (9,11 dideoxy-11α,9α-epoxymethano prostaglandin $F_{2\alpha}$, Sigma, St. Louis, MO) was dissolved in 100% ethanol at a concentration of 10 mg/ml and was diluted in 0.9% saline. The thromboxane A_2 mimic was then infused into the perfused lobar artery with a Harvard infusion pump at rates (50–320 ng/min) required to raise lobar arterial pressures to values of 32–38 mm Hg.

Arterial blood gases and pH were measured with a Corning model 178 analyzer and were in the normal range. All hemodynamic data are expressed in absolute units and are presented as mean ± S.E. Responses represent peak changes, and data were analyzed using a one-way analysis of variance and Scheffe's *F*-test or a paired *t*-test (Snedecor and Cochran, 1980). A P value of less than 0.05 was used as the criterion for statistical significance.

3. Results

Responses to intravenous injections of diethylamine/NO (0.3–10 μg), diethylenetriamine/NO (10–100 μg), spermine/NO (10–100 μg), sulfite/NO (10–100 μg), and angeli's salt (10–100 μg) were compared in the pulmonary vascular bed of the cat, and these results are summarized in Fig. 1. Under conditions of controlled blood flow, when lobar arterial pressure was at normal resting conditions, injections of the NO donors produced no significant change in lobar arterial pressure (data not shown). When tone in the pulmonary vascular bed was raised to a high steady level with a constant infusion of U-46619, injections of the diazeniumdiolate class of NO donors into the perfused lobar artery produced dose-related decreases in lobar arterial pressure without altering left atrial pressure (Fig. 1). Diethylamine/NO decreased systemic arterial pressure when injected into the perfused lobar artery in doses of 3–10 μg, whereas the other diazeniumdiolates had no systemic effect (Fig. 1). Diethylamine, diethylenetriamine, spermine, and ammonium sulfite in the absence of an attached NO moiety produced no significant change in lobar arterial pressure (data not shown). In terms of relative vasodilator activity in the pulmonary vascular bed, when doses of the NO donors required to decrease lobar arterial pressure 4 mm Hg (ED_4 mmHg) [diethylamine/NO 2.5 nmol, *S*-nitroso-*N*-acetylpenicillamine 7.2 nmol, diethylenetriamine/NO 380 nmol, spermine/NO 380 nmol, sulfite/NO 590 nmol, angeli's salt 380 nmol] were compared, diethylamine/NO was more potent than *S*-nitroso-*N*-acetylpenicillamine and significantly more potent than diethylenetriamine/NO, spermine/NO, angeli's salt which were not significantly dif-

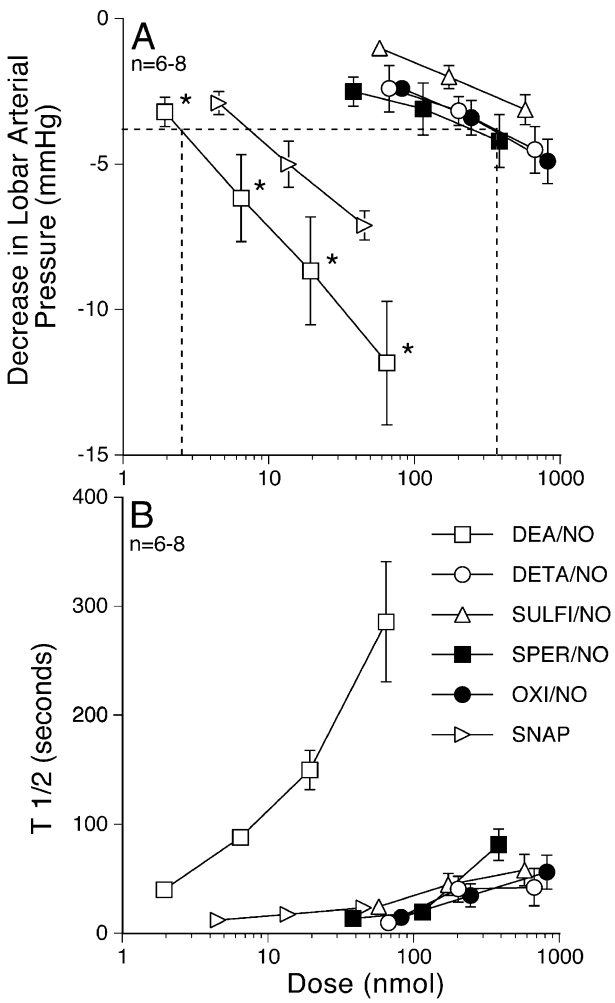

Fig. 2. (A) Dose–response curves comparing decreases in lobar arterial pressure in response to diethylamine/NO (DEA/NO), diethylenetriamine/NO (DETA/NO), spermine/NO (SPER/NO), sulfite/NO (SULFI/NO), and angeli's salt (OXI/NO). The dashed vertical lines represent the approximate dose required to decrease lobar arterial pressure 4 mm Hg (ED_4 mm Hg). The asterisk indicates that the responses to all four doses of diethylamine/NO were significantly greater ($P < 0.05$) than the responses to diethylenetriamine/NO, spermine/NO, and angeli's salt. In addition, the responses to all three doses of sulfite/NO were significantly less than the response to the same doses of diethylenetriamine/NO, spermine/NO, and angeli's salt. (B) Dose–response curves showing the half-life ($T_{1/2}$) of the decrease in lobar arterial pressure in response to diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt. The compounds were injected into the perfused lobar artery in small volumes in a randomized sequence, while baseline pressure in the perfused lobar artery was raised to a high steady level (32–38 mm Hg) with an infusion of U-46619; *n* indicates the number of experiments.

ferent from each other (Fig. 2A). Sulfite/NO, was significantly less potent than diethylamine/NO, *S*-nitroso-*N*-acetylpenicillamine, diethylenetriamine/NO, spermine/NO and angeli's salt (Fig. 2A).

The half-lives ($T_{1/2}$), as measured by 50% response recovery time, of the decreases in lobar arterial pressure in response to diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, angeli's salt and *S*-nitroso-*N*-acetylpenicillamine are compared in Fig. 2B. The $T_{1/2}$ of the vasodilator responses to diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt was similar for doses with similar magnitudes of vasodilator responses (ED_4 mm Hg; Fig. 2B), while the $T_{1/2}$ of the vasodilator response to *S*-nitroso-*N*-acetylpenicillamine was significantly less than the diazeniumdiolates investigated.

4. Discussion

Results of the present investigation demonstrate that the diazeniumdiolate NO donors diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt cause dose-related decreases in lobar arterial pressure when tone in the pulmonary vascular bed was raised to a high steady level. Inasmuch as blood flow and left atrial pressure were unchanged, the decreases in lobar arterial pressure reflect decreases in pulmonary lobar vascular resistance. In terms of relative vasodilator activity in the pulmonary vascular bed, the dose of diethylamine/NO required to decrease lobar arterial pressure 4 mm Hg (ED_4 mm Hg) was significantly smaller than the ED_4 mm Hg for *S*-nitroso-*N*-acetylpenicillamine, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt.

These results are consistent with previous observations demonstrating a high potency of diethylamine/NO in vivo as well as in vitro (Diodati et al., 1993). The larger decrease in pulmonary and systemic arterial pressure by diethylamine/NO when injected into the perfused lobar artery is most likely the result of both its faster rate of NO release ($T_{1/2} = 2$ min) and its large amount of NO released. Diethylamine/NO is reported to release 1.5 mol of NO per diazeniumdiolate group, while diethylenetriamine/NO and spermine/NO each release 2, angeli's salt releases 0.5, and sulfite/NO releases 0 (Keefer et al., 1996); the $T_{1/2}$ values of the NO donors diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt in aqueous media are 2 min, 20 h, 39 min, 7 min, and 2 min, respectively (Keefer et al., 1996). Combining these data as previously described for an in vitro setting (Maragos et al., 1991), the expected relative potencies of the agents studied here can be seen to agree well with the present observations. The larger decrease in pulmonary and systemic arterial pressure by diethylamine/NO cannot be explained by an effect of the nucleophile residue, as diethylamine did not alter pulmonary arterial pressure.

Compounds that are now known to release NO have been utilized clinically for decades (Janero, 2000). Nitroglycerin and sodium nitroprusside have long been used to treat angina. The effects of these organic nitrates are mediated through the enzymatic and non-enzymatic release of NO. It has been suggested that thiol-containing intermediates may be involved in the activation of organic nitrates (Ignarro et al., 1981, 1991). *S*-nitroso-*N*-acetylpenicillamine is an example of a nitrosothiol derivative of NO. The results of the present investigation support the concept that *S*-nitroso-*N*-acetylpenicillamine is a potent vasodilator in the pulmonary vascular bed and demonstrate the recovery half-life of *S*-nitroso-*N*-acetylpenicillamine was significantly less than the diazeniumdiolates investigated (De Witt et al., 1994).

Diazeniumdiolates containing the $[N(O)NO]^-$ functional group are seeing increasing use both as biomedical research tools and as lead compounds for potential clinical applications (Fitzhugh and Keefer, 2000). A primary basis for their utility is that many of them decompose spontaneously at predictable rates in aqueous media to release the critical bioregulatory species, NO (Fitzhugh and Keefer, 2000). In contrast to differences in vasodilator potency, there was not a significant difference in the $T_{1/2}$ response of the diazeniumdiolates in the pulmonary vascular bed of the cat, as measured by the vasodilatory response return to 50% control value, at doses which produce similar vasodilation (ED_4 mm Hg). The reason for this is unknown. The observation of a pronounced systemic effect of diethylamine/NO, but not of any of the other agents is also difficult to explain, but it is not without precedent. Aerosolized diethylamine/NO showed a larger depression in systemic arterial pressure than sodium nitroprusside in an ovine model of U-46619-induced pulmonary hypertension (Adrie et al., 1998). Similar experiments comparing an anionic and zwitterionic (i.e., no net charge) diazeniumdiolate showed that only the former caused a reduction in systemic blood pressure (Brilli et al., 1997); diethylamine/NO's systemic effect in the present experiments may be a result from its obligatorily anionic character.

Changes in the structure of the carrier nucleophile residue have been reported to alter the NO release parameters (Keefer et al., 1996; Maragos et al., 1991). Sulfite/NO, which contains the same $[N(O)NO]^-$ functional group as the other NO donors used in the present study, was the least potent of the diazeniumdiolates investigated. This result is consistent with previous observations demonstrating a decrease in release of NO from the sulfite/NO moiety (Morley et al., 1993).

Substances that release NO have been shown to be potent vasodilators and are mediated through the activation of soluble guanylyl cyclase and production of cyclic 3′, 5′-guanosine monophosphate (cGMP) in the pulmonary

vascular bed (Cheng et al., 1994; De Witt et al., 1997; Ignarro and Kadowitz, 1985). The observation that diazeniumdiolates have dilator activity in the pulmonary vascular bed of the cat also lends support to the concept that NO may play a role in regulating vascular tone. However, recently the mechanism of vasodilatory action of diazeniumdiolates has been debated (Homer et al., 1999; Homer and Wanstall, 2000). In rat pulmonary artery, spermine/NO was shown to have relaxation that was mediated, in part, by activation of Na^+/K^+-ATPase, sarcoendoplasmic reticulum, Ca^{2+}-ATPase, Ca^{2+}-activated K^+ channels and independent of cGMP. These data suggests that spermine/NO, and perhaps other diazeniumdiolates, may produce vasodilation by multiple mechanisms; however, this mechanism does not appear to be mediated by the nucleophile residue, as each of the nucleophiles used as controls did not alter pulmonary vascular tone. Therefore, the mechanism of action of diazeniumdiolate NO releasing substances in the feline pulmonary vascular bed requires further investigation.

In conclusion, the results of the present investigation demonstrate that diethylamine/NO, diethylenetriamine/NO, spermine/NO, sulfite/NO, and angeli's salt have potent vasodilator activity in the pulmonary vascular bed of the cat. Furthermore, intralobar injections of diethylamine/NO produced dose-dependent decreases in systemic arterial pressure. These data suggest that diazeniumdiolates may be useful adducts in the treatment of vascular disorders sensitive to NO.

Acknowledgements

This work was supported by a grant from the American Heart Association-Louisiana Affiliate and National Institutes of Health grant HL-62000.

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