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# Dual impact of a nitric oxide donor, GEA 3175, in human pulmonary smooth muscle

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**Abstract**

Nitric oxide (NO) donors could constitute an alternative to inhaled NO as treatment in some patients with pulmonary hypertension. Therefore, the present study investigated the relaxation mechanisms of a novel NO donor, 3-(3-chloro-2-methylphenyl)-5-[[4-methylphenyl)sulphonyl]amino]-)hydroxide (GEA 3175) in segments of human pulmonary arteries and bronchioles, which were mounted in microvascular myographs. GEA 3175 induced concentration-dependent relaxations and was more potent in pulmonary arteries than in bronchioles. A blocker of soluble guanylyl cyclase, 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ), and iberiotoxin, a blocker of large-conductance calcium-activated K channels, both reduced relaxations induced by GEA 3175 in pulmonary arteries and bronchioles. Combining of ODQ and iberiotoxin did not produce additional inhibition. GEA 3175 relaxation is mediated through guanylyl cyclase-dependent mechanisms followed by activation of large-conductance calcium-activated $K^+$ channels. The dilatation of both pulmonary small arteries and airways by GEA 3175 seems advantageous, if it is considered administered as inhalation therapy for pulmonary hypertension.


*Keywords:* Human pulmonary artery; Human bronchiole; Nitric oxide donor; Soluble guanylyl cyclase; Large-conductance calcium-activated potassium channel

## 1. Introduction

Inhaled nitric oxide (NO) has been shown to be a selective pulmonary vasodilator with minimal systemic effects (Frostell et al., 1991), and is an applicable treatment in some types of pulmonary hypertension (Cuthbertson et al., 1997). Some of the disadvantages of authentic gaseous NO administration are that it has a short half-life and therefore requires continuous administration. Moreover, specialized equipment is required to avoid exposure of patients to toxic levels of NO and nitrogen dioxide, a toxic by product of NO. These factors may limit the use of inhaled NO gas, especially in groups of patients who require long-term treatment (Cuthbertson et al., 1997). New approaches to modulate the NO cyclic guanosine monophosphate (GMP) pathway either by administration of L-arginine or inhibition of phosphodiesterase type 5 with sildenafil have shown promising results for treatment of pulmonary hypertension (Morris et al., 2003; Sastry et al., 2004). In addition to NO, a range of nitrosothiols as well as sodium nitroprusside and authentic NO proved to be more potent than traditional vaso- and bronchodilators such as pinacidil and theophylline (Gaston et al., 1994; Wanstall et al., 1997b), and a few studies have shown a positive effect of NO donors in man (Haraldsson et al., 1998; Palhares et al., 1998; Lee et al., 2001). Therefore, NO donors may also serve as possible alternatives to NO gas in the treatment of pulmonary hypertension.

Soluble guanylyl cyclase has been established as the main mediator of NO-induced broncho- and vasodilatation in human systemic arteries and proximal airways (Corompt et al., 1998; Lovren and Triggle, 2000; Yuan et al., 1996). In human bronchi, both cyclic GMP-dependent and independent mechanisms have been shown in 3-mopholinosydnoni-

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mine (SIN-1) and S-nitroso-*N*-acetylcysteine (SNAP)-evoked relaxations (Janssen et al., 2000), whereas the involvement of large-conductance calcium-activated $K^+$ channels in bronchodilatation is more controversial (Corompt et al., 1998). The involvement of different $K^+$ channels is particularly important in relation to pulmonary hypertension, because certain $K^+$ channels which are normally present in large amounts in human pulmonary artery smooth muscle cells (Peng et al., 1996) have been shown to be negatively affected in pulmonary hypertension (Peng et al., 1997, 1998). It is therefore important to establish which type of K channel the NO donor affects.

Most studies addressing the effect of NO or NO donors have been carried out in either animal tissue (rat, guinea pig) or in proximal arterial (Vaali et al., 1996; Wanstall et al., 1997a) and bronchial preparations (Paakkari et al., 1995; Johansson et al., 1997; Vaali et al., 1996), and no studies have compared the two types of pulmonary smooth muscle from humans. However, when NO is administered by inhalation, both airways and pulmonary arteries are affected alike (Cases et al., 1996; Schindler et al., 1995). Finally, pharmacodynamic behaviour differs between the proximal pulmonary arteries and airways and the more distally located resistance arteries and bronchioles (Chopra et al., 1994), that contribute to the resistance in the pulmonary circulation and in the bronchial tree. Therefore, the present study aimed to investigate the relaxation mechanisms of a novel NO donor, GEA 3175, and compare the effect with that of authentic NO in isolated human pulmonary resistance arteries and bronchioles.

## 2. Methods

### 2.1. Tissue preparation, dissection and mounting

Human lung tissues were obtained from patients (16 males and 15 females) who had undergone surgery for lung carcinoma at the Department of Thoracic Surgery, Aarhus University Hospital, Skejby, Aarhus. Mean age of the patients examined was 66.5 years (51–79 years), and all individuals were smokers. The research has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and the Local Ethics Committee approved the study. Each patient was informed about the purpose and nature of the project and gave informed consent.

Bronchioles and pulmonary arteries were isolated from identical locations within the tissue, dissected free from adjoining connective tissue and lung parenchyma, placed in physiological salt solution (for composition see below) and maintained at 4 °C. All tissues were used within 3–12 h postsurgery. Segments of the bronchioles and pulmonary arteries (2 mm long) were mounted on two wires with a diameter of 40 μm in microvascular myographs for isometric tension recording as earlier described (Elmedal et al., 2004; Hernandez et al., 1998). The viability of the bronchial preparations was examined by exposing the bronchioles initially twice and at the end of each experiment once to potassium-rich physiological salt solution, which was physiological salt solution with KCl exchanged for NaCl on an equimolar basis giving a final concentration of 125 mM $K^+$. Preparations in which the final response to 125 mM $K^+$ was reduced compared to the 2 initial responses to 125 mM $K^+$ were excluded from the study. The pulmonary arteries were stretched to a passive load of 2.4 kPa (18 mm Hg), exposed twice to 125 mM $K^+$, and subsequently the endothelial function was examined by a contraction to the thromboxane analogue, U46619 ($10^{-8}$ M) and relaxation to acetylcholine ($10^{-5}$ M). Preparations in which the acetylcholine-induced relaxation was less than 50% of initial precontraction level were excluded. The rest of the protocol was performed in normal physiological salt solution containing a cyclooxygenase inhibitor, indomethacin ($3\times10^{-6}$ M), with the aim of preventing the development of basal spontaneous tonus due to prostaglandins.

### 2.2. Experimental protocols

Initially, bronchiolar preparations were contracted to histamine ($10^{-5}$ M), acetylcholine ($3\times10^{-4}$ M), or U46619 ($10^{-8}$ M). For subsequent experiments, arterial and bronchiolar preparations were contracted with U46619 ($10^{-8}$ M), and when the tone was stable, concentration relaxation curves were obtained for the nitric oxide donor, GEA 3175 ($10^{-10}$ to $10^{-5}$ M), the $\beta_2$ adrenoceptor agonist, salbutamol ($10^{-10}$ to $10^{-4}$ M), and authentic nitric oxide (NO, $10^{-10}$ to $10^{-5}$ M). To investigate the mechanisms involved in the GEA 3175- and NO-induced relaxations a first concentration relaxation curve was constructed, the bath solution changed several times, and the preparation allowed to equilibrate for 30 min before they were incubated for another 30 min with either vehicle, a soluble guanylyl cyclase inhibitor, ODQ ($3\times10^{-6}$ M), a blocker of large-conductance calcium-activated $K^+$ channels, iberiotoxin ($10^{-8}$ M), or the combination of ODQ ($3\times10^{-6}$ M) and iberiotoxin ($10^{-8}$ M), respectively. Then contraction levels were matched by increasing the concentrations of U46619 stepwise from $10^{-9}$ up to $10^{-8}$ M, and a second concentration–response curve for either NO or GEA 3175 was constructed.

In order to ensure that distal airways were used, histological confirmation of the absence of cartilage and glands was performed in a series of the first preparations used.

### 2.3. Drugs and solutions

The pulmonary arteries and bronchioles were dissected, mounted, and held relaxed in physiological salt solution of the following composition (mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $NaHCO_3$ 25, $KH_2PO_4$ 1.18, glucose 5.5, $CaCl_2$ 2.5 and ethylenediaminetetraacetic acid (EDTA) 0.026. $Ca^{2+}$ free solution had the same composition as physiological salt solution except that $CaCl_2$ was replaced with EGTA (0.1 mM).

The following drugs were used: acetylcholine hydrochloride, histamine dihydrochloride, iberiotoxin, salbutamol hemisulphate, indomethacin, and 9,11-dideoxy-9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619) were obtained from Sigma (U.S.A.); GEA 3175 (3-(3-chloro-2-methylphenyl)-5-[[4-methylphenyl)sulphonyl]amino]-)hydroxide) was obtained from GEA Ltd. (Copenhagen, Denmark); 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ) and iberiotoxin was supplied by Tocris Cookson (MO, USA). All drugs were dissolved in distilled water except for U46619, which was dissolved in 96% ethanol, and GEA 3175 and ODQ

which were dissolved in dimethyl sulphoxide (DMSO). The solvents, ethanol and DMSO, did not in the concentrations used influence the contractile state of the preparations. NO solutions were prepared as a saturated water solution as previously described and administered through gastight syringes (Hernandez et al., 1998).

### 2.4. Analysis of data

The mechanical responses of preparations in myographs were measured as changes in force and expressed as wall tension, $\Delta T$, which is the increase in measured force, $\Delta F$, divided by twice the segment length (Mulvany and Halpern, 1976). Relaxations are expressed as percentage of U46619 contraction just before construction of the concentration–relaxation curves. By using a computer program (GraphPad, Institute for Scientific Information, San Diego, CA, USA), the concentration–response curves to the different relaxant agents were fitted to the classical "Hill-equation": $R/R_{max}=A(M)^n/(A(M)^n+EC_{50}(M)^n)$, where $R/R_{max}$ is the relative response to the effective concentration of drug, $A(M)$, and $EC_{50}(M)$ is the concentration of agonist required to give half maximal inhibition ($R_{max}$) of the precontraction, when $A(M)$ and $EC_{50}(M)$ are given in molar concentrations. $n$ is a curve fitting parameter or "Hill-coefficient". $EC_{50}$ values are expressed as the negative log molar concentration, $pD_2=-\log(EC_{50})$.

The results are expressed as mean±S.E.M. Means of multiple groups were compared by one-way analysis of variance (ANOVA) followed by Bonferroni post-tests. The concentration–response curves were evaluated by calculation of area under curve followed by one-way ANOVA, and in case of significance $t$-tests were applied with Bonferroni correction for number of comparisons. Probability levels less than 5% were considered significant.

## 3. Results

### 3.1. GEA 3175- and NO-evoked relaxation of pulmonary arteries

Subsequent to equilibration of the human pulmonary arteries (internal normalized diameter 742±41 μm, $n=28$) to a passive tension of 2.4±0.1 kPa (18 mm Hg), 125 mM $K^+$ contracted the preparations to 2.0±0.2 N/m. Acetylcholine ($10^{-5}$ M) relaxed U46619-contracted preparations 79±3% ($n=28$).

In human isolated pulmonary arteries contracted with U46619 ($10^{-8}$ M), GEA 3175, slowly induced relaxations, which reached a plateau after 3–6 min (Fig. 1A). In contrast, NO-induced relaxations reached a peak value after 10–20 s (Fig. 1B). GEA 3175 and NO both evoked concentration-dependent relaxations (Fig. 2A, B). GEA 3175 was more potent than NO, while salbutamol even at the highest concentration caused less than 50% relaxation. The maximal relaxation induced by the compounds was of the following order: GEA 3175=NO>salbutamol (Table 1).

In pulmonary arteries, an inhibitor of soluble guanylyl cyclase, ODQ ($3\times10^{-6}$ M), and iberiotoxin ($10^{-8}$ M), an inhibitor of the large-conductance calcium-activated $K^+$ channels, did not change basal tone, and U46619-induced contraction was similar in the presence of ODQ, iberiotoxin, and the combination of ODQ and iberiotoxin (data not shown). Both ODQ and iberiotoxin significantly reduced GEA 3175 relaxation, but there was no additive effect of a combination of ODQ and iberiotoxin (Fig. 3A), although a minor ODQ- and iberiotoxin-insensitive GEA 3175 relaxation remained (Fig. 3A).

NO-induced relaxation of pulmonary arteries was significantly reduced in the presence of ODQ at the lower concentrations of NO ($10^{-8}$ to $10^{-6}$ M) whereas the highest concentration of NO ($10^{-5}$

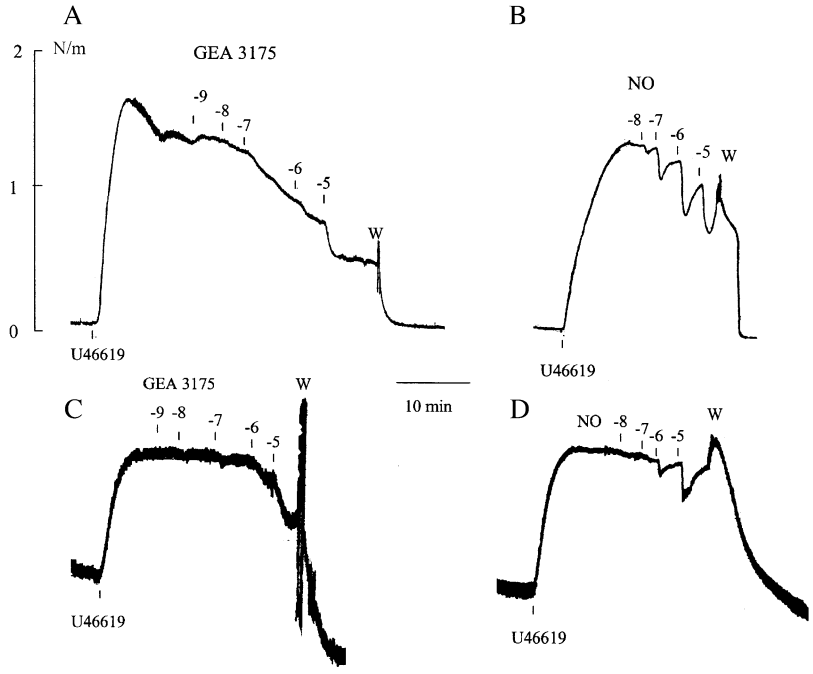


Fig. 1. Effect of GEA 3175 and authentic nitric oxide on human isolated pulmonary arteries and bronchioles. Isometric force recordings in an isolated human pulmonary artery with an effective lumen diameter of 548 μm (A, B) and a human bronchiole of 989 μm (C, D). Addition of U46619 ($10^{-8}$ M) and concentration-dependent relaxations evoked by (A, C) GEA 3175 and (B, D) NO. Vertical bar shows tension in N/m and horizontal bar time in minutes. W—wash out.

M) was insensitive to ODQ. Also iberiotoxin reduced the NO relaxation in this preparation. Combining ODQ and iberiotoxin had no additive effect. An ODQ- and iberiotoxin-insensitive component persisted for the highest concentrations of NO (Fig. 3B).

### 3.2. GEA 3175- and NO-induced relaxation of bronchioles

Transverse sections of the human bronchioles showed that the epithelium represented a major component and in media sparse, i.e., 1–2 layers, circularly orientated smooth muscle were observed ($n=4$). Subsequent to equilibration of human bronchioles (internal normalized lumen diameter 1110±87 μm), 125 mM $K^+$ contracted the preparations by 0.6±0.1 N/m ($n=27$).

As was the case for the pulmonary arteries, GEA 3175 also evoked slowly developing relaxations, while NO-induced relaxations were faster in human bronchioles (Fig. 1C, D). In U46619-contracted bronchioles, GEA 3175 induced concentration-dependent relaxations with $pD_2$-values and maximal relaxations of, respectively, 5.34±0.36 and 63±7% ($n=27$). In the same preparations contracted with acetylcholine ($3\times10^{-4}$ M) or histamine ($10^{-5}$ M) to, respectively, 1.1±0.2 N/m ($n=5$) and 1.4±0.2 N/m ($n=4$), GEA 3175-evoked maximal relaxations were lowered to, respectively, 14±5% and 20±18%. In U46619-contracted preparations only GEA 3175 caused more than 50% relaxation (Table 1).

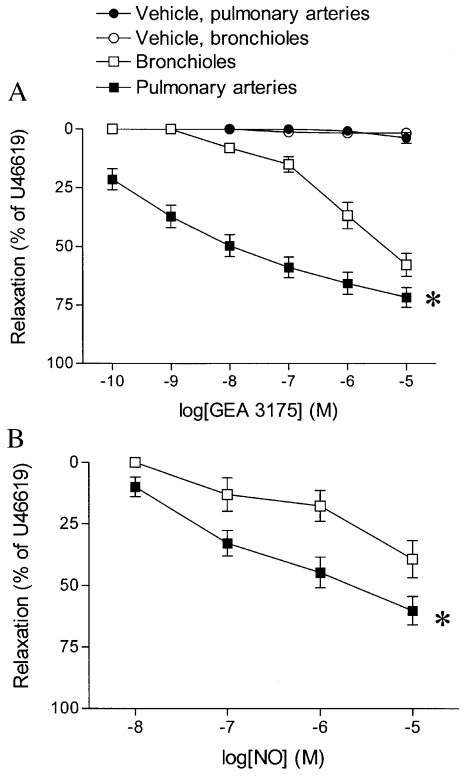


Fig. 2. Average relaxations induced by (A) GEA 3175, vehicle for GEA 3175, dimethyl sulphoxide (DMSO), and (B) NO in pulmonary arteries and bronchioles. The relaxations are expressed as percentage of U46619-induced contraction. Results represent means and vertical lines S.E.M. of 21–27 preparations. Differences in area under concentration–response curves were evaluated by one-way analysis of variance (ANOVA) followed by Students $t$-test. *$P<0.05$, concentration–response curves for GEA 3175 and NO in pulmonary arteries were significantly different from corresponding curves in bronchioles.

Table 1
Relaxations induced by GEA 3175, NO or salbutamol in U46619-contracted human pulmonary arteries and bronchioles

| | $n$ | Contraction (N/m) | $pD_2$ | $R$ (%) |
|---|---|---|---|---|
| *Pulmonary arteries* | | | | |
| GEA 3175 | 28 | 1.34±0.2 | 8.90±0.26 | 71.7±4.2 |
| NO | 22 | 1.47±0.2 | 6.98±0.20* | 60.2±5.8 |
| Salbutamol | 6 | 0.83±0.2 | ND | 24.2±6.3*,** |
| *Bronchioles* | | | | |
| GEA 3175 | 27 | 1.25±0.3 | 5.34±0.36 | 62.5±6.6 |
| NO | 15 | 0.59±0.1 | ND | 39.2±7.6 |
| Salbutamol | 6 | 0.68±0.1 | ND | 32.3±11.5 |

Values are mean±S.E.M. of $n$ number of preparations examined. Differences were evaluated by one-way analysis of variance (ANOVA) followed by a posteriori Bonferroni $t$-test in case of significance. $R$ (%) is the relaxation obtained at the highest concentration applied of each drug: GEA 3175 $10^{-5}$ M, NO $10^{-5}$ M, salbutamol $10^{-4}$ M. ND—not determined.
* $P<0.05$ compared to GEA 3175.
** $P<0.05$ compared to NO.

In U46619-contracted preparations, GEA 3175 was significantly more potent in human pulmonary arteries compared to human bronchioles ($P<0.05$, Students $t$-test, $n=27$, Table 1), whereas the maximally induced relaxations in both preparations were similar (Fig. 2A). NO-induced relaxations were also more pronounced in human pulmonary arteries than in bronchioles, but with similar maximal relaxations (Fig. 2B, Table 1).

In bronchioles, incubation with ODQ, iberiotoxin, or the combination of ODQ and iberiotoxin only inhibited the response to the highest concentration of GEA 3175 (Fig. 3C). Relaxation of human bronchioles evoked by the highest concentration of NO was significantly reduced in the presence of ODQ, but it was not changed in the presence of iberiotoxin. Combining ODQ and iberiotoxin equaled the effect of ODQ alone (Fig. 3D).

## 4. Discussion

The present study shows that GEA 3175, a novel NO donor, slowly induces potent long-lasting relaxations of isolated human pulmonary arteries and bronchioles, which were more potent than authentic NO and salbutamol, and significantly more potent in human pulmonary arteries than in bronchioles. In pulmonary arteries, both GEA 3175- and NO-induced relaxations appear to involve a soluble guanylyl cyclase-dependent opening of large-conductance calcium-activated $K^+$ channels.

Compounds designed for treating diseases of the lung such as pulmonary hypertension are best given by inhalation, thereby minimizing systemic side-effects. There is a close functional relation between bronchioles and pulmonary arteries. Thus, arterial rings from patients with a positive bronchodilator test exerted hyperresponsiveness to contractile agonists compared to control preparations (Cases et al., 1996), and both bronchial

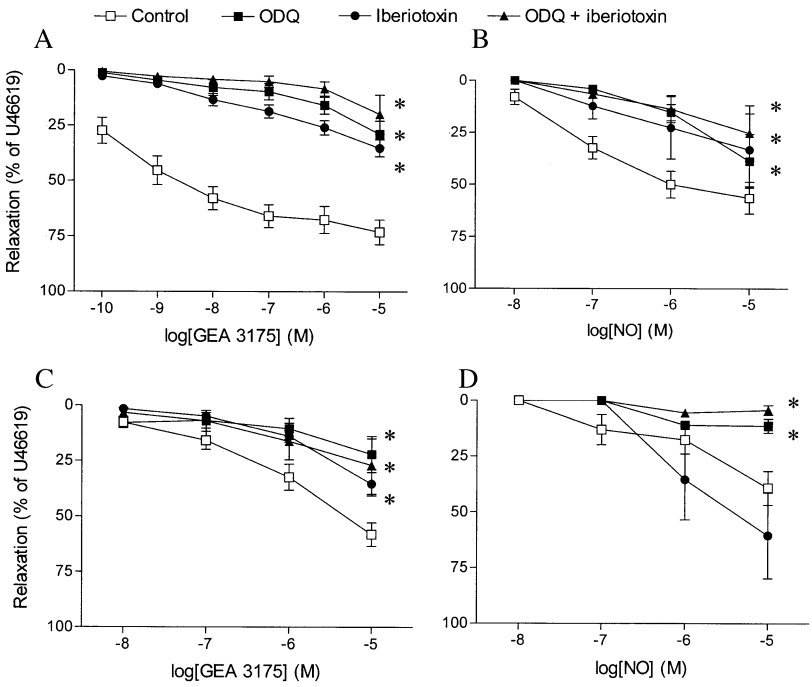


Fig. 3. ODQ and iberiotoxin abolish GEA 3175-induced relaxations of human isolated pulmonary arteries. Concentration–relaxation curves for (A) GEA 3175 and (B) NO in human isolated pulmonary arteries, and (C) GEA 3175 and (D) NO in human isolated bronchioles in the absence ($n=14-17$) and in the presence of ODQ ($3\times10^{-6}$ M, $n=7-8$), iberiotoxin ($10^{-8}$ M, $n=4-9$), or the combination of ODQ and iberiotoxin ($n=7-8$). Results represent mean±S.E.M. of $n$ preparations. Differences in area under concentration–response curves were evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni method: $^{*}P<0.05$ vs. vehicle control.

and vascular smooth muscle from patients suffering from pulmonary hypertension exhibited hypertrophy (Schindler et al., 1995). These studies suggest a close functional correlation between the two types of smooth muscle. NO donors may improve or worsen ventilation-perfusion matching depending on the relative relaxant effect in airway and arterial smooth muscle. We found that GEA 3175 relaxed both pulmonary arteries and bronchioles with a more pronounced effect on the arteries. If the compound was administered by inhalation, this profile would be advantageous with respect to ventilation–perfusion matching, as the major effect would be seen on the circulation, but also less ventilated areas would be opened. The preparations examined in our study were isolated from the peripheral airways with accompanying pulmonary artery. The nature of the bronchial preparations was established using histological examination. The present approach constitutes a technique where the distal resistance parts of the bronchial tree and pulmonary circulation can be examined, and allows evaluation of the selectivity towards either airway or arterial smooth muscle for a given compound.

Authentic NO has previously been shown to induce more potent relaxations in arterial than in airway smooth muscle. Most previous work has, however, been carried out in animal (rat or guinea pig) or in proximal airway (bronchial and tracheal) preparations (Johansson et al., 1997; Vaali et al., 1996; Paakkari et al., 1995; Wanstall et al., 2001). In the present study, we found that whereas GEA 3175 was highly selective towards pulmonary arteries, authentic NO showed a less favourable effect towards the pulmonary arteries. The slower and longer lasting nature of GEA 3175 induced relaxations may be beneficial, as a constant delivery of the compound may not be necessary as it is presently customary with inhaled NO, and in addition possibly toxic peak effects may be avoided.

In most studies, NO and NO donor-induced effects are mediated through activation of soluble guanylyl cyclase (Cohen et al., 1999). In the present study, we found that GEA 3175-evoked vaso- and bronchorelaxations as well as NO-evoked bronchorelaxations were inhibited by ODQ. GEA 3175 has been shown to increase cyclic GMP content of guinea pig trachea and human platelets (Correll et al., 1994; Kankaanranta et al., 1996). Other structurally related GEA NO donors have been demonstrated to induce relaxations of guinea pig and rat tracheal preparations through a cyclic GMP-dependent involvement of iberiotoxin sensitive $K^{+}$ channels (Vaali et al., 1996). Taken together, these data indicate that GEA 3175-evoked vaso- and bronchorelaxation as well as NO-evoked bronchorelaxation is mediated through a soluble guanylyl cyclase-dependent pathway.

NO has also been shown to induce cyclic GMP-independent vasorelaxation (Bolotina et al., 1994). We found that relaxations of pulmonary arteries to high concentrations of authentic NO were unaffected by

ODQ. It could be argued that either the concentration of ODQ or the incubation time applied was insufficient to inhibit NO-evoked vasorelaxations. In previous studies, we found that the incubation time and concentration of ODQ used in this study was sufficient to inhibit both GEA 3175- and NO-evoked relaxations of bovine isolated bronchioles, and increasing the concentration of ODQ had no additional effect (Hernandez et al., 1998). As GEA 3175-evoked vaso- and bronchorelaxations as well as NO-evoked bronchorelaxations were inhibited by this application of ODQ, this contradicts the hypothesis that soluble guanylyl cyclase inhibition was ineffective. However, in addition to NO which is considered an electrically neutral free radical as it possess an unpaired electron, different NO donors also generate different NO related species such as an oxidized form ($NO^+$) and an a reduced form ($NO^-$) of NO (Feelisch and Stamler, 1996). Moreover, it was found that $NO^+$ induced cyclic GMP-independent relaxation of airway smooth muscle probably through calcium-dependent activation of $K^+$ channels, whereas NO evoked cyclic GMP-dependent relaxation without changes in intracellular calcium (Janssen et al., 2000). However, it is unlikely that the observed difference between GEA 3175- and NO-evoked vasorelaxation depends on different NO species released, as oxatriazole derivatives apparently only release NO in their decomposition process (Feelisch and Stamler, 1996; Holm et al., 1998). Therefore, the findings suggest that relaxations evoked by high concentrations of authentic NO can probably be ascribed to different kinetics and/or amounts of NO release. In the present study, authentic NO-induced relaxations were more rapid and transient compared to the relaxations induced by GEA 3175 in bovine bronchioles. In contrast to authentic NO, GEA 3175 requires the presence of tissue for release of NO, and therefore, these findings suggest that different kinetics and/or mechanisms of NO release by the two compounds play a role for the signal transduction.

Large-conductance calcium-activated $K^+$ channels have been identified in human pulmonary arterial smooth muscle cells, and NO has been shown to activate this channel through cGMP-dependent protein kinase mediated phosphorylation (Peng et al., 1996; Snetkov and Ward, 1999; Miura et al., 1992). In the present study, we found a consistent effect of iberiotoxin on GEA 3175-evoked vaso- and bronchorelaxations as well as NO-evoked vasorelaxations. Combining ODQ and iberiotoxin had no additive effect. In a previous study, we found that neither glibenclamide, an inhibitor of ATP-sensitive $K^+$ channels, nor apamin, an inhibitor of small conductance calcium-activated $K^+$ channels, had any effect on GEA 3175- and NO-evoked relaxations in bovine bronchioles, whereas iberiotoxin consistently inhibited GEA 3175- and NO-evoked bronchorelaxations (Hernandez et al., 1998). Taken together, these data indicate that GEA 3175 vaso- and bronchorelaxation as well as NO-evoked vasorelaxation involve soluble guanylyl cyclase-dependent activation of large-conductance calcium-activated $K^+$ channels. Human pulmonary hypertension has been associated with a reduced amount and altered reactivity of another type of $K^+$ channel, the voltage-dependent $K^+$ channels ($K_v$ channels). Exerting relaxations through the large-conductance calcium-activated $K^+$ channels therefore seems an advantageous aspect of GEA 3175 when considering its potential use in treating pulmonary hypertension.

The present investigation suggests that the slow releasing NO donor, GEA 3175, induces potent relaxations of both isolated human pulmonary arteries and bronchioles. GEA 3175 and NO relaxations are mediated through cyclic GMP-dependent mechanisms, which in case of GEA 3175 also lead to activation of large-conductance calcium-activated $K^+$ channels. The dual impact of GEA 3175 on both pulmonary arteries and bronchioles, favouring the arteries, could be advantageous when administered by inhalation, although in vivo studies are needed in order to elucidate the therapeutic potential of GEA 3175 in pulmonary hypertension.

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