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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 (3×10^{-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 (3×10^{-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 β_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 (3×10^{-6} M), a blocker of large-conductance calcium-activated K^+ channels, iberiotoxin (10^{-8} M), or the combination of ODQ (3×10^{-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