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Differential Relaxant Responses of Guinea-pig Lung Strips and Bronchial Rings to Sodium Nitroprusside: A Mechanism Independent of cGMP Formation

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Abstract

The biochemical mechanism subserving smooth muscle relaxant effects of sodium nitroprusside was examined on U46619, 9,11-dideoxy-9α,11α-methanoepoxy $PGF_{2\alpha}$, precontracted guinea-pig lung strips and hilar bronchial rings.

Lung strips were resistant to the relaxant action of sodium nitroprusside or sodium nitrite ($NaNO_2$), whereas they markedly relaxed to 8-bromo-cyclic GMP (8-Br-cGMP), a membrane permeable analogue of cGMP. Precontracted bronchial rings completely relaxed to sodium nitroprusside, $NaNO_2$, or 8-Br-cGMP in a concentration-dependent manner. Sodium nitroprusside (10 μM) substantially raised tissue cGMP level in lung strips. Conversely, sodium nitroprusside had no detectable effect on cGMP levels in bronchial rings. In the presence of 10 μM dipyridamole, an agent which preferentially inhibits cGMP-specific phosphodiesterase, cGMP levels in lung strips treated with sodium nitroprusside was significantly enhanced, but sodium nitroprusside demonstrated no relaxant effect on the preparations. However, dipyridamole potentiated sodium nitroprusside-induced precontracted bronchial ring relaxation without affecting the bronchial tissue cGMP level. In the presence of 10 μM LY83583 (6-anilino-5,8-quinolinedione), a specific cGMP concentration-lowering agent, sodium nitroprusside-mediated elevation of cGMP level in lung strips was significantly reduced with no effect on the functional response. LY83583 demonstrated no inhibitory effect on either relaxation or cGMP level in bronchial rings treated with sodium nitroprusside.

Our results suggest that precontracted smooth muscle in lung strips and in hilar bronchi respond distinctly to sodium nitroprusside. Furthermore, sodium nitroprusside mediates bronchial smooth muscle relaxation by mechanisms unrelated to cGMP.

Sodium nitroprusside is a well-known vascular smooth muscle relaxant that generates nitric oxide (NO) which subsequently mediates cGMP formation (Ignarro & Kadowitz 1985). Studies on the pharmacologic effect of sodium nitroprusside or related nitro-compounds on intrapulmonary bronchi or lung parenchymal strips are relatively scant (Dale & Obianime 1985; Gruetter & Lemke 1985). Predominant findings with sodium nitroprusside on airway smooth muscle were obtained from trachealis (Gruetter et al 1989; Jansen et al 1992) and a few from hilar bronchus (Gruetter et al 1989). The present study stemmed from our previous work (Wong et al 1992a) showing that U46619-precontracted guinea-pig lung strips were completely relaxed by theophylline, papaverine or salbutamol, but resistant to sodium nitroprusside. In contrast, sodium nitroprusside totally reversed U46619-precontracted guinea-pig pulmonary artery and hilar bronchus (Wong et al 1992a). Dale & Obianime (1985) showed that sodium nitroprusside had little effect on histamine contraction of guinea-pig lung strips and actually increased the phorbol myristate acetate-induced spasm. Other studies demonstrated that sodium nitroprusside or organic nitrate such as glyceryl trinitrate, exhibited diminishing relaxant effects on bovine airway preparations as the airway sizes decreased from trachea, hilar bronchus, intrapulmonary bronchus to lung strips (Gruetter & Lemke 1985; Gruetter et al 1989). These studies did not concomitantly measure lung tissue cGMP levels after sodium nitroprusside treatment. Although there is substantial evidence supporting a central role of cGMP as the relaxant mediator of sodium nitroprusside, findings contrary to or inconsistent with this putative mechanism have also been reported (Nakatsu & Diamond 1989; Clapp & Gurney 1991; Bolotina et al 1994).

The purpose of the present study was to determine whether the inability of lung strips to relax to sodium nitroprusside is related to tissue levels of cGMP. Guinea-pig hilar bronchi were also studied to provide comparative effects of sodium nitroprusside on a different region of the airway tree.

Materials and Methods

Preparations of lung tissues

Male Hartley guinea-pigs (Charles River, Portage, MI), 400–500 g, were killed by CO_2 asphyxiation and subsequent decapitation. After thoracotomy, heart and lung were excised en bloc and perfused with 50 mL Krebs-bicarbonate solution via the pulmonary artery. Lung strips were isolated along the distal edge of the lower lung lobes. Bronchial rings (~3 mm in length) were obtained from the hilar bronchi and cleaned of any parenchyma. Both lung strips and bronchial rings were then suspended isometrically under an optimum resting load of 1 g and 2 g, respectively, in

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organ baths containing 10 mL Krebs-bicarbonate solution aerated with 95% O_2–5% CO_2 at 37°C of the following composition (mM): NaCl, 118·2; KCl, 4·6; $NaHCO_3$, 24·8; $CaCl_2 \cdot 2H_2O$, 2·5; KH_2PO_4, 1·2; $MgSO_4 \cdot 7H_2O$, 1·2 and dextrose 10·0. Contractile responses were monitored using force-displacement transducers (Grass FT-03) coupled to a Grass model 7E polygraph recorder (Grass Instrument Co., Quincy, MA).

Functional studies

After equilibration, lung strips and bronchial rings were contracted twice with 60 mM KCl; an interval of 60 min was interposed between the two responses. After repeated washing, active tension returned to baseline level. Tissues were then contracted with 1 μM U46619 (9,11-dideoxy-9α,11α-methanoepoxy $PGF_{2\alpha}$), a thromboxane A_2 (TXA_2) mimetic. This concentration was previously shown to elicit an 80% maximal contraction in lung parenchymal strips and generate a sustained contraction for more than 90 min (Wong et al 1992a). After the contraction had reached a plateau, lung strips were relaxed with either sodium nitroprusside (1 nM-100 μM), sodium nitrite ($NaNO_2$, 1 μM-100 mM), or 8-bromo-cyclic GMP (8-Br-cGMP, 1 μM-3 mM) in increasing concentrations. To help determine the role of endogenous cGMP, sodium nitroprusside-mediated relaxation was examined in the presence and absence of 10 μM dipyridamole, an inhibitor of cGMP-specific phosphodiesterase (Lugnier et al 1986; Torphy & Undem 1991), or 10 μM LY83583 (6-anilino-5, 8-quinolinedione), a specific cGMP concentration lowering agent (Schmidt et al 1985; Diamond 1987; Mulsch et al 1988). Relaxation was expressed as percent maximum tension of the U46619-induced peak contraction of the lung tissues.

cGMP measurements

Lung strips and bronchial rings were precontracted with 1 μM U46619 and, at the plateau, relaxed by a single concentration of sodium nitroprusside (10 μM). Lung strips were removed from the organ baths at 1, 5, 10, and 15 min after sodium nitroprusside treatment, and immediately frozen between a pair of metal clamps precooled in dry ice. Bronchial rings were removed and frozen at 1, 2, 5, and 10 min after sodium nitroprusside. Relaxation was expressed as percent U46619-induced peak contraction.

To evaluate the effect of phosphodiesterase activity on sodium nitroprusside-induced cGMP formation and relaxation in U46619-precontracted lung tissues, bronchial rings and lung strips were incubated with 10 μM dipyridamole for approximately 30 min before addition of 1 μM and 10 μM sodium nitroprusside, respectively. A lower concentration of sodium nitroprusside was chosen for bronchial rings because, in our preliminary study, a substantial potentiation of relaxation in dipyridamole-treated bronchial rings was observed. To examine the cGMP-lowering effect of LY83583 on sodium nitroprusside-mediated cGMP formation and relaxation in lung tissues, lung strips and bronchi were pre-incubated with 10 μM LY83583 for 30 min before the administration of 10 μM sodium nitroprusside. Lung strips and bronchi were immediately frozen at 10 min and 5 min, respectively, after sodium nitroprusside addition, and stored at −70°C until assay. They were then homogenized in 1 mL 5% trichloroacetic acid and centrifuged at 3000 *g* for 30 min. Aliquots (100 μL) of each supernatant fraction were acetylated with acetic anhydride in the presence of 4 M KOH, and determined for acetylated-cGMP level using enzyme immunoassay (EIA) (Pradelles et al 1989). The precipitable fraction was used for protein assay. Protein concentration was determined with the bicinchoninic acid protein assay (Pierce Chemical Co., Rockford, IL) using bovine serum albumin as standard. cGMP concentration was expressed as fmol cGMP (mg protein)$^{-1}$.

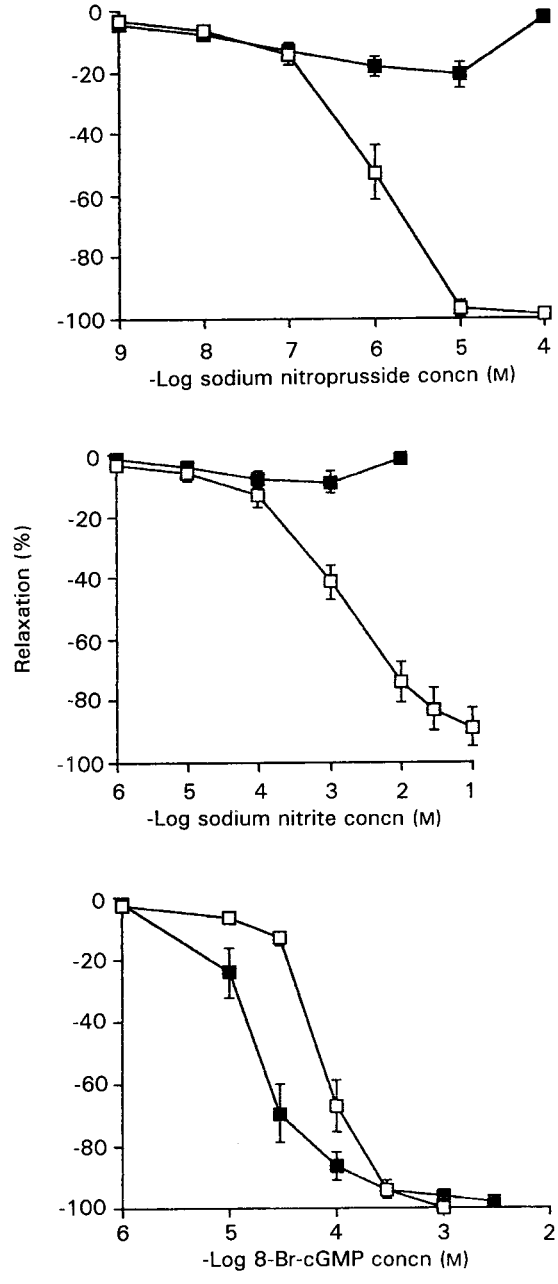

FIG. 1. Log concentration-relaxation curves of bronchial rings (□) and lung strips (■) precontracted with 1 μM U46619 in response to sodium nitroprusside, sodium nitrite and 8-bromo-cGMP. Results are expressed as percent reduction of peak contraction. Each point represents the mean ± s.e.m. of 5–7 animals.

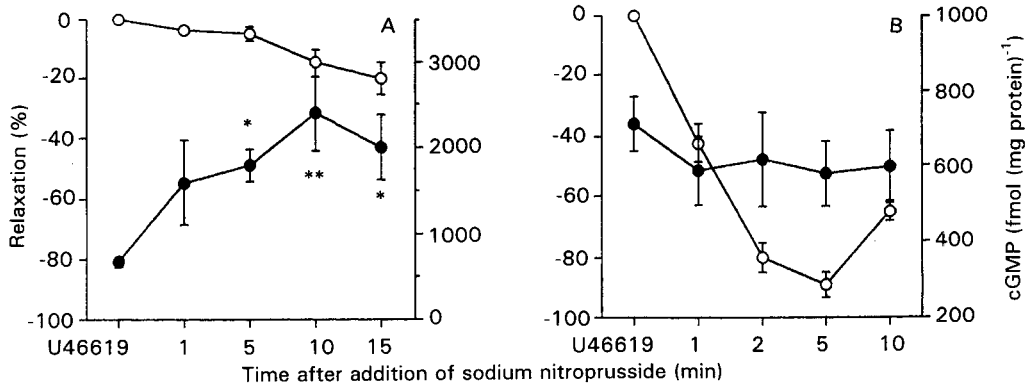

FIG. 2. Time course study of effects of 10 μM sodium nitroprusside on relaxation (○) and cGMP (●) formation in 1 μM U46619-precontracted lung strips (A) or bronchial rings (B). Relaxation was expressed as percent reduction of peak contraction. Each point represents the mean ± s.e.m. of 4–6 animals. Significant differences in cGMP levels after sodium nitroprusside treatment are indicated as * $P < 0.05$ and ** $P < 0.01$.

Data analysis

All data are presented as mean ± s.e.m. EC50 values were determined at 50% maximal responses for each smooth muscle relaxant using a 4-parameter logistics model of nonlinear regression analysis (De Lean et al 1978) and expressed as -log molar EC50. Statistical differences among means were analysed using one-way analysis of variance. Significance was determined at a level of $P < 0.05$.

Materials

Sodium nitroprusside, 8-bromoguanosine 3′,5′-cyclic monophosphate, U46619, dipyridamole, and bovine serum albumin were purchased from Sigma Chemical Co. (St Louis, MO, USA), sodium nitrite from J. T. Baker Chemical Co. (Phillipsburg, NJ), acetic anhydride and trichloroacetic acid from EM Science (Gibbstown, NJ), bicinchoninie protein assay reagent from Pierce Chemical Co. (Rockford, IL), and LY83583 from Eli Lilly and Co. (Indianapolis, IN). cGMP EIA kits were obtained from Cayman Chemical Co. (Ann Arbor, MI). Dipyridamole was dissolved in ethanol and U46619 in methyl acetate, and they were further diluted in distilled water. Final concentration of ethanol was 0·05%. All other reagents were dissolved in distilled water.

Results

Lung strips and bronchial rings contracted to 60mM KCl with peak tension reaching 0·49 ± 0·01 g (n = 25) and 2·89 ± 0·12 g (n = 28), respectively. Contraction of the lung strips and the bronchial rings evoked by 1 μM U46619 was 67% and 86%, respectively, of their corresponding maximal KCl response. U46619 produced sustained contraction of these airway preparations for at least 60 min with negligible decline in tension (Wong et al 1992a). Fig. 1 demonstrates that sodium nitroprusside or $NaNO_2$ slightly relaxed (10–20%) U46619-precontracted lung strips; whereas, 8-Br-cGMP totally reversed lung strip contraction in a concentration-dependent manner with a-log molar EC50 value of 4·8 ± 0·2. On the other hand, sodium nitroprusside, $NaNO_2$, or 8-Br-cGMP completely relaxed U46619-evoked bronchial ring smooth muscle contraction with -log molar EC50 values of 6·0 ± 0·1, 2·8 ± 0·1, and 4·1 ± 0·1, respectively.

Time course studies showed that, whereas 10 μM sodium nitroprusside exhibited minimal relaxant effect on U46619-precontracted lung parenchymal strips, tissue cGMP levels in the lung strips were significantly elevated ($P < 0.01$) with a peak level of 2·4 pmol (mg protein)$^{-1}$ at 10 min as compared with 0·63 pmol (mg protein)$^{-1}$ before sodium nitroprusside (Fig. 2A). Bronchial rings relaxed by 40% in 1 min and 90% in 5 min in response to 10 μM sodium nitroprusside; however, tissue cGMP levels remained constant at about 0·6 pmol (mg protein)$^{-1}$ throughout the exposure to this relaxant (Fig. 2B).

To examine the effect of a cGMP phosphodiesterase inhibitor on sodium nitroprusside-induced airway smooth muscle relaxation and cGMP levels, lung strips and bronchial rings were pre-incubated with 10 μM dipyridamole

Table 1. Effects of sodium nitroprusside on relaxation and cGMP levels in U46619-precontracted guinea-pig bronchial rings[a].

	Tension (%)			cGMP (fmol (mg protein)$^{-1}$)		
Treatments	Control	Dipyridamole	LY83583	Control	Dipyridamole	LY83583
Basal				392·1 ± 33·8	441·0 ± 39·0	416·7 ± 64·0
U46619	100	100	100	409·9 ± 30·1	475·9 ± 66·8	357·9 ± 63·9
U46619 + sodium nitroprusside (1 μM)	73·0 ± 3·5	20·3 ± 4·8*		338·4 ± 32·6	484·7 ± 67·5	
U46619 + sodium nitroprusside (10 μM)	14·8 ± 5·4		23·1 ± 4·8	499·3 ± 28·8		483·7 ± 40·0

[a] Bronchial rings were frozen at 5 min after sodium nitroprusside addition (see Materials and Methods).
* $P < 0.001$ indicates significant difference from the corresponding control. Each value represents the mean ± s.e.m. of 4–5 animals.

Table 2. Effects of sodium nitroprusside on relaxation and cGMP levels in U46619-precontracted guinea-pig lung strips[a].

Treatments	Tension (%)			cGMP (fmol (mg protein)$^{-1}$)		
	Control	Dipyridamole	LY83583	Control	Dipyridamole	LY83583
Basal				1185·7 ± 198·6	2608·8 ± 499·9	1153·3 ± 156·1
U46619	100	100	100	1473·6 ± 154·5	2886·5 ± 954·0	820·8 ± 65·9
U46619 + sodium nitroprusside (10 μM)	93·7 ± 1·8	88·9 ± 3·6	93·8 ± 1·0	3288·7 ± 537·3°	8301·3 ± 973·2†	1360·1 ± 37·1*

[a] Lung strips were frozen at 10 min after sodium nitroprusside addition (see Materials and Methods). * $P < 0.001$ indicates significant difference from the corresponding control. ° $P < 0.05$ and † $P < 0.001$ denote significant differences in cGMP levels after sodium nitroprusside treatment. Each value represents the mean ± s.e.m. of 4–5 animals.

before addition of U46619 and sodium nitroprusside. The concentration of dipyridamole used has been shown to have a minimum effect on cAMP levels whereas it substantially inhibited the cGMP-specific phosphodiesterase activity in isolated bovine aorta (Lugnier et al 1986). Dipyridamole alone did not affect the contractile responses of lung strips and bronchial rings to 1 μM U46619. The agent did, however, potentiate bronchial ring smooth muscle relaxation to 1 μM sodium nitroprusside by sixfold as compared with control with -log molar EC50 values shifting from 5·9 ± 0·1 to 6·7 ± 0·1. Despite this enhanced ($P < 0.001$) relaxation, dipyridamole did not facilitate a sodium nitroprusside-mediated elevation of cGMP (Table 1). Conversely, dipyridamole did not potentiate sodium nitroprusside-induced relaxation of lung strips whereas tissue cGMP levels were substantially elevated ($P < 0.001$, Table 2).

Pretreatment with LY83583 at a concentration shown to attenuate cGMP production in guinea-pig lung fragments by at least 50% (Schmidt et al 1985), did not attenuate the relaxant effect of sodium nitroprusside on bronchial rings (data not shown). Moreover, LY83583 did not alter the bronchial tissue cGMP levels in response to sodium nitroprusside (Table 1). Nevertheless, LY83583 significantly impaired tissue cGMP levels in lung strips in response to sodium nitroprusside by 49% ($P < 0.001$). Sodium nitroprusside exhibited no relaxant effect on precontracted lung strips either with or without LY83583 (Table 2).

Discussion

The present investigation examined the relationship between relaxation and tissue cGMP levels induced by sodium nitroprusside on guinea-pig lung strips and hilar bronchial rings. Our results show that U46619-precontracted lung strips were resistant to the relaxant action of sodium nitroprusside or $NaNO_2$. Sodium nitroprusside previously failed to relax lung strips precontracted with KCl, histamine or phorbol myristate acetate (Dale & Obianime 1985; Gruetter & Lemke 1985). Hence, the inability of sodium nitroprusside to relax precontracted lung strips is independent of the agonist used to contract the tissue. Other studies reported that sodium nitroprusside or glyceryl trinitrate exhibited gradual reduction of relaxing efficacy on the airway smooth muscle as the airway sizes decreased from trachea, hilar bronchi, intrapulmonary bronchi to lung parenchyma (Gruetter & Lemke 1985; Gruetter et al 1989). Since U46619-precontracted lung strips were completely relaxed by increasing concentrations of exogenously applied 8-Br-cGMP, a membrane permeable analogue of cGMP, the lack of relaxant response of lung strips to sodium nitroprusside could not be explained by potential unresponsiveness of this tissue to endogenously formed cGMP. In contrast, lung strip cGMP levels were significantly increased in response to sodium nitroprusside, confirming the stimulatory action of this NO-releasing agent on guanylate cyclase and subsequent formation of cGMP (Ignarro & Kadowitz 1985). Lack of correlation between relaxation and cGMP elevation was further observed in lung strips pre-incubated with dipyridamole, or LY83583. Although the former potentiated and the latter attenuated sodium nitroprusside-mediated elevation of cGMP formation in lung strips, sodium nitroprusside failed to relax the precontracted lung strips with or without either of the inhibitors. Dissociation between relaxation and tissue cGMP elevation in response to sodium nitroprusside or glyceryl trinitrate has also been observed in rat myometrium, rat vas deferens and guinea-pig taenia coli (Nakatsu & Diamond 1989). Recent studies identified layers of smooth muscle in the lung pleura which contribute substantially to the net contraction of guinea-pig lung strips to U46619, histamine, ovalbumin, A23187 (Wong et al 1992a, b), and platelet activating factor (Halonen et al 1990). The increase in lung strip cGMP in response to sodium nitroprusside is probably due to cellular sources other than pleural smooth muscle such as alveolar epithelium, interstitial contractile cells, on the microvasculature since the pleural smooth muscle clearly was not relaxed by sodium nitroprusside.

Unlike the lung strips, U46619-precontracted bronchial rings were effectively relaxed by either sodium nitroprusside or $NaNO_2$, which is consistent with our previous findings (Wong et al 1992a). These results imply intrinsic pharmacologic differences of airway smooth muscle preparations isolated from different regions of the lung (Gruetter & Lemke 1985; Gruetter et al 1989; Wong et al 1992a, b). Similar to the effect observed with lung strips, 8-Br-cGMP exhibited complete reversal of the contraction of guinea-pig hilar bronchial rings in a concentration-dependent manner. Relaxation of the precontracted bronchial rings to sodium nitroprusside was not accompanied by elevation of tissue cGMP levels. This finding suggests a signal-transducing molecule other than cGMP as being responsible for sodium nitroprusside-induced relaxation of the bronchial

smooth muscle. Bolotina et al (1994) has recently shown that NO can relax vascular smooth muscle by directly activating calcium-dependent potassium channels without requiring cGMP. Sodium nitroprusside has been shown to lower cytosolic Ca^{2+} by inhibiting Ca^{2+} influx and promoting Ca^{2+} uptake into the sarcoplasmic reticulum (Clapp & Gurney 1991). Our data did not reveal an increase in bronchial tissue cGMP level in the presence of dipyridamole, despite a marked potentiation of relaxation of U46619-precontracted bronchial rings to sodium nitroprusside. Moreover, bronchial rings were relaxed by sodium nitroprusside to the same extent in the presence and absence of LY83583, and tissue cGMP levels were not affected by LY83583. These results further suggest a relaxation mechanism for sodium nitroprusside independent of cGMP.

In conclusion, precontracted bronchial rings, but not lung strips, markedly relaxed in response to sodium nitroprusside; whereas cGMP levels in lung strips but not in bronchial rings rose in response to sodium nitroprusside. The dissociation between relaxation and cGMP formation manifested in lung strips and bronchial smooth muscle exposed to sodium nitroprusside implies a relaxation mechanism other than cGMP formation (Nakatsu & Diamond 1989; Clapp & Gurney 1991; Bolotina et al 1994).

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