Soluble guanylate cyclase-dependent relaxation is reduced in the adult rat bronchial smooth muscle

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Soluble guanylate cyclase-dependent relaxation is reduced in the adult rat bronchial smooth muscle. Am J Physiol Lung Cell Mol Physiol 292: L699–L703, 2007. First published November 17, 2006; doi:10.1152/ajplung.00108.2006.—Cyclic nucleotides are relaxants of the airway smooth muscle, yet most of the available data were obtained in adult animals. The expression and activity of cyclases have been reported to be developmentally regulated in the lung, and little is known about the age-related changes in their bronchial muscle relaxation potential. We evaluated and compared the newborn and adult rat bronchial smooth muscle response to cyclic AMP- and GMP-dependent agonists in isometric mounted bronchial rings. In acetylcholine-precontracted bronchial muscle, the relaxant response to the cAMP agonist forskolin was not age dependent, but the relaxant response to the nitric oxide (NO) donor sodium nitroprusside (SNP) was significantly greater (P < 0.01) in the newborn. To further evaluate the cGMP pathway, we stimulated the soluble guanylate cyclase (sGC) with the specific agonists BAY 41-2272 and YC-1. In keeping with the SNP dose-response curves, the sGC agonists significantly relaxed the newborn, but not the adult bronchial muscle. Protein expression of the sGC α1- and β1-subunits were significantly lower (P < 0.01) in the adult compared with the newborn bronchial tissue. Consistent with these results, the NO-stimulated sGC activity was significantly greater in the newborn compared with the adult (P < 0.01). In conclusion, the bronchial smooth muscle cGMP-, but not cAMP-dependent, relaxant response is developmentally regulated and significantly reduced in the adult rat. bronchodilation; lung; cAMP; cGMP; salbutamol

DEVELOPMENTALLY, it is well known that the airway muscle is capable of contraction at any stage of postnatal life, and bronchoconstriction is observed even in premature neonates. It has been suggested that childhood asthma is in relaxation potential (8). Limited maturational data regarding the airway smooth muscle relaxant response are presently available, and therapeutically, bronchodilators are often chosen based on their proven efficacy later in life.

The main cyclic nucleotides involved in airway smooth muscle relaxation are the cAMP and cGMP. Commonly therapeutically used β2-adrenoceptor agonists act via cAMP to induce bronchodilation (12). The rat tracheal smooth muscle relaxant response to the adenylate cyclase stimulator forskolin does not change with age (11). Yet, the β-adrenergic receptor density in the rat lung is developmentally regulated and progressively increases from fetal to postnatal life (22).

cGMP-mediated airway smooth muscle relaxation is present in several, if not all mammalian animal species, and nitric oxide (NO) is considered to be a weak bronchodilator based on data derived from adult animal and human studies (12). This contrasts with the important role of NO in the regulation of pulmonary vascular smooth muscle tone and the pathogenesis of pulmonary hypertension (23). The extent to which the limited NO-dependent bronchodilation relate to maturational differences in the protein expression and activity of soluble guanylate cyclase (sGC) merits further investigation.

Therefore, the purpose of the present study was to evaluate the newborn and adult bronchial cAMP- and cGMP-modulated relaxation response in the rat. A significant age-dependent difference for the latter, but not former, was documented that correlated with the higher expression of the two isoforms and greater activity of the sGC in the newborn rat bronchial tissue.

METHODOLOGY

Newborn aged 2–5 days (n = 22) and adult (2–4 mo old; n = 18) Sprague-Dawley rats were used for this study (Charles River). The animals were killed with an overdose of pentobarbital sodium (50 ml/kg ip), the chest open and the lungs removed for immediate dissection. The left lung intralobar fourth (newborn) or fifth (adult) generation bronchi were identified and dissected free from the surrounding lung tissue. The isolated bronchi were either mounted fresh for the functional studies or fast-frozen for further processing for sGC protein extraction. This protocol followed the guidelines for humane experimentation with animals and was approved by the Hospital for Sick Children Animal Care Committee.

Functional studies. The airway rings (average diameter 70–100 μm, and length = 2 mm) were mounted on a wire myograph (Danish Myo Technology). Isometric changes were digitized and recorded online (Myodaq, Danish Myo Technology). Tissues were bathed in Krebs–Henseleit buffer (115 mM NaCl, 25 mM NaHCO3, 1.38 mM NaHPO4, 2.51 mM KCl, 2.46 mM MgSO4·7H2O, 1.91 mM CaCl2, and 5.56 mM dextrose), bubbled with air/6% CO2 and maintained at 37°C.

After 1 h of equilibration, the optimal resting tension of the tissue was determined by repeated stimulation with 128 mM KCl until maximum active tension was reached. All subsequent force measurements were obtained at optimal resting tension. Contractile responses were normalized to the tissue cross sectional area as (width × diameter) × 2, expressed as milli-Newtons per square millimeter, as previously described (3).

To study the relaxation response, the airways were precontracted with acetylcholine (EC75 for newborn and adult tissue = 10−5 M). This resulted in a stable contraction for up to 45 min. The relaxant agonist response was expressed as a percentage...
change from the initial precontracted force and a second order polynomial curve line fitted around the concentration-specific data. The NO-dependent relaxation was evaluated with the donor sodium nitroprusside. The sGC stimulation was induced with the BAY 41-2272 and YC-1 compounds. To test the cAMP-mediated relaxation, we used forskolin.

All drugs were dissolved in Krebs-Henseleit solution, except for the YC-1 in DMSO. All drugs were purchased from Sigma (Sigma, Oakville, Ontario, Canada), except for YC-1 (Cayman Chemical).

Preparation of bronchi homogenates. The frozen bronchi were minced in liquid nitrogen and homogenized using a Teflon pestle of a Potter-Elvehjem homogenizer with 10 strokes in 4% wt/vol of tissue extraction buffer (50 mM Tris HCl, pH 7.5, 500 mM NaCl, 10 mM MgCl₂, 5 mM DTT). EDTA-free complete protease inhibitor tablets (Roche) were added to the tissue extraction buffer to prevent proteolysis. The homogenate was incubated for 1 h stirring on ice and centrifuged at 17,000 g for 10 min at 4°C. The protein concentrations were determined by the method of Bradford using bovine serum albumin as standard.

Western blot analysis. Laemmlí sample buffer was added to 15 μg of protein samples, and the proteins were resolved on 10% SDS polyacrylamide electrophoresis gels. After the transfer of proteins to nitrocellulose membranes, the membrane was reversibly stained with Ponceau S, and the nonspecific protein-binding sites were blocked for 1 h in TBST buffer (10 mM Tris HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) containing 5% nonfat dry milk. The following antibodies (Sigma, Oakville, Ontario, Canada) against the housekeeping protein actin, β₁, and β₂ sGC subunits were used for detection: actin (1:500), β₁ (1:5,000), and β₂ (1:4,000); and these were incubated for 1.5 h in TBST buffer at room temperature. The membranes were washed three times for 10 min with TBST and subsequently incubated for 1 h with horseradish peroxidase-conjugated anti-rabbit IgG antibody (1:2,000; Cell Signaling). After three washes with TBST buffer the membranes were processed with the enhanced chemiluminescence Western blotting detection system according to the manufacturer’s recommendations (Perkin Elmer).

Guanylate cyclase activity assay. Guanylate cyclase activity was measured as described by Koglin et al. (16). The activity of the obtained protein samples (10–100 μg/assay tube) was determined by incubation for 10 min at 37°C in the presence of 50 mM triethanolamine-HCl buffer, pH 7.4, containing 3 mM dithiothreitol, 1 mM 3-isobutyl-1-methylxanthine, 1 mM cGMP, 5 mM creatine phosphate, 10 units per tube creatine phosphokinase, 0.5 mM [α-32P]GTP (~1 mCi), and 3 mM MgCl₂ with or without 100 μM 2-(N,N-diethylamino)-diazenolate-2-oxide (DEA-NO) in a total volume of 0.1 ml. The incubation was stopped by the addition of 500 μl of NaHCO₃ (120 mM) and 500 ml zinc acetate (125 mM). The radiolabeled cGMP was isolated according to the method of Jakobs et al. (13) using aluminum oxide columns.

Statistical analysis. Data of the functional studies were evaluated by one- or two-way analysis of variance (ANOVA) and the Tukey-Kramer test was used for multiple comparisons. The guanylate cyclase activity data were analyzed by the
RESULTS

The bronchial smooth force generation in response to KCl (128 mM) was 6.9 ± 0.8 in the newborn and significantly increased to 12.8 ± 1.7 in the adult (P < 0.01).

To evaluate the maturational changes in the cyclic nucleotide-dependent bronchial relaxation, we first tested the cAMP pathway. Forskolin, a cAMP-mediated agonist, induced a similar bronchial muscle relaxation of newborn, compared with the adult bronchial smooth muscle (Fig. 1).

A clear maturational difference in the cGMP-mediated relaxation was observed for the rat bronchial smooth muscle. In response to the NO donor sodium nitroprusside (Fig. 2), the adult bronchi relaxed to a significantly lesser extent when compared with the newborn (P < 0.01). To further ascertain as to whether the NO-dependent airway muscle relaxation age difference was related to maturational changes in the sGC activity and/or content, we evaluated the response to specific stimulants of this enzyme.

Whereas in the newborn bronchi the sGC stimulants BAY 41-2272 and YC-1 induced a significant relaxation (P < 0.01), in the adult, either no response or contraction (YC-1 at 3 × 10⁻⁵ M or higher concentration; P < 0.01) was observed (Fig. 3). A relaxant response of the adult bronchi was only observed with BAY 41-2272 at a concentration of 3 × 10⁻⁵ M (37 ± 11% relaxation; n = 4; P = 0.03).

Expression of the α₁- and β₁-subunits of sGC in bronchi was significantly (P < 0.01) reduced in the adult tissue (Fig. 4). The newborn and adult bronchial tissue basal sGC activity was similar (Fig. 4). Yet, in keeping with the maturational differences in NO-mediated relaxation, the bronchial tissue sGC activity under NO-stimulated condition was significantly (P < 0.03) reduced in the adult when compared with the newborn values (Fig. 4).

DISCUSSION

It is well known that the airway ability to bronchoconstrict is already present in the immediate neonatal period and even as early as during fetal life (18). As shown in this study, when compared with the newborn, the adult airway smooth muscle generates greater force in response to agonist stimulation (21).

Since under isotonic conditions the smooth muscle potential for force development translates into airway narrowing, this age difference implies that the potential for bronchoconstriction is greatest in the adult.

To offset the tendency for bronchoconstriction and to minimize the airway resistance under physiological conditions, the bronchial smooth muscle is maintained in a relaxed state via distinct mechanisms. The most important are the cGMP- and cAMP-mediated responses and K⁺ channel openers, although the latter play only a minor role in humans (1).
Limited data are available on the maturational changes in bronchial smooth muscle relaxation. Chitano et al. (6) have recently shown that airway smooth muscle relaxation potential increases with age, and these maturational-dependent changes in airway smooth muscle relaxation may have important implications in the pathogenesis of asthma (7).

Salbutamol, a β2-agonist that relaxes airway smooth muscle via cAMP stimulation, has been shown to have a greater response in the newborn, compared with the adult rat trachea (10). Maturational differences in cAMP-mediated bronchial muscle relaxation do not account for the age-dependent salbutamol effect since the forskolin-induced response was similar for the newborn and adult rat. This apparent discrepancy between the salbutamol and forskolin age-related relaxation response has been shown by Fayon et al. (10) to be secondary to the greater muscarinic type 2 receptor expression in the adult airway resulting in functional antagonism between the cAMP-induced relaxation and muscle contraction.

Maturational changes in NO-mediated airway muscle relaxation have been reported by others. In the rat, Mhanaa et al. (19) showed that substance P-induced, NO-mediated relaxation decreases with age. In this study, we have also documented a greater relaxation in the newborn, compared with the adult rat bronchial muscle, in response to the NO donor sodium nitroprusside. In addition, we further confirmed this age difference to be sGC-related, given the similar response pattern to the enzyme stimulants BAY 41-2272 and YC-1. In fact, YC-1 in high doses induced airway smooth muscle contraction in the adult but not newborn for reasons that are not presently clear. The YC-1 site of action on sGC is not known (17), and possibly the contraction responses are unrelated to their effect on this enzyme.

Based on our previous observation of greater sGC protein expression in the newborn, compared with the adult lung (2), we speculated and further demonstrated in this study developmental changes in the expression and activity of this enzyme in the rat. sGC is a key enzyme responsible for the NO-triggered cGMP-dependent smooth muscle relaxation. This heterodimeric heme-containing enzyme consists of four subunits named α1, α2, β1, and β2. The α1- and β1-subunits are present in the lung (9, 14, 15), whereas the α2- and β2-subunits are expressed in other organs (5). In the Sprague-Dawley (4) and Wistar (2) rat, lung tissue sGC activity is highest in neonates and decreases with age.

The lung is composed of vascular, airway, and parenchymal compartments, and each populated by different cells expressing sGC. Therefore, in the present study, we measured sGC expression and activity in the same generation airway tissue used for the functional studies. We documented that the sGC α1- and β1-subunits protein expression and enzyme activity are markedly reduced in the adult rat bronchi, accounting for the lesser cGMP-dependent relaxation later in life.

Although the present data was obtained from rat airways, the clinical relevance of NO-mediated airway smooth muscle relaxation needs to be revisited with attention to the developmental changes in sGC expression. Most of the published studies comparing the airway muscle relaxation potential of different agonists were carried out in animal tissue. Based on these mostly adult tissue data, NO is considered to have a potency intermediate between the β-adrenergic agonist and the phosphodiesterase inhibitor theophylline (12). In contrast, the data from this study indicate that NO, the β-adrenergic agonist salbutamol, and the adenylate cyclase activator forskolin have similar bronchial smooth muscle relaxation potential in the newborn rat.

In addition, it has been recently reported that asthma-like disease in adult mice is associated with reduction in sGC activity, thus further limiting the potential therapeutic benefit of cGMP-mediated bronchodilation in adults (20). Taken together and to the extent that extrapolations can be made to humans, the evidence from ours and other studies suggest that cAMP-mediated bronchodilation should be preferentially sought in adults, whereas for the newborn airway, muscle relaxation is equally effective when either cyclic nucleotide is stimulated.

In summary, we documented a significant reduction in the α1- and β1-subunit expression and NO-stimulated sGC activity in adult rat bronchial tissue. This finding was associated with a significantly reduced cGMP-mediated relaxation in the adult rat airway muscle. Further comparative maturational studies of the adenylate and guanyl cyclase airway expression and activity are warranted to better address the pharmacological selection of bronchodilators to treat airway reactivity in humans.

GRANTS

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REFERENCES


