Effects of salbutamol and Ro-20-1724 on airway and parenchymal mechanics in rats

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Peták, Ferenc, J anet L. Wale, and Peter D. Sly.Effects of salbutamol and Ro-20-1724 on airway and parenchymal mechanics in rats. J. Appl. Physiol. 87(4): 1373–1380, 1999.—We investigated the effects of a selective β2-adrenergic agonist, salbutamol, and of phosphodiesterase type 4 inhibition with 4-(3-butoxy-4-methoxy benzyl)-2-imidazolidinone (Ro-20-1724) on the airway and parenchymal mechanics during steady-state constriction induced by MCh administered as an aerosol or intravenously (iv). The wave-tube technique was used to measure the lung input impedance (ZL) between 0.5 and 20 Hz in an anesthetized, paralyzed, open-chest adult Brown Norway rat. To separate the airway and parenchymal responses, a model containing an airway resistance (Raw) and inertance (law), and a parenchymal damping (G) and elastance (H), was fitted to ZL spectra under control conditions, during steady-state constriction, and after either salbutamol or Ro-20-1724 delivery. In the Brown Norway rat, the response to iv MCh infusion was seen in Raw and G, whereas continuous aerosolized MCh challenge produced increases in G and H only. Both salbutamol, administered either as an aerosol or iv, and Ro-20-1724 significantly reversed the increases in Raw and G when MCh was administered iv. During the MCh aerosol challenge, Ro-20-1724 significantly reversed the increases in G and H, whereas salbutamol had no effect. These results suggest that, after MCh-induced changes in lung function, salbutamol increases the airway caliber. Ro-20-1724 is effective in reversing the airway narrowings, and it may also decrease the parenchymal constriction.

Recent studies have established that the lung periphery, including the small peripheral airways and the pulmonary parenchyma, is the predominant site of constriction in patients with chronic airflow obstruction (26, 34). Furthermore, despite the general view that constrictor agents predominantly alter the airway caliber, numerous studies have revealed the importance of the altered viscoelastic properties of the pulmonary parenchyma in response to exogenous constrictor stimuli (12, 18, 21, 25, 30) and after an allergic reaction (24). For instance, the lung tissue resistance (Rti) has been demonstrated to contribute significantly to the increase in total lung resistance after methacholine (MCh) (25, 27, 30) or histamine (12) challenge.

Although a thorough understanding of how bronchodilators alter pulmonary mechanics would be of therapeutic importance, the effects of these agents on airway and parenchymal mechanics have been examined with far lower intensity. This was therefore the primary purpose of the present study. Clinical studies indicate that, after the induction of constriction, salbutamol (a selective β2-adrenergic agonist) causes increases in forced expiratory lung volumes (13) and decreases in high-frequency respiratory resistance (16, 17, 23, 29); i.e., it increases the airway caliber. Most of the previous studies, however, also report a significant increase in respiratory compliance (which describes the elasticity of the respiratory tissues) after intravenous (iv) (16) or aerosolized salbutamol (17, 23, 29). Recently, Kaczka et al. (14) separated the airway and tissue responses after inhalation of another β2-agonist, albuterol, in asthmatic patients. They reported significant decreases in airway and lung tissue mechanical parameters after β2-agonist-induced bronchodilation. Although β2-adrenoceptors have been demonstrated autoradiographically to be widely distributed in the lungs and abundant in the epithelium, submucosal glands, airway smooth muscle, and alveoli, the direct action of salbutamol on lung tissue has been frequently questioned, and alternative mechanisms have been proposed, such as the changes in tissue properties being related to the opening of previously occluded terminal airways, and, hence, to an increase in effective lung volume (4, 7).

Theophylline, a nonselective phosphodiesterase (PDE) inhibitor, has also been used as a bronchodilator in the treatment of asthma for many years. The development of PDE inhibitors with selectivity for one or more of the PDE families of isoenzymes offers an opportunity for the application of such compounds as new drugs in the treatment of asthma. These drugs possess both anti-inflammatory and bronchodilator properties (1). Evidence has been put forward that PDE type 4 (PDE4) activity is tightly coupled to the adenylyl cyclase enzyme stimulated by β2-adrenergic agonists (32).

The present work was performed to determine the predominant site of the dilator activity of salbutamol in anesthetized rats during moderate MCh-induced constriction. If parenchymal β2-adrenoceptors play a physiological role in determining the lung tissue viscoelasticity, a dilator effect of salbutamol on parenchymal parameters will be apparent in vivo. Because the pattern of changes in the airway and parenchymal mechanical parameters is different when constrictor agents are administered in aerosol form or iv (25, 27), we were also interested in whether this was the case with salbutamol. In rats, PDE4 inhibition with 4-(3-butoxy-4-methoxy benzyl)-2-imidazolidinone (Ro-20-1724) results in antispasmogenic activity against MCh-induced constriction (unpublished observations), and this isoenzyme appears to be closely linked to the β2-adrenoceptors (31). Therefore, we further investigated whether the dilator activity of this agent on...
airway and parenchymal components to reverse MCh-induced constriction resembled that of salbutamol.

METHODS

Animal Preparation

Thirty-one adult male Brown Norway rats (214–294 g) were anesthetized (12 mg/kg xylazine and 40 mg/kg ketamine intramuscularly, supplemented iv every 40 min) and placed in the supine position. Tracheostomy was performed, and a metal cannula (2-mm ID) was inserted into the distal trachea. A femoral vein was cannulated for drug delivery. The other femoral vein was also cannulated in the rats receiving MCh infusion. Paralysis was accomplished with pancuronium bromide (1 mg/kg initial dose, supplemented with 0.3 mg/kg every 40 min), and the rats were mechanically ventilated (model 683, Harvard Apparatus) with a tidal volume of 9 ml/kg at a frequency of 90 breaths/min. The thorax was opened by means of a midline thoracotomy, and the ribs were widely retracted. After the chest opening, the positive end-expiratory pressure was set to 2.5 cmH_2O to keep the transpulmonary pressure constant at end expiration. The pressure in the box chambers was set to 2.5 cmH_2O to keep the transpulmonary pressure constant during measurements. The loudspeaker generated a small-amplitude pseudorandom signal (15 noninteger multiples between 0.5 and 21 Hz) through a 120-cm-long, 2-mm-inner-diameter polyethylene tube. Two identical pressure transducers (ICS model 33NA002D) were used to measure the lateral pressures at the loudspeaker (P_1) and at the tracheal end (P_2) of the wave tube. The P_1 and P_2 signals were low-pass filtered (5th-order Butterworth, 25-Hz corner frequency) and sampled with an analog-to-digital board of an IBM-compatible computer at a rate of 128 Hz. Fast Fourier transformation with a 4-s time windows and 95% overlapping was used to calculate the pressure transfer functions (P_1/P_2) from the 6-s-long recordings. Z_L was calculated as the load impedance of the wave tube

\[
Z_L = \frac{Z_0 \sinh(\gamma L)}{[\gamma P_1/P_2] - \cosh(\gamma L)}
\]

where L is the length, Z_0 is the characteristic impedance, and \(\gamma\) is the complex propagation wave number of the wave tube. The latter two parameters are determined by the geometric data and the material constants of the tube wall and the air.

Estimation of Airway and Parenchymal Parameters

To separate airway and parenchymal mechanics, a model containing a frequency-independent airway resistance (Raw) and inertance (Iaw) in series with a constant-phase tissue model \((\alpha, h)\), including damping (G) and elastance (H), was fitted to the \(Z_L\) spectra by minimizing the differences between the measured and modeled impedance values

\[
Z_L = \text{Raw} + jo \text{law} + (G - jH)/\omega^\alpha
\]

where j is the imaginary unit, \(\omega\) is the angular frequency \((2\pi f)\), and \(\alpha = 2\pi \arctan(H/G)\) is not an independent parameter. Impedance data at frequencies coinciding with the heart rate and its harmonics were omitted from the model fitting if cardiac activity caused low signal-to-noise ratio at these frequency components.

Study Protocols

MCh deliveries. Steady-state constriction was generated by the administration of MCh either by iv infusion \((n = 19)\) or as an aerosol \((n = 12)\). Before administration of the constrictor agent, the lungs were hyperinflated by superimposing two inspiratory cycles to standardize the volume history.

IV DELIVERY. After four to six successive \(Z_L\) recordings to establish the baseline, infusion of MCh was started at 2 \(\mu\)g kg\(^{-1}\) min\(^{-1}\) \((\text{flow rate: } 25 \mu\text{ml/min})\). Because our purpose was to generate a definite constriction, the MCh infusion rate was doubled successively until an approximately twofold increase in Raw was seen.

AEROSOL DELIVERY. A plateau response was achieved by continuous inhalation of nebulized MCh at a concentration of 1 mg/ml. A nebulizer (Parry LC-plus) driven by air with a constant flow of 5 l/min was connected into the inspiratory port of the respirator. This nebulizer delivers particles \(\sim 5 \mu\text{m}\) in size. Steady-state constriction, i.e., no change in the pressure transfer function, was obvious after 8–12 min.

Dilator deliveries. After the establishment of a stable level of constriction, iv bolus (0.2 \(\mu\)g/kg) or aerosolized salbutamol (1 \(\mu\)g/ml for 90 s) or iv bolus of Ro-20-1724 (200 \(\mu\)g/kg) was administered, as detailed in Table 1. Because Ro-20-1724 was dissolved in 95% ethanol, a bolus injection of the same volume of 95% ethanol was administered as a control where appropriate.

In each protocol, \(Z_L\) was measured at 1-min intervals during the MCh challenge. Monitoring of the mechanical parameters with this timing was resumed 2 min after the dilator administration and was continued for at least 15 min thereafter. This procedure resulted in a 25- to 35-min total MCh administration time.

In all of the five protocol groups, four to six \(Z_L\) spectra were ensemble averaged at the baseline and during plateau constriction. After salbutamol or Ro-20-1724 administration the individual \(Z_L\) curves were fitted, and the peak dilation responses in each model parameter were used for further analysis. If there was no obvious response in any of the parameters, i.e., none of the model parameters deviated by \(>5\%\) from that obtained at the MCh plateau after salbutamol or Ro-20-1724 administration, averaged parameter values observed after administration of the dilator agent are reported.

Materials

The following materials were used: xylazine (Bayer), ketamine (Troy Laboratories), pancuronium bromide (Astra Pharmaceuticals), Ro-20-1724 (a gift from Roche Products and Dee Why, NSW, Australia), salbutamol (Allen & Hanburys), MCh chloride, and ascorbic acid (Sigma Chemical). MCh was dissolved in saline. Dilutions of salbutamol were made with

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>MCh</th>
<th>Dilator Agent</th>
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<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>iv Salbutamol aerosol</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>iv Salbutamol iv</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>iv Ro-20-1724 iv</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Aerosol Salbutamol iv</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>Aerosol Ro-20-1724 iv</td>
</tr>
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MCh, methacholine; iv, intravenous; Ro-20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone.
saline containing ascorbic acid (10 µg/ml) and kept on ice. Ro-20-1724 was dissolved in 95% ethanol.

Statistical Analysis

Scatters in the parameters were expressed in SE values. Repeated measures of one-way ANOVA with the Student-Newman-Keuls multiple-comparison procedure was used to assess the effect of MCh, salbutamol, and Ro-20-1724 on airway and parenchymal mechanical parameters. Statistical tests were performed with a significance level of \( P < 0.05 \).

RESULTS

The real (i.e., \( R_L \)) part of \( Z_L \) and the pulmonary elastance spectra (\( E_L = -\omega X_L \), where \( X_L \) is the lung reactance) obtained in a representative rat, and the corresponding model fits for the baseline, during steady-state constriction induced by 4 \( \mu \)g·kg\(^{-1}\)·min\(^{-1} \) iv MCh infusion, and after aerosolized salbutamol administration, are demonstrated in Fig. 1. Data points representing the baseline and the constriction were obtained by averaging five measurements. The low variabilities during MCh infusion indicate very stable plateau response. MCh-induced increases can be seen in \( R_L \) at all frequencies. Increases in the high-frequency range of \( R_L \) indicate a significantly higher \( R_{aw} \), whereas the proportionally greater increase in the low-frequency range of \( R_L \) suggests an elevated \( G \). Nebulized salbutamol administration during MCh infusion caused a marked decrease in \( R_L \): a decrease in the high-frequency range indicates a significant fall in \( R_{aw} \), whereas a decrease in the low-frequency gradient suggests a reduction in \( G \). In general, changes in \( R_L \) were associated with minor changes in the low-frequency \( E_L \) for any agent; however, both agents induced significant changes in the frequency dependence of \( E_L \) at higher frequencies.

Representative \( R_L \) and \( E_L \) spectra obtained in the control condition, during steady-state constriction generated by aerosolized MCh, and after iv 4-(3-butoxy-4-methoxy benzyl)-2-imidazolidinone (Ro-20-1724; \( \square \)) administration. Lines denote corresponding model fits. Open symbols, data points corrupted by cardiac artifacts and therefore omitted from model fits.

iv Infusion of MCh

MCh responses. Figure 3 summarizes the airway and tissue responses in the rats receiving iv MCh. In agreement with our previous finding (27), MCh induced marked and statistically significant increases in Raw (262 ± 28, 227 ± 64, and 239 ± 77% above the baseline in groups 1, 2, and 3, respectively) and in G (207 ± 37, 161 ± 41, and 147 ± 17%), decreases in Iaw (−40 ± 5,
Salbutamol. Compared with the levels established during MCh infusion, salbutamol caused immediate and statistically significant decreases in Raw ($-49.5 \pm 1.6$ and $-21 \pm 2.2\%$ from the MCh plateau response in groups 1 and 2, respectively) and in G ($-41.8 \pm 3.6$ and $-23.5 \pm 3.9\%$) and significant increases in Iaw ($68.4 \pm 18$ and $62 \pm 43\%$), whereas H remained fairly constant or even slightly further increased ($0.8 \pm 1.1$ and $8.8 \pm 2.1\%$). The parameter values returned toward the MCh plateau level within 15 min when salbutamol was administered iv but remained depressed over this period of time after aerosol administration.

Ro-20-1724. Similar to the salbutamol responses observed during iv MCh infusion, PDE4 inhibition with Ro-20-1724 significantly and immediately (within 2 min) decreased Raw ($-54 \pm 8.3\%$) and G ($-42 \pm 3\%$), whereas Iaw displayed a marked increase ($57 \pm 9\%$). A slight but statistically not significant further increase was found in H ($7.9 \pm 1.9\%$). Ro-20-1724 was not administered in aerosol form, because delivery of its solvent (95% ethanol) into the airways is likely to cause significant and irreversible damage to the lung epithelium.

Inhalation of MCh

MCh responses. Data obtained under control conditions, during steady-state MCh-induced constriction, and after administration of the dilator agents to rats receiving aerosolized MCh are presented in Fig. 4. MCh inhalation induced marked and statistically significant increases in G ($183 \pm 12$ and $145 \pm 15\%$ above the baseline for groups 4 and 5, respectively) and in H ($89 \pm 4$ and $78 \pm 4\%$). In contrast to the responses observed during iv MCh infusion, the aerosol MCh challenge generated only slight plateau increases in Raw ($4.9 \pm 4.6$ and $26 \pm 12\%$) and in Iaw ($4.9 \pm 2.4$ and $4.8 \pm 2.3\%$). It is noteworthy that a transient change was seen in the airway parameters after the onset of aerosolized MCh administration (data not shown).

Salbutamol. None of the parameters underwent a statistically significant change when iv salbutamol was administered during aerosolized MCh-induced steady-state constriction ($-1.7 \pm 2.3$, $0.3 \pm 1.5\%$, $-1.6 \pm 0.8$, and $-3.0 \pm 1.8\%$ changes relative to MCh plateau response in Raw, Iaw, G, and H, respectively). It was not technically feasible to obtain data on inhaled salbutamol during the aerosol administration of MCh as the delivery of two aerosols simultaneously would produce unpredictable and unreliable results.

Ro-20-1724. We found a slight but statistically not significant immediate decrease in Raw ($-16 \pm 5\%$) and no change in Iaw ($1.3 \pm 1.1\%$) when Ro-20-1724 was administered during steady-state constriction induced by MCh inhalation. In contrast to the lack of a tissue response after salbutamol administration, Ro-20-1724 induced an immediate, marked, and statistically significant decrease in G ($-25 \pm 9\%$), which was accompanied by a moderate and statistically significant decrease in
(−16 ± 7%). The duration of action of Ro-20-1724 was similar to the airway and parenchymal responses, where changes were seen in both parameters. The response generally returned toward the steady-state constriction level within 15 min, although in some experiments it exhibited a further slight recovery beyond this period of time.

The solvent of Ro-20-1724 (95% ethanol) did not provoke a significant change in any parameter during MCh-induced steady-state constriction, with changes always <5%.

DISCUSSION

In the present study, we investigated the effects of the β₂-adrenoceptor agonist salbutamol and of PDE4 inhibition with Ro-20-1724 on the airway and parenchymal mechanics during plateau constrictor responses induced by iv or aerosol administration of MCh. As we have previously shown (27), iv MCh produces a predominantly airway response that may be accompanied by an increase in G, presumably because of ventilation inhomoogeneities (21), whereas inhaled MCh results primarily in increases in both parenchymal parameters (G and H). Salbutamol administered either iv or as an aerosol reversed the MCh-induced changes in airway mechanics when the MCh was administered iv but had no effect on the elevated lung tissue parameters when the steady-state constriction was generated by aerosolized MCh. However, Ro-20-1724 significantly reversed the MCh-induced increases in both airway and the parenchymal parameters independently of the MCh delivery route.

Effects of Salbutamol

Salbutamol during iv MCh infusion. In agreement with our previous results (27), we obtained significant increases in Raw and G after iv MCh challenge in the present study, whereas H remained at the baseline level. Because the parenchymal parameters of a single-compartment, constant-phase model may be biased by airway heterogeneities, we used gas mixtures with different densities and viscosities in our earlier study, as suggested by Lutchen et al. (21), to elucidate the possible interactions between airway and tissue model parameters. In accordance with the findings of Lutchen et al., we concluded that iv MCh induces markedly inhomogeneous constriction of the airways and that the entire increase in G can be attributable to the increased ventilation inhomogeneity (27). Because iv MCh induced the same changing pattern in the mechanical parameters (i.e., significant increases in Raw and G, but no change in H; Fig. 3), the data of the present study related to iv MCh challenge suggest the same phenomenon, i.e., airway constriction with enhanced ventilation inhomogeneities.

Although the presence of an inhomogeneous airway constriction during iv MCh infusion is reinforced by recent simulation work of Lutchen and Gillis (20), the results of their study also raise the possible involvement of a significant central airway wall shunting. They demonstrated that shunting would shift Rₐ up
fairly uniformly with frequency, whereas the increases in EL are more pronounced above 1–2 Hz and extend to higher frequencies. In the present study, iv MCh changed the frequency dependence of RL, indicating the presence of an inhomogeneous peripheral airway constriction. Furthermore, MCh infusion also caused a marked increase in Raw and frequency-dependent increases in EL with essentially no change at the very-low-frequency range (Fig. 1). Thus the responses in our impedance spectra and model parameters during iv MCh suggest substantial airway constriction throughout the periphery, which may have invoked airway wall shunting, whereas the intrinsic viscoelastic properties of the parenchyma remained unchanged.

Under these circumstances, the salbutamol-induced marked decreases in Rl were associated with a reduction of the frequency dependence of EL (Fig. 1). These changes in the impedance spectra resulted in decreased Raw and G with no change in H (Fig. 3). The decrease in G suggests that salbutamol reversed the MCh-induced airway heterogeneities. The bronchial pathways that were most constricted by MCh are likely to dilate to a greater degree in response to salbutamol. This response decreases the inhomogeneities in the periphery and hence produces a significant decrease in G. As a consequence of the dilated peripheral airways, shunting had less effect after salbutamol. This phenomenon further advanced the returns of the impedance spectra and the model parameters toward the baseline.

Salbutamol during aerosolized MCh. During plateau constriction, aerosolized MCh caused increases in G and H, whereas the airway parameters remained at the baseline level (Fig. 4). This finding is at variance with the results of our previous study, in which the aerosolized MCh-induced increases in G and H were associated with significant elevations in Raw (27). Beside the fact that different rat strains were studied, methodological differences between the studies may explain this discrepancy. The peak responses in Raw after short (1-min) aerosolized MCh administration were reported in the previous study (27), whereas plateau responses during a prolonged (0.5-h) aerosolized MCh challenge were analyzed in the present experiments. Indeed, Raw exhibited a transient increase after the onset of the MCh aerosol (data not shown), but this effect diminished when the constriction became steady state.

The most obvious interpretation of the increased tissue parameters with no change in the Raw is that continuous aerosol MCh challenge induced an intrinsic parenchymal constriction, whereas the airways did not respond at the dose used. In this case, the significant increases in the frequency-dependent component of RL would reflect real increases in Rti. This, together with less pronounced frequency-dependent increases in the EL spectra, could be a manifestation of altered hysteresis. Indeed, the parenchymal hysteresis has been shown to increase after a constrictor challenge even in parenchymal strips (8), where the confounding influence of the inhomogeneous bronchocstriction is minimized. Alternatively, without any MCh-induced change in the intrinsic tissue properties, such a changing pattern in the impedance spectra, and consequently in G and H, could have resulted from an acutely inhomogeneous airway constriction with a few highly constricted or closed airways. The possible involvement of this phenomenon can be substantiated from experimental results by Nagase et al. (25), demonstrating significant tissue distortions after aerosolized MCh, or from the results of a recent simulation study by Lutchen and Gillis (20) revealing marked frequency-dependent increases in RL and EL spectra. Although our data obtained during inhaled MCh do not allow one to distinguish between these interpretations, the lack of a salbutamol-induced response may give some insight into the underlying mechanisms. Our finding that salbutamol reversed the increased Raw after iv MCh and decreased G shows that salbutamol produces airway dilatation, and, as a consequence, airway inhomogeneities decrease as well (see above). If the increases in G and H during aerosolized MCh administrations were also due to an even more heterogeneous airway constriction, it would be not clear why these constricted airways are not dilated in any detectable degree by iv salbutamol (Fig. 4, ●). Accordingly, the hypothesis that a continuous aerosol MCh challenge induced real constriction of the pulmonary parenchyma, and that iv salbutamol was not able to reverse the parenchymal constriction to any appreciable degree, seems to be better supported by our experiments. Consequently, our results indicate that salbutamol acts on the airway compartment only and has no effect on parenchymal mechanics in rats.

Autoradiographic studies show that β-adrenoceptors are widely distributed in the lungs. They are abundant in the epithelium, the submucosal glands, the airway smooth muscle, and the alveoli (4, 7). In rats, β-adrenoceptors are diffusely distributed, being present even in the alveolar septa (7). Indeed, Conner and Reid (5) found that 97% of the β-adrenoceptors reside in alveolar tissue. Moreover, the 3:1 overall proportion of β2- to β1-adrenoceptors in the rat parenchyma reveals a predominance of β2-adrenoceptors (6, 22). In vitro, β-adrenoceptor agonists have been observed to be effective dilators in tracheal, bronchial, and parenchymal preparations from a number of species, when either spontaneous tone (8, 9, 19) or a contraction induced by exposure to a spasmogen (3, 10) was used. Although it is likely that β-adrenoceptor agonists act on the airway smooth muscle in tracheal and bronchial preparations, the site of action of these agents in parenchymal strips is less predictable; the possibilities include the smooth muscle in the terminal airways, the vascular smooth muscle, and the alveolar wall.

There have been only a few studies separating the airway and parenchymal responses to β-adrenoceptor agonists in the whole lungs. Vettermann et al. (33) used alveolar capsules to partition RL into Raw and Rti in nonconstricted excised canine lungs and showed that another β-agonist, isoproterenol, altered both airway and tissue mechanics. More recently, Kaczka et al. (14) partitioned the lung response into airway and parenchymal components before and after inhalation of al-
buterol on the basis of low-frequency Zl spectra in asthmatic patients. They observed decreases in Raw and G; however, significant decreases in H were also reported in the case of severely asthmatic patients. Although the reasons for the inconsistency between the results of the present study and those reported previously are not completely clear, the difference in the species or the different initial conditions before dilator administrations may play a role. With regard to the latter, it is possible that the β-agonist-induced decreases in the tissue parameters obtained in excised dog lobes or in asthmatic patients can be attributable to an increase in the effective lung volume due to reopening of terminal air spaces rather than to a decrease in the hysteresis of the airway and alveolar duct smooth muscles.

Ro-20-1724 Responses

The selective PDE inhibitors for one or more PDE families of isoenzymes possess anti-inflammatory and/or bronchodilator properties and therefore offer an opportunity for the application of such compounds as new drugs in the treatment of asthma. These agents act via inhibition of the hydrolysis of second-messenger cyclic nucleotides cAMP and cGMP, and they are the second messengers for β2-adrenoceptor agonists and nitric oxide, respectively. In the lungs, increases in either nucleotide result in relaxation of bronchial smooth muscles. Recently, Turner et al. (32) presented evidence that PDE4 activity is tightly coupled to the adenylyl cyclase enzyme stimulated by β2-adrenoceptor agonists. Using preparations from different species, and therefore with differing proportions of β1- to β2-adrenoceptors, Tomkinson et al. (31) demonstrated a close association between the PDE4 isoenzyme and lung β2-adrenoceptors in vitro.

Because the pattern of the reversal of constriction by Ro-20-1724 was identical to that by salbutamol when the constrictor tone was generated by iv MCh (Fig. 3), it can be concluded that PDE4 inhibition with Ro-20-1724 significantly decreases the overall Raw. However, in contrast to salbutamol, this agent also caused significant reversal of the increased parenchymal G and H when the MCh was aerosolized (Fig. 4). One possible interpretation of this dilator response would be that Ro-20-1724 reversed the highly inhomogeneous airway constriction developed during aerosolized MCh by distending the constricted airways and reopening some of the closed pathways. Thus the decreases in G and H would be attributable to the increased lung air volume and the decreased inhomogeneities, whereas the intrinsic lung tissue properties do not change. Alternatively, our data may suggest that Ro-20-1724 acts directly on the lung tissues, for the following reason. The unchanged Raw and the lack of any salbutamol response during aerosolized MCh, whereas it had a significant dilator effect during iv constrictor challenge, suggest the presence of parenchymal constriction. Accordingly, the reversal of the increases in both G and H, and the rather frequency-independent decreases in E\textsubscript{L} in response to Ro-20-1724 can also be attributable to a true action on the lung tissues. Our data therefore indicate that Ro-20-1724 is able to produce a significant dilation of the airways and may also reduce the parenchymal constriction considerably.

Ro-20-1724 is highly selective for the inhibition of cAMP hydrolysis (27). The tissue levels of this second messenger are increased by smooth muscle relaxants, including β2- and β3-adrenoceptor agonists PGE\textsubscript{2} and vasoactive intestinal protein (2, 15). It is possible, therefore, that the tissue cAMP levels are raised by non-β2-adrenoceptor mediators, if this is the mechanism by which Ro-20-1724 acts on the peripheral lung. Because isoprenaline does not alter the tissue parameters during MCh inhalation (data not shown), it is unlikely that β2-adrenoceptors are involved. The failure of salbutamol to reverse the MCh-induced parenchymal constriction, under the same circumstances in which Ro-20-1724 did reverse these changes, suggests that either 1) stimulation of β-adrenoceptors in the lung parenchyma does not result in an increase in intracellular cAMP levels or 2) Ro-20-1724 is acting to reverse the MCh-induced changes in tissue mechanics via a mechanism independent of its PDE inhibition. In either case, the data from the present study support the conclusion of Hantos et al. (12), who used histamine to induce constriction, that the mechanisms by which airways and parenchyma respond to constrictor agonists are different.

Finally, it is possible that prolonged administration of aerosolized MCh might result in fluid accumulation in the lung periphery either as a direct consequence of the long aerosolized fluid administration or due to microvascular leak. These phenomena may lead to a decrease in lung volume and thus could conceivably contribute to the parenchymal response to inhaled MCh reported here. Elevations in G and H under these conditions may not be readily reversed by dilator drugs. The contribution of fluid accumulation in the periphery due to increased microvascular leakage to the observed tissue responses during aerosolized MCh cannot be fully excluded. Nevertheless, if this phenomenon had played a significant role, gradual and continuous increase in the parenchymal parameters would have been expected. Regardless of the mechanisms for the observed increases in G and H after inhaled MCh, Ro-20-1724 was able to reverse this process, whereas salbutamol had no effect. To test the possible role of peripheral fluid accumulation as a consequence of the long inhalation procedure itself, we aerosolized normal saline for a period of 30 min in two rats. As no significant change in the pulmonary mechanics in either rat was seen, this mechanism seems unlikely to contribute to the measured parenchymal response to inhaled MCh.

In summary, the results of the present study demonstrate that MCh-induced changes in airway mechanics can be reversed by either salbutamol or the selective PDE4 isoenzyme inhibitor Ro-20-1724, suggesting that an increase in cAMP in the airway smooth muscle cells is responsible. In contrast, true MCh-induced changes in lung tissue mechanics can be reversed by Ro-20-1724...
and not by salbutamol, suggesting that MCh may act differently on airway and lung tissues. Furthermore, the effects of salbutamol were independent of the route by which it was delivered to the lungs.

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