Rosiglitazone elicits in vitro relaxation in airways and precision cut lung slices from a mouse model of chronic allergic airways disease

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THERE IS INCREASING EVIDENCE that rosiglitazone (RGZ) and other agonists of peroxisome proliferator-activated receptor-γ (PPARγ) may offer therapeutic benefit in asthma. Numerous studies suggest that PPARγ ligands can inhibit allergen-induced inflammation and the development of airflow remodeling and airway hyperresponsiveness (AHR) in vivo (reviewed in Ref. 8), as well as exert direct dilator effects on airway smooth muscle (ASM) in vitro (3, 12).

PPARγ expression is increased in bronchial biopsies from patients with asthma, including in the epithelial and ASM layers (2). In vitro, human ASM cytokine secretion and proliferation have been shown to be suppressed by the PPARγ agonists RGZ and ciglitazone (CGZ) (10, 27, 30, 34, 37). However, only some of these anti-inflammatory and antiremodeling activities were inhibited by the selective PPARγ antagonist GW9662, suggesting both PPARγ-dependent and -independent mechanisms (30, 34).

The effects of chronic treatment with PPARγ ligands have also been examined in mouse models of allergic airway disease (AAD), where sensitization and nebulization with ovalbumin (OVA) causes airway inflammation, airway wall remodeling, and AHR to acetyl-β-methylcholine chloride (MCh). In BALB/c mice, treatment with either RGZ or CGZ inhibited OVA-induced airway inflammation and fibrosis as well as the development of AHR (14, 32, 35), with the effects of the synthetic PPARγ ligands mimicked by AdPPARγ (15, 17) and abrogated by GW9662 (14, 15, 17, 35). Although RGZ also reduced the development of AHR in OVA-challenged C57BL/6 mice, the increase in BAL inflammatory cells in this mouse strain was not affected, suggesting that RGZ may also modulate AHR by a mechanism that is independent of inhibition of inflammatory cell recruitment to the airway (33).

In addition to these inhibitory effects in models of chronic AAD, RGZ has been shown to elicit acute relaxation of mouse tracheal segments and intrapulmonary airways in precision cut lung slices (PCLS) precontracted with muscarinic agonists in vitro (3, 12). In PCLS, RGZ elicited complete relaxation under conditions of impaired β-adrenoceptor agonist responsiveness, opposing contraction by inhibiting calcium oscillations and sensitivity (3). However, these novel direct dilator actions have yet to be confirmed when large and small airway reactivity to contractile agonists is altered by allergen challenge.

The aim of the current study was to further characterise dilator responses to RGZ relative to β-adrenoceptor agonists in isolated trachea from mice and guinea pigs to demonstrate its efficacy in both large and small airways from a mouse model of chronic AAD where in vitro responsiveness to MCh is altered (6). Here, we confirmed that RGZ elicits tracheal bronchodilation, albeit at lower potency than either salbutamol hemisulphate salt (SALB) or isoprenaline (ISO). However, in contrast to these β-adrenoceptor agonists, both the potency and complete relaxation to RGZ were maintained in maximally contracted trachea. Despite increased in vitro responsiveness to
with ketamine (200 μg OVA per 0.4 mg aluminum potassium sulfate adjuvant (alum) in 0.5 ml saline ip) or equivalent volumes of adjuvant on days 0 and 14 followed by nebulized OVA (2.5% wt/vol saline) or nebulized saline three times per week between days 21 and 63 as previously described (20).

**Methods**

**Chronic AAD model.** For the chronic AAD model, female BALB/c mice were administered grade V chicken egg OVA [10 μg OVA per 0.4 ml Krebs-Henseleit buffer (pH 7.4, 37°C, aerated with 95% O₂-5% CO₂)] or equivalent volumes of adjuvant on days 0 and 14 followed by nebulized OVA (2.5% wt/vol saline) or nebulized saline three times per week between days 21 and 63 as previously described (20).

**Myograph and organ bath experiments.** Experimental details are as previously described (6). Mice were killed by a slow-fill asphyxiation method (80% CO₂-20% O₂) or by cardiac puncture under anesthesia with ketamine (200 μg/g) and xylazine (10 μg/g). Guinea pigs were concussed by a blow to the head before exsanguination. Tracheas were removed, immersed in Krebs-Henseleit buffer (59 mM NaCl, 2.3 mM KCl, 0.69 mM MgSO₄·7H₂O, 2.5 mM CaCl₂·H₂O, 0.6 mM KH₂PO₄, 10 mM EDTA, 25 mM NaHCO₃, and 6 mM glucose, containing 3 μM indomethacin for guinea pig tissues only), and dissected into 2- to 4-mm segments. In some experiments, the tracheal epithelium was denuded by gently rubbing a cocktail stick inside the lumen. Epithelial removal was confirmed histologically and by a reduced dilator response to Substance P as previously described (1).

Airway segments were mounted under static conditions in temperature-controlled myograph baths (mouse; Danish MyoTechnology, Aarhus, Denmark) or standard organ baths (guinea pig) containing Krebs-Henseleit buffer (pH 7.4, 37°C, aerated with 95% O₂-5% CO₂) for continuous recording of changes in isometric tension (ΔN) using Power Lab and Chart software (AD Instruments). An optimal resting tension was determined based on the maximum response to depolarizing potassium solution (DKS; containing 123.7 mM K⁺) before generation of a full concentration-response curve to MCh. Tissues were then recontracted to 20, 75, or 100% of the maximal MCh response, followed by cumulative addition of PPARγ agonists [RGZ, PGZ, and 15-deoxyΔ12,14-prostaglandin J₂ (15-deoxy-PGJ₂)] or nonselective cyclooxygenase (COX) inhibitor [nitra-t-arginine: 10 μM, 10 min] or a nonselective cyclooxygenase (COX) inhibitor (indomethacin: 10 μM, 15 min) on RGZ-induced relaxation were also determined. Comparisons of dilator responses to RGZ and ISO were made between tracheas from saline- and OVA-challenged mice.

**PCLS experiments.** PCLS were prepared as previously described (3, 6, 9). Mice were killed with an overdose of sodium pentobarbitone (0.45 ml, 60 mg/ml ip). Tracheas were cannulated and the lungs were inflated with warm ultra pure low melting point agarose (~1.2 ml of 2% wt/vol in Hank’s balanced salt solution supplemented with 40 mM HEPES (sHBSS); Invitrogen). A small bolus of air was then used to push the agarose into alveolar spaces. After the agarose solidified (20 min, 4°C), a single lobe was mounted in cold HBSs in a vibratome (VT 1000S; Leica Microsystems) to cut 150-μm slices commencing at the lung periphery. Slices were cultured overnight in DMEM supplemented with 1% penicillin-streptomycin solution (37°C, 5% CO₂).

Individual slices were then placed in a custom-made perfusion chamber between two cover glasses (~100-μl vol) and covered in fine wire mesh (Small Parts) with a small hole cut over a single airway (150- to 400-μm diameter). Viability was confirmed by observation of an intact epithelial layer displaying ciliary activity under phase contrast microscopy using ×10 objective lens, zoom adaptor, reducing lens, and camera (Nikon Eclipse Ti-U; Pulnix CCD Camera Model TM-62EX). Digital images (744 × 572 pixels) recorded in time lapse (0.5 Hz) using image acquisition software (Video Savant; IO Industries,) were converted to TIFF files and analyzed using the PC software NIH/Scion (Scion). With the use of an appropriate grey scale threshold to distinguish between the airway lumen and surrounding tissue, the lumen area in each image could be calculated by pixel summation.

Comparisons of constrictor and dilator responses were made in perfused PCLS from saline- and OVA-challenged mice. In matched PCLS from the same mice, full concentration-response curves to MCh were generated or airways were precontracted with MCh (100 nM) followed by cumulative addition of RGZ or SALB, delivered at a constant rate under anesthesia with ketamine (200 μg/g) and xylazine (10 μg/g).

**Drugs and reagents.** MCh, indomethacin, ISO, nito-t-arginine, penicill-streptomycin solution, SALB, PGZ, GW9662, and T0070907 were from Cayman. Ultra pure low melting point agarose, indomethacin, nitro-L-arginine, PGZ, RGZ, and T0070907 were from Cayman; ultra pure low melting point agarose, 100 mg/ml, 60 mg/ml ip).

**Results.** RGZ causes relaxation of isolated mouse and guinea pig tracheal segments precontracted with MCh. Dilator responses to RGZ were compared with the β-adrenoceptor agonists SALB and ISO (Fig. 1). A typical trace from an isolated mouse tracheal segment demonstrates bronchodilator responses to RGZ at concentrations >1 μM (Fig. 1A). After contraction to...
75% of the maximum response to MCh, cumulative addition of RGZ caused concentration-dependent and complete relaxation. Responses to either SALB or ISO were more rapid than RGZ, with plateau responses to each drug addition occurring within 2 min for the β-adrenoceptor agonists (not shown) vs. 10 min for RGZ (Fig. 1A). When measured after similar MCh precontraction, (Fig. 1B), the potency of RGZ in mouse tracheal segments was lower than either β-adrenoceptor agonist. However, only RGZ was able to elicit complete relaxation (Fig. 1C; Table 1). In guinea pig tracheal segments, both β-adrenoceptor agonists were also more potent than RGZ, with all dilators fully reversing the matched MCh-induced contraction in this species (Fig. 1, D and E).

**Relaxation to RGZ, but not β-adrenoceptor agonists, is maintained with increasing contraction of mouse tracheal segments.** To assess the influence of the level of precontraction on dilator potency and efficacy, responses to RGZ (Fig. 2A) were compared with SALB and ISO (Fig. 2, B and C) in mouse tracheal segments precontracted to low (20% MCh maximum), submaximal (75% MCh maximum), or high (100% MCh maximum) tone. Representative precontractions to MCh are shown (Fig. 2D, before ISO).

RGZ maintained both its potency and capacity to cause near complete relaxation even in maximally contracted tissues (Fig. 2A; Table 1). With increasing tone, both the potency and partial relaxation for SALB were decreased (Fig. 2B; Table 1). Although ISO potency was not significantly reduced, the complete relaxation to ISO at low tone was reduced by >80% in tissues contracted to high tone (Fig. 2, C and D; Table 1).

**Relaxation to RGZ is additive with β-adrenoceptor agonists in mouse tracheal segments.** To determine if RGZ had additive effects with β-adrenoceptor agonists, tracheas were precontracted with MCh to 75% maximum before assessment of SALB-mediated relaxation in the absence or presence of RGZ (Fig. 3A). After a submaximal relaxation to RGZ was established (% relaxation to 10 μM RGZ alone: 31.4 ±
RGZ- and CGZ-induced relaxation is independent of PPARγ activation. To explore potential PPARγ dependence, the effects of RGZ were compared with two other PPARγ ligands, the structurally related CGZ and the putative endogenous agonist 15-deoxy-PGJ₂ (Fig. 4A). In mouse tracheal segments precontracted with MCh to similar levels, CGZ and RGZ elicited relaxation at similar potency and efficacy while 15-deoxy-PGJ₂ had no effect (Fig. 4A).

The effect of preincubation with either of the PPARγ antagonists T0070907 or GW9662, at concentrations previously shown to inhibit PPARγ activation (16, 18) on relaxation to RGZ and CGZ was then tested. Both the potency and maximal agonist effect of these dilators were maintained, with both causing near-complete relaxation (Fig. 4, B–D).

**RGZ-induced relaxation is epithelium-independent.** The potential role of epithelial-derived factors in the dilator response to RGZ was assessed. The level of MCh-induced precontraction was similar in matched tracheal segments, irrespective of preincubation with inhibitors or the absence of an intact epithelium (data not shown). RGZ-induced relaxation was maintained in the presence of the COX inhibitor indomethacin and the NOS inhibitor nitro-L-arginine (Fig. 5, A and B). RGZ also still elicited complete relaxation, with no loss of potency, in preparations where epithelium-denudation was confirmed by a significant reduction in relaxation to the epithelium-dependent bronchodilator Substance P (Fig. 5, C and D).

**Contraction of isolated trachea and PCLS to methacholine are differentially altered in a model of chronic AAD.** To confirm our previously reported effects of allergen challenge on contractile responses (6), concentration-response curves to MCh were prepared in tracheal segments and PCLS from saline- and OVA-challenged mice. Trachea from OVA-challenged mice were hyperresponsive to MCh (Fig. 6A), with a >50% increase in the maximum force of contraction (*P < 0.05 vs. SAL, unpaired *t*-test). In PCLS following OVA challenge, MCh potency was decreased approximately threefold (Fig. 6B;
RGZ-induced relaxation of isolated mouse trachea is maintained in a model of chronic AAD. Comparisons of RGZ- and ISO-induced relaxation were made in trachea from saline- and OVA-challenged mice. Tissues were precontracted to 75% of the maximal MCh response in same preparation (Fig. 6A), such that precontraction was relatively higher after allergen challenge, before addition of RGZ (Fig. 7A and B). RGZ was equally effective at causing complete relaxation with no loss of potency in trachea after chronic exposure to OVA (Fig. 7B; Table 2). ISO-mediated relaxation was also maintained under these conditions but failed to fully overcome the precontraction to MCh in tracheal segments from either saline- or OVA-challenged mice (Fig. 7C and D).

RGZ potency is increased in small airways in PCLS from a mouse model of chronic AAD. Dilator responses to RGZ and SALB were compared in perfused PCLS from saline- and OVA-challenged mice, measuring changes in airway lumen area. Reduced MCh potency was established in PCLS from the same mice after OVA challenge (Fig. 6B). The average sub-maximal precontraction with MCh before RGZ was also reduced, but the reduction in airway area in response to this single MCh concentration was variable between PCLS in both groups (Fig. 8A).

RGZ induced complete relaxation in small airways from both saline- and OVA-challenged mice at higher potency than trachea in both groups (Fig. 8B; Table 2). As shown in traces from PCLS from saline- and OVA-challenged mice matched for precontraction, and also in combined results where MCh contraction was relatively lower, RGZ showed an approximately threefold higher potency in PCLS after chronic OVA challenge (Fig. 8, B and C; Table 2). In contrast, SALB failed to fully reverse the MCh-induced contraction in either group (Fig. 8, E and F), while subsequent perfusion with 100 μM RGZ in the same preparations caused complete relaxation (Fig. 8F).

DISCUSSION AND CONCLUSIONS

While inhaled β-adrenoceptor agonists remain the gold standard treatment for relief of asthma symptoms, their efficacy may be limited during a severe asthma attack and reduced by receptor desensitization associated with frequent use (11, 24, 25). There has been increasing recent interest in identifying novel bronchodilators that target alternative mechanisms to overcome some of these limitations.

We have previously demonstrated that RGZ mediates relaxation of small airways in perfused mouse PCLS under conditions of reduced β-adrenoceptor responsiveness, inhibiting...
MCh-induced calcium oscillations and sensitivity (3, 7). In the current study, we have explored dilator responses to RGZ in both trachea and small airways and confirmed its in vitro efficacy after chronic allergen challenge. In summary, we showed that tracheal relaxation to RGZ was not mediated by PPARγ, was independent of epithelial-derived relaxing factors, and unlike β-adrenoceptor agonists was maintained with increasing contraction. Critically, RGZ overcame the hyperresponsiveness to MCh that persisted in vitro in tracheal segments from a mouse model of chronic AAD. Of note, responses to RGZ, but not SALB, were increased in small airways in PCLS from this model. These combined findings demonstrate acute PPARγ-independent dilator responses to RGZ in inflamed airways that are superior to β-adrenoceptor agonists. These actions are in addition to its previously reported inhibitory effects on chronic allergen-induced inflammation, airway wall remodeling, and AHR (reviewed by Ref. 8).

In establishing the therapeutic potential of novel dilator agents, it is critical to characterize their in vitro efficacy relative to existing therapy and to define their underlying mechanisms of action. Our previous studies have described bronchodilator responses to RGZ in small airways in situ in perfused PCLS, opposing contraction to MCh, serotonin, and endothelin-1, with greater efficacy than SALB (3, 7). More recently, we have shown that RGZ and the bitter taste receptor agonist chloroquine, but not SALB or ISO, maintained their potency and efficacy following β-adrenoceptor desensitization. However, only pretreatment with RGZ, but not chloroquine or the β-adrenoceptor agonists, also inhibited the development of MCh-induced contraction and reduced MCh potency in PCLS (7).

We have now extended these studies to confirm an isolated report of dilator actions of RGZ in mouse trachea (12). We demonstrate that RGZ elicits full relaxation in both mouse and guinea pig tracheal segments, albeit at lower potency than the β-adrenoceptor agonists. In mouse trachea where β1-adrenoceptors are known to play a major role in mediating relaxation (13), increasing the level of MCh precontraction markedly compromised the partial relaxation to both SALB and ISO. Consistent with our previous findings in PCLS (3), RGZ was able to maintain potency and elicit marked relaxation in maximally contracted trachea, overcoming the functional antagonism shown to limit β-adrenoceptor responsiveness (19).

Fig. 5. Relaxation to RGZ is not inhibited by INDO, nitro-L-arginine (NOLA), or epithelial removal. Mouse tracheal segments were precontracted to 75% of the maximal MCh response for the same tissue before cumulative addition of RGZ. Responses were compared in the absence or presence of INDO (10 μM) (A) or NOLA (10 μM) (B) or between epithelial-intact and epithelial-denuded tracheal segments (C) in which dilator responses to Substance P (1 μM) were also assessed (D). Relaxation responses (means ± SE) are expressed as %relaxation of the corresponding MCh contraction (ΔmN) for n = 4–8 tracheal segments from different mice. **P < 0.01 vs. control, paired t-test.

Fig. 6. Contraction to MCh is increased in mouse trachea but decreased in precision cut lung slices (PCLS) following ovalbumin (OVA) challenge. Concentration-response curves to MCh were prepared in tracheal segments from saline (SAL)- and OVA-challenged mice (n = 11, 13) (A), expressed as change in force (ΔmN) and PCLS from SAL- and OVA-challenged mice (n = 4, 5) (B), expressed as %initial airway area. Contraction responses are means ± SE.
Dilator responses mediated through β2-adrenoceptors in guinea pig trachea may be of greater relevance to human airways (23, 36). The higher potency and efficacy of SALB in tracheal segments from guinea pigs relative to mice are consistent with this, while the finding of marked relaxation to RGZ at similar potency in both species is suggestive of potential benefit independent of β-adrenoceptor density.

We then explored the potential contribution of PPARγ activation to RGZ-mediated tracheal relaxation. We observed that the rates of relaxation to both RGZ and CGZ, another thiazolidinedione PPARγ agonist, were relatively rapid compared with the time likely to be required for genomic effects via PPARγ. In addition, although both RGZ and CGZ mediated relaxation, a structurally unrelated PPARγ ligand, 15-deoxy-PGJ2, failed to elicit bronchodilation. Critically, relaxation to both RGZ and CGZ was maintained in the presence of two structurally distinct and selective PPARγ antagonists, T0070907 and GW9662 (16, 18). These combined findings suggest that relaxation to both RGZ and CGZ in mouse tracheal segments is independent of PPARγ activation, as we have reported for RGZ in PCLS (3).

We have previously explored mechanisms underlying relaxation to RGZ in PCLS, utilizing the unique capacity of this approach to assess regulation of calcium signaling within ASM by dilator drugs. In this setting, RGZ inhibited the amplitude and frequency of MCh-induced calcium oscillations and reduced the MCh-induced calcium sensitivity of ASM cells when relaxing intrapulmonary airways in PCLS (3). However, the possible contribution of RGZ-mediated release of epithelial-derived relaxing factors to relaxation could not be assessed in perfused PCLS, as this approach precludes the accumulation of factors such as prostaglandin E2 (PGE2) or NO. In the current study, the relatively slow reversal of an established contraction by RGZ in isolated tracheal segments in a static organ bath was suggestive of a mechanism whereby accumulation of epithelial-derived relaxing factors might play a role. Previously, RGZ-mediated relaxation of mouse tracheal segments was associated with increased PGE2 levels and attributed to RGZ-mediated inhibition of PGE2 breakdown rather than its increased synthesis (12). However, we did not find any evidence to support this epithelial-dependent mechanism, since relaxation to RGZ was maintained in the presence of the COX inhibitor indomethacin and also with the NOS inhibitor nitro-L-arginine and following epithelial denudation.

Further assessment of the relative dilator efficacy of RGZ and β-adrenoceptor agonists was conducted when contractile reactivity was altered by allergen challenge. Tracheal preparations and PCLS were prepared from an established model of chronic AAD characterized by in vivo inflammation, epithelial remodeling, airway fibrosis, and AHR (20). We and others

Table 2. Comparison of relaxation responses to rosiglitazone in mouse trachea and in small airways in lung slices from a mouse model of chronic allergic airways disease

<table>
<thead>
<tr>
<th>Tissue/Group (n)</th>
<th>pEC50</th>
<th>Maximal Agonist Effect, %relaxation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (6)</td>
<td>4.4 ± 0.1</td>
<td>103.7 ± 6.7</td>
</tr>
<tr>
<td>OVA (7)</td>
<td>4.4 ± 0.02</td>
<td>100.4 ± 4.4</td>
</tr>
<tr>
<td>Small airway</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (6)</td>
<td>5.5 ± 0.1*</td>
<td>103.0 ± 6.0</td>
</tr>
<tr>
<td>OVA (7)</td>
<td>6.3 ± 0.3‡*</td>
<td>109.1 ± 4.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Average pEC50 and maximal agonist effect values obtained from fitted concentration-response curves from each tissue were compared by unpaired t-tests, as appropriate. *P < 0.05 vs. same treatment in trachea. ‡P < 0.05 vs. saline treatment in small airways.

Fig. 7. RGZ, but not ISO, elicits complete relaxation in trachea from a mouse model of chronic allergic airways disease (AAD). Mouse tracheal segments from saline- and OVA-challenged mice were precontracted to 75% of the maximal MCh response for the same tissue (A and C) before cumulative addition of RGZ (B) or ISO (D). Relaxation responses (means ± SE) are expressed as %relaxation of the corresponding MCh contraction (ΔmN) for n = 3–8 tracheal segments from different mice. **P < 0.05 vs. saline, unpaired t-test.
Fig. 8. RGZ, but not SALB, elicits complete relaxation at higher potency in small airways from a mouse model of chronic AAD. Small airways in PCLS from saline- and OVA-challenged mice were precontracted with MCh (100 nM) (A and D) before cumulative addition of RGZ (B) or SALB (E). Representative traces obtained in slices from saline- and OVA-challenged mice are shown for RGZ alone (C) and SALB followed by 100 µM RGZ (F). Relaxation responses (means ± SE) are expressed as %relaxation of the corresponding MCh contraction (% reduction in airway lumen area) for n = 4–6 PCLS from different mice. *P < 0.05 vs. saline, unpaired t-test.
have previously reported that in vivo hyperresponsiveness to MCh is maintained in isolated trachea from OVA-challenged mice and rats when measured as a greater increase in force (6, 22). In contrast, we have recently shown that MCh is less potent in small airways in after OVA challenge when measured in situ as a change in area in PCLS (6). In this latter setting, we proposed that MCh-induced narrowing in PCLS was opposed by altered interactions of remodeled airways with parenchymal tissue after allergen challenge.

Despite the increased tracheal contraction to MCh after allergen challenge, RGZ elicited complete relaxation, with no loss of potency. This was consistent with its ability to overcome functional antagonism in maximally contracted trachea from naïve mice. Previous studies have shown reduced β-adrenoceptor-mediated relaxation in rat and guinea pig trachea after allergen challenge (4, 5, 21). However, we did not find evidence to support this, with partial relaxation to ISO maintained, although the level of precontraction to MCh was not significantly increased in this subset of OVA-challenged mice.

An increase in small airway sensitivity to RGZ was observed in PCLS from OVA-challenged mice relative to saline-challenged mice. The increased potency of RGZ was evident in traces from single PCLS matched for precontraction from the two groups, as well as group data in which the average contraction to MCh was relatively reduced in PCLS from OVA-challenged mice. These findings were unexpected since RGZ potency in either trachea or PCLS (3) from naïve mice was similar irrespective of the level of precontraction. Relaxation to RGZ may have been increased because the airway narrowing to MCh had been limited by OVA-induced remodeling and altered airway-parenchymal interactions within PCLS. Alternatively, OVA challenge may have had a selective effect on an as-yet unidentified mechanism underlying RGZ-mediated relaxation in small airways. A possible explanation for the failure to see a similar increase in sensitivity to SALB may be that the reduced functional antagonism (dependent on the level of MCh precontraction) was negated by an allergen-induced decrease in β-adrenoceptor density in the peripheral lung, although this was not tested here. Nevertheless, our current and previous findings have now shown in both trachea and PCLS from naïve, saline-, and OVA-challenged mice that RGZ potency is independent of the level of precontraction, with consistently greater efficacy than β-adrenoceptor agonists (3).

The implications of these findings for the treatment of asthma should be considered. An exploratory clinical trial for RGZ has been conducted in smokers with asthma, a difficult-to-treat group that shows resistance to current therapies (31). In this cohort, treatment with RGZ (8 mg, once daily, oral) over 4 wk produced improvements in lung function compared with inhaled beclometasone dipropionate, despite the absence of detectable anti-inflammatory actions. These authors suggested that the improvements in forced expiratory flow values (FEV1) with RGZ treatment may reflect reduced small airway obstruction. More recently, treatment with RGZ (3 mg, twice daily for 28 days, oral) was associated with a modest (15%) reduction in the late asthmatic reaction in the allergen challenge model of asthma (28). Of note, a 12-wk treatment with progressively increasing oral doses of RGZ (2 mg for 4 wk, then 4 mg for 4 wk, then 8 mg for 4 wk) decreased responsiveness to MCh (2.5-fold increase in PC20), albeit without effects on exhaled NO or FEV1 (29). However, these findings have yet to be extended to assess responses to acute inhalation of PPARγ agonists. This route of administration and/or identification of more potent agents eliciting airway relaxation by the same mechanisms as RGZ would minimize the reported adverse cardiovascular effects that have limited the systemic use of RGZ in diabetes (26) and would achieve the higher local concentrations that may be required to exert direct effects on airway contraction.

In conclusion, this study provides additional evidence of dilator efficacy of RGZ under conditions of impaired or limited β-adrenoceptor responsiveness. RGZ was able to oppose increases in MCh-induced contraction and OVA-induced AHR in trachea and showed increased potency in small airways after allergen challenge. The use of RGZ may provide a novel therapeutic approach targeting both large airways and small airways in the distal lung to improve clinical outcomes in patients with severe asthma whose symptoms are poorly controlled with β2-adrenoceptor agonists.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS

REFERENCES
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