**RESPIRATORY DISORDERS**

**Targeted inhibition of Gq signaling induces airway relaxation in mouse models of asthma**

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Obstructive lung diseases are common causes of disability and death worldwide. A hallmark feature is aberrant activation of Gq protein–dependent signaling cascades. Currently, drugs targeting single G protein (heterotrimeric guanine nucleotide–binding protein)–coupled receptors (GPCRs) are used to reduce airway tone. However, therapeutic efficacy is often limited, because various GPCRs contribute to bronchoconstriction, and chronic exposure to receptor-activating medications results in desensitization. We therefore hypothesized that pharmacological Gq inhibition could serve as a central mechanism to achieve efficient therapeutic bronchorelaxation. We found that the compound FR900359 (FR), a membrane-permeable inhibitor of Gq, was effective in silencing Gq signaling in murine and human airway smooth muscle cells. Moreover, FR both prevented bronchoconstrictor responses and triggered sustained airway relaxation in mouse, pig, and human airway tissue ex vivo. Inhalation of FR in healthy wild-type mice resulted in high local concentrations of the compound in the lungs and prevented airway constriction without acute effects on blood pressure and heart rate. FR administration also protected against airway hyperreactivity in murine models of allergen sensitization using ovalbumin and house dust mite as allergens. Our findings establish FR as a selective Gq inhibitor when applied locally to the airways of mice in vivo and suggest that pharmacological blockade of Gq proteins may be a useful therapeutic strategy to achieve bronchorelaxation in asthmatic lung disease.

**INTRODUCTION**

G protein (heterotrimeric guanine nucleotide–binding protein)–coupled receptors (GPCRs) acting via heterotrimeric G proteins are important drug targets for treating a number of disorders. In the airways, Gq-coupled GPCRs are thought to play an important role in controlling the tone of airway smooth muscle. The individual or combined activation of many of these receptors induces an increase in airway resistance, which is the hallmark of airway hyperresponsiveness (1). In addition, increased expression of Gq and Gi proteins has been reported in animal models of asthma (2, 3). Elevated Gq-coupled receptor agonists such as acetylcholine, thromboxane, or histamine have also been found in the airways of patients with asthma (4).

Current treatment regimens for obstructive airway disease such as application of β2 adrenergic agonists or muscarinic antagonists have limitations, because these compounds target only single GPCRs requiring combination therapy in more severely affected patients (5). Moreover, there have been concerns over the longer-term use of β2 agonists because of adverse effects or poor disease control (6). In addition, there exists a group of patients who are partial or complete nonresponders, demonstrating a lack of efficacy for all current therapies targeting GPCRs (7). Although many patients respond well to anti-inflammatory treatment, there remains a subgroup of patients with more severe disease whose asthma control remains suboptimal despite medication. We therefore sought to explore the impact and selectivity of the recently reported Gq protein inhibitor FR900359 (FR) on the regulation of airway tone in health and disease. Direct G protein inhibitors are currently unavailable with the exception of the Gi/o protein inhibitor pertussis toxin, which is a valuable tool for the analysis of Gi function in vitro and ex vivo. Severe side effects have precluded its routine application in vivo, in particular for clinical purposes. We have recently shown that FR is a cell membrane–permeable pan-Gq (Gq, G11, and G14) inhibitor (8). The compound is characterized by high metabolic stability, making it a suitable pharmacological candidate for in vivo use. In addition, the potential for local application makes FR an interesting compound to study airway physiology and disease given that multiple GPCRs converge on Gq in the pathophysiology of airway obstruction.

Herein, we demonstrate that FR specifically inhibited Gq-dependent signaling in airway smooth muscle cells and that this compound strongly reduced airway tone in rodent, pig, and human airways ex vivo. In addition, inhalation of FR effectively and persistently prevented airway constriction in both healthy and allergen-sensitized mice without causing acute cardiovascular side effects.

**RESULTS**

FR is a specific Gq inhibitor in mammalian airway smooth muscle cells in vitro

First, we analyzed the expression of Gq protein isoforms in isolated whole murine lung and trachea using polymerase chain reaction (PCR) and detected all three Gq family members Gq11, and G14 in these tissues. Because airway smooth cells play a key role in regulating airway resistance, we also investigated murine tracheal smooth muscle cells (mTSMCs) and found Gq and G11 expression (fig. S1A). These data were confirmed by immunostaining with strong pan-Gq expression detected in mTSMCs (fig. S1B). Next, we tested the action of the pharmacological Gq inhibitor FR in these cells and discovered that pretreatment of cells with 1 μM FR entirely prevented the increase in intracellular free calcium ion concentration ([Ca2+]i) induced by the thromboxane analog U46619 (fig. S1, C to E). In contrast, FR was
ineffective when intracellular Ca^{2+} was raised by a G_{q/11}-independent mechanism using cyclopiazonic acid (CPA) (Fig. S1, C and D). Next, we investigated the effect and specificity of FR in human bronchial smooth muscle cells (hBSMCs); immunostaining confirmed G_{q} expression in this cell type (Fig. 1A). We used label-free readouts, namely, receptors are known to signal via G_{q/11}, we used HOE140, a B2 receptor known indicator for G_{q/11}-dependent signaling. Bradykinin induced IP1 responses in single mTSMCs, pretreatment with FR (Fig. 1I). We also probed the effect of FR in hBSMCs, demonstrating that these signals were B2-mediated (fig. S1, M).

To further corroborate FR-mediated G_{q/11} inhibition in hBSMCs, we took advantage of an inositol-1-phosphate (IP1) assay, which is a well-established dynamic mass redistribution (DMR) and bioimpedance measurements (Figs. 1, B and C) and largely diminished effects of histamine (Fig. 1D). Similar findings were obtained when FR was applied in bioimpedance measurements before stimulation with bradykinin (Fig. 1E). To show that the bradykinin-induced signals in DMR and bioimpedance measurements were B2 receptor-dependent (B2 receptors are known to signal via G_{q/11}), we used HOE140, a B2 receptor antagonist. Preincubation of hBSMCs with HOE140 (0.1 to 10 μM) resulted in a dose-dependent reduction in bradykinin-induced signals in hBSMCs, demonstrating that these signals were B2-mediated (fig. S1, F and G). Additionally, FR did not have any effect on G_{s} adenyl cyclase, and G_{i} signaling in DMR (fig. S1, H and I) and adenosine 3′,5′-monophosphate (cAMP) accumulation assays (Fig. 1, F to H). These results in label-free biosensor and canonical second messenger assays suggested that FR specifically inhibited G_{q/11} signaling in hBSMCs. To further corroborate FR-mediated G_{q/11} inhibition in hBSMCs, we took advantage of an inositol-1-phosphate (IP1) assay, which is a well-known indicator for G_{q/11}-dependent signaling. Bradykinin induced IP1 production in a dose-dependent manner, and this effect could be abrogated by pretreatment with FR (Fig. 1I). We also probed the effect of FR at the single-cell level using Ca^{2+} imaging. Congruent with our observations in single mTSMCs, pretreatment with the pharmacological G_{q/11} inhibitor FR abolished bradykinin-induced [Ca^{2+}]_i elevation in hBSMCs (96.3 ± 1.7%, n = 10,233 cells), whereas CPA-induced Ca^{2+} release was preserved (Fig. 1, J to L); basal [Ca^{2+}]_i was unaltered upon addition of FR. These experiments indicated that FR is a selective G_{q/11} inhibitor in mouse and human airway smooth muscle cells.

**FR induces dose-dependent relaxation of murine, porcine, and human airways ex vivo**

Next, we tested the impact of FR on airway contraction ex vivo using isometric force measurements. In murine trachea, methacholine induced a dose-dependent elevation in tone (Fig. 2, A and C) that could be abrogated completely by FR (1 μM) pretreatment (Fig. 2, B and C); FR had no prominent effect on basal tone (P > 0.05). When mouse tracheas were preconstricted with methacholine first, cumulative application of FR caused a dose-dependent reversal of the constriction (pEC50: 6.2 ± 0.8, n = 7) (Fig. 2, D and E); neither application of vehicle or FR after constriction of mouse tracheas by G_{q/11}-independent depolarization with KCl solution had such effects (Fig. 2F). A single dose of FR (1 μM) induced complete airway relaxation of mouse tracheae after methacholine-mediated preconstriction, whereas the vehicle did not have any effect (Fig. 2, G and H). These findings show that FR is an effective bronchorelaxant in murine trachea smooth muscle ex vivo.

Because small intrapulmonary airways are also of pathophysiological relevance in obstructive airway disease, we assessed the potential of FR to modulate their tone in murine (precision cut) lung slices. Methacholine application induced a strong reduction in airway lumen area indicative of bronchoconstriction (Fig. 2, I and J). When FR (1 μM) was applied after the agonist, the airways were found to relax completely, returning to basal levels (Fig. 2, I and K), whereas the vehicle had no effect (Fig. 2, J and K). These data demonstrated that FR can reverse G_{q/11}-mediated bronchoconstriction in mouse small intrapulmonary airways ex vivo.

We next examined the efficacy of FR in pig airways, which are known to be similar to human airways. A very low concentration (30 nM) of FR reduced force production in pig airways ex vivo in response to a range of concentrations of carbachol in a time-dependent fashion (Fig. 3A). Similarly, after preconstriction of pig airways with carbachol, FR induced airway relaxation in a dose-dependent manner with effects evident at concentrations of 100 nM or higher (pEC50: 7.1 ± 0.1, n = 4) (Fig. 3, B and C). This effect was persistent, because even after repetitive washout after different durations of FR treatment, only weak contractions upon subsequent carbachol exposure (10 μM) were observed (Fig. 3D). Preincubation with FR (30 nM) also reduced force increases induced by histamine, another important G_{q/11}-coupled bronchoconstrictor, in pig and human airway tissue ex vivo (Fig. 3E). When using low concentrations, the extent of relaxation proved to be strongly time-dependent as FR (300 nM) applied after preconstriction with histamine reached maximal effectiveness after about 45 min (Fig. 3F). Finally, we examined the effect of FR on smooth muscle tone in human airways ex vivo. Human small intrapulmonary airways were preconstricted with histamine, followed by application of increasing doses of FR from 10 nM to 1 μM. As with porcine airways, a strong bronchorelaxation was observed in human airways, starting at 10 nM of FR (pEC50: 7.2 ± 0.2, n = 5) leading to about 80% relaxation at 1 μM FR (Fig. 3G). Thus, FR is a powerful bronchorelaxant in mouse, pig, and human airways ex vivo.

**Inhalation of FR results in prominent airway relaxation without systemic side effects in vivo**

Given the effectiveness of FR for bronchorelaxation ex vivo, we next examined whether FR also exerted strong airway relaxation in vivo. To limit potential systemic side effects of G_{q/11} inhibition such as hypertension (12), we applied FR directly to the lungs of mice either by intratracheal instillation or as an aerosol. We measured lung mechanics and investigated the effect of FR on airway resistance with the low-frequency forced oscillation technique in anesthetized healthy wild-type mice. As would be expected with this readout, we found that increasing concentrations of methacholine strongly elevated respiratory system resistance and that this response was attenuated by inhalation of salbutamol (10 mg/ml, 25 μl), the β2 adrenergic receptor agonist that is the current first-line therapy for the treatment of reversible airflow obstruction (Fig. 4A). Inhalation of FR as an aerosol (0.1 mg/ml, 25 μl, 2.5 μg per animal) virtually extinguished the methacholine-induced elevation of airway resistance but had no effect on basal airway resistance, P > 0.05 FR versus control (Fig. 4A). To determine the duration of the pharmacological effect, we applied a single dose of FR (0.1 mg/ml, 50 μl) into the mouse trachea and measured respiratory system resistance in response to increasing methacholine concentrations 24 hours later. Our results demonstrated that FR was still active at this time point and prevented the increase in respiratory system resistance induced by methacholine at most concentrations (Fig. 4B). Thus, FR can be administered as an aerosol and is a persistent bronchorelaxant in vivo.

Although we used the direct intrapulmonary application route to minimize systemic side effects, we also investigated potential adverse effects in the mouse cardiovascular system. Because we found FR to
Fig. 1. FR inhibits Gq protein signaling in hBSMCs. (A) Immunohistochemical staining of Gq proteins (red) in hBSMCs. Scale bar, 50 µm. (B) Original traces of DMR measurements in response to bradykinin with or without FR pretreatment (quantified as change in reflected wavelength in picometers). (C) DMR measurements for a bradykinin dose-response curve with or without FR. AUC, area under the curve. (D) DMR measurements for a histamine dose-response curve with or without FR. (E) Bioimpedance measurements for a bradykinin dose-response curve with or without FR pretreatment (quantified as change in impedance of extracellular current (dZ)). (F) Induction of cAMP production by isoprenaline with or without FR pretreatment. (G) Induction of cAMP production by forskolin with or without FR pretreatment. (H) Inhibition of cAMP production by methacholine after forskolin stimulation with or without FR pretreatment. (I) IP1 production induced by bradykinin with or without FR pretreatment. (J) Original traces of single-cell Ca2+ imaging experiments in hBSMCs without FR treatment. CPA served as control. (K) Original traces of single-cell Ca2+ imaging experiments in hBSMCs with FR treatment. Each line represents one cell. (L) Statistical analysis of single-cell Ca2+ imaging experiments in hBSMCs without (-) FR (n = 11,252 cells in total) or with (+) FR (n = 10,233 cells in total). (L) ***P < 0.001, unpaired two-tailed t test.
act with fast kinetics after application in our in vivo preparations, blood pressure and heart rate were monitored in anesthetized healthy wild-type mice with a Millar catheter in the carotid artery for 10 min after inhalation of FR (2.5 μg per animal, which is the amount that proved to strongly reduce airway resistance; see Fig. 4, A and B). Our experiments revealed that neither FR nor control solution affected blood pressure (Fig. 4C) or heart rate (fig. S2A) when applied as an aerosol. As a positive control, we administered high aerosol concentrations of the
nitric oxide donor S-nitroso-N-acetyl-penicillamine (SNAP) (100 mM, 50 µl or 200 mM, 20 µl) and found, as expected (13), that it induced a rapid drop in blood pressure (Fig. 4C), underscoring the efficacy of our inhalation application route. Although we could not detect overt acute cardiovascular side effects upon local application of FR to the mouse lungs, we investigated in more detail its effects in the cardiovascular system by injecting FR directly into the jugular vein and monitoring carotid blood pressure and heart rate. We found that intravenous application of 2.5 µg of FR did not alter cardiovascular characteristics (Fig. 4D and fig. S2B), whereas injection of a five times higher dose of FR (12.5 µg per animal) induced a marked drop in blood pressure (Fig. 4D and fig. S2B). We also assessed the biodistribution of FR upon aerosol application by measuring FR concentrations in the plasma and different organs with liquid chromatography coupled to mass spectrometry (LC-MS) 10 min after FR administration. These experiments revealed at least 10 times higher concentrations of the Gq inhibitor FR in the mouse lungs compared to all other tissues, including blood (Fig. 4E). Thus, inhalation of a low amount of FR (2.5 µg) prevented airway constriction without acute effects on the cardiovascular system.

**FR abolishes airway hyperreactivity after ovalbumin-induced sensitization in mice**

The fact that FR induced strong bronchorelaxation ex vivo and in vivo without obvious systemic side effects pointed to its therapeutic potential. We tested this in the well-established murine ovalbumin...
Fig. 4. Local application of FR provides prolonged protection against Gq-dependent airway constriction in normal and OVA-sensitized mice in vivo. (A) Dose-response curves of respiratory system resistance (Rrs) in response to methacholine after inhalation of FR, salbutamol, or vehicle in healthy CD1 wild-type mice. (B) Dose-response curves for respiratory system resistance in response to methacholine treatment 24 hours after intratracheal application of FR in healthy CD1 wild-type mice. (C) Systemic systolic blood pressure (P) in healthy CD1 wild-type mice before (−) and 1 or 10 min after (+) inhalation of 2.5 µg of FR, control solution, or 5 µmol of the NO donor SNAP. (Note that 2.5 µg of FR was the amount that was used for aerosol application and that reduced respiratory system resistance.) (D) Systemic systolic blood pressure in healthy CD1 wild-type mice before (−) and after (+) intravenous injection of 2.5 or 12.5 µg of FR. (E) Tissue and plasma concentrations of FR 10 min after inhalation of 2.5 µg of FR by healthy CD1 wild-type mice. (F) Methacholine dose-response curves for respiratory system resistance after inhalation of vehicle or FR by OVA-treated Balb/c mice. *P < 0.05, **P < 0.01, ***P < 0.001. (A, B, and F) Two-way ANOVA, Bonferroni’s multiple comparison test. (C and E) One-way ANOVA, Tukey’s multiple comparison test (FR, control). (C and D) Paired two-tailed t test (SNAP). *P < 0.05, **P < 0.01, ***P < 0.001 FR versus control (A, B, and F).
We found that, as would be expected, this allergen induced peribronchial, perivascular, and interstitial infiltration of inflammatory cells (Fig. 5, A and B), as well as enhanced mucin expression in mouse airway epithelial cells (Fig. 5, D and E). In this model, inflammation and mucin production could not be prevented by FR (Fig. 5, C and F). Similarly, FR did not reduce the number of eosinophils or macrophages in BAL of mice exposed to the HDM allergen (Fig. 5G). However, intratracheal application

Fig. 5. FR reduces airway hyperresponsiveness in the HDM mouse model of asthma but has no effect on pulmonary inflammation. (A to C) Hematoxylin and eosin staining of lung sections from control (A), HDM-exposed (HDM) (B), and FR-treated HDM-exposed (C) Balb/c mice. (D to F) Periodic acid–Schiff staining of lung sections from control (D), HDM-exposed (E), and FR-treated HDM-exposed (F) Balb/c mice. Scale bar, 50 µm. (G) Cell counts in BAL fluid for control, HDM-exposed, and FR-treated HDM-exposed Balb/c mice. Macro, macrophages; Eosino, eosinophils; Neutro, neutrophils; Lympho, lymphocytes. (H) Dose-response curves for respiratory system resistance in response to inhaled methacholine in control and FR-treated Balb/c mice exposed to HDM. (I to K) Sirius red staining of lung sections from control (I), HDM-exposed (J), and FR-treated HDM-exposed (K) Balb/c mice to assess collagen deposition. Scale bar, 20 µm. (L) Quantitation of collagen deposition in lung sections from control, HDM-exposed, and FR-treated HDM-exposed mice. Collagen area per micrometer length of cell basement membrane is displayed. *P < 0.05, ***P < 0.001. (H) Two-way ANOVA, Bonferroni’s multiple comparison test. (L) One-way ANOVA, Tukey’s multiple comparison test.
of FR before administration of the allergen was highly effective at preventing airway hyperresponsiveness (Fig. 5H). Given that FR has been reported to reduce airway smooth muscle growth in vitro (15), we also investigated airway remodeling and potential modulatory effects of FR in this asthma mouse model. Quantitation of collagen deposition revealed a strong increase around airways in mice administered with HDM allergen, an indication of early airway remodeling. We found that FR could prevent this increase in collagen deposition in the HDM mouse model of asthma (Fig. 5, I to L). Next, we investigated airway smooth muscle mass, an indicator of chronic airway remodeling, but could not detect differences in lung sections from control mice (1.5 ± 0.1 μm²/μm, n = 5, phosphate-buffered saline (PBS)), mice treated with HDM allergen (1.9 ± 0.3 μm²/μm, n = 6, P > 0.05 versus controls), and FR-treated mice exposed to allergen (1.8 ± 0.1 μm²/μm, n = 5; P > 0.05 versus allergen). Thus, our data demonstrate that intratracheal application of FR does not affect airway inflammation, whereas it is a powerful inhibitor of bronchoconstriction in allergen-sensitized mice in vivo and also prevents early stages of airway remodeling.

**DISCUSSION**

Herein, we demonstrate that the Gq inhibitor FR selectively abrogated Gq-dependent signaling and induced bronchodilation in rodent, pig, and human airway tissue when tested ex vivo. When applied in healthy and sensitized mice in vivo, we found that inhalation of FR prevented the elevation of airway resistance without acute effects on blood pressure or heart rate. In addition, FR also suppressed airway hyperreactivity and even early airway remodeling, but not inflammation in mouse models of airway hyperresponsiveness.

Earlier studies targeting the Gq pathway used either genetic approaches (12, 16, 17) or compounds that interact with GPCRs (15, 18) or Gq downstream signaling (19). A Gq inhibitor isolated from *Chromobacterium* sp. QS3666 (YM-254890) illustrated the therapeutic potential of pharmacological G protein inhibition, because it promoted antithrombotic and vasodilatory effects in mice, rats, and monkeys (20–23). However, in these studies, the Gq inhibitor was often administered as a bolus injection directly into the bloodstream, yielding depression of the cardiovascular system. We could recapitulate this effect upon intravenous injection of a five times higher FR dose than that required for airway relaxation; this was comparable to the quantity of YM-254890 that was reported to lower systemic blood pressure (21). We postulated that the lung is an ideal organ for the therapeutic use of a pharmacological Gq inhibitor, first because of the prominent role of Gq proteins in the regulation of airway tone in health and disease, and second, because it is feasible to administer drugs locally via inhalation (24). We, therefore, tested FR in human airway smooth muscle cells as well as in mouse, pig, and human tissue ex vivo and found that it is a highly selective and powerful inhibitor of Gq signaling and a strong agent for inducing bronchodilation. In addition, FR can be applied locally via inhalation, yielding much higher concentrations of the compound in lung tissue compared to other organs or plasma. Although we could not detect any cardiovascular adverse effects using the inhalation route, longer-term studies will need to be performed to investigate the bioavailability and the distribution of FR; these experiments will also serve to rule out potential side effects upon long-term application of the compound.

Because relevant GPCRs inducing bronchoconstriction converge on Gq (4), the airway-relaxing effect of a Gq inhibitor would be expected to be more powerful than that of the single GPCR agonists or antagonists that are currently used for the pharmacotherapy of obstructive lung disease. Our in vivo experiments underscored this, because inhalation of FR more effectively reduced airway resistance than did a high concentration of the β2 agonist salbutamol, the standard bronchodilator used for treating obstructive airway disease (7). Moreover, FR completely prevented and reversed Gq agonist–induced airway constriction, suggesting that Gq-dependent signaling rather than a receptor phosphorylation–dependent pathway (25) was primarily responsible for airway constriction ex vivo and in vivo. In our earlier study, we reported the long-lasting action of FR in human embryonic kidney 293 cells and in vascular tone regulation of the murine tail artery ex vivo (8). Here, we also observed a long-lasting effect of FR on rodent, pig, and human airways ex vivo. Single-dose inhalation of FR led to persistent protection against Gq-dependent airway constriction for at least 24 hours in mice in vivo. This direct airway relaxation effect was the reason for FR’s protective effect against airway hyperresponsiveness. Inflammation proved to be unaltered upon intratracheal application of FR before OVA or HDM allergen administration. Our study has some limitations that need to be addressed in the future. In vivo airway resistance measurements only permitted determination of the effects of bronchodilators applied before bronchoconstriction, whereas in patients, airway relaxants are administered after the onset of bronchoconstriction. Although we could exclude acute cardiovascular side effects of FR, future work will need to investigate longer-term FR effects on other organ systems and during chronic application of FR. Furthermore, our experiments have been restricted to acute asthma mouse models. More studies in large animal models are warranted to explore the therapeutic potential of FR for the treatment of chronic obstructive airway diseases. These studies will also provide information on the impact of FR to prevent or reverse airway remodeling in chronic lung disease. For that purpose, different timings and application routes of FR (inha- lation versus systemic) need to be tested. Our data demonstrate a dominant role of Gq signaling in the control of airway resistance and identify FR as a promising new bronchodilator agent in animal models.

**MATERIALS AND METHODS**

**Study design**

The research objective of the study was to demonstrate that the pharmacological Gq inhibitor FR is a specific Gq inhibitor in rodent and human airway smooth muscle cells and can be used to relax airways ex vivo and in vivo. The hypothesis was that local pulmonary application of the compound would induce a reduction of airway resistance in vivo and would show no overt cardiovascular side effects. The airway relaxation properties of FR were demonstrated in two mouse models of airway hyperreactivity. All experiments were performed with appropriate controls. All cells, tissues, and animals were randomly assigned to the experimental groups. For nebulizer measurements, animals starting spontaneous breathing during the recording were excluded because this compromised the quality of the recording. Buffer controls in fig. S1F were also included in Fig. 1C. In label-free DMR and impedance measurements, only some FR concentrations were tested in each experiment because of the limited number of airway smooth muscle cells.

**Label-free DMR measurements**

The DMR assay used a label-free biosensor for tracking the dynamic redistribution of cellular matter after cell stimulation. The DMR signal thereby represented a shift in resonant wavelength indicated in
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5′-ACGAGGTAGGAATGCTGAGC-3′ (forward) and 5′-CCATCCTGAAGATGTTCTCC-3′ (reverse); and G14, 5′-TCACCTGACCTCTGAGAACC-3′ (forward) and 5′-GACATCTGTGTTGTCGTTG-3′ (reverse). PCR products were separated by 2% agarose gel electrophoresis. DNA bands were visualized by ethidium bromide staining of the gel, and the size of the fragments was confirmed by a DNA marker.

**cAMP and IP1 accumulation assays**

Changes of the intracellular second messengers cAMP and IP1 were quantified with the homogeneous time-resolved fluorescence (HTRF)–cAMP dynamic kit and the HTRF-IP1 kit, respectively (CisBio International), on a Mithras LB 940 reader (Berthold Technologies) according to the manufacturer’s instructions and as described elsewhere in detail.

For analysis, the AUC values of DMR signals between 0 and 3600 s were used to calculate agonist activity. AUC values were transformed into relative AUC units to give equivalent baseline optical recordings for all dose-response curves from any given assay plate. Data were normalized and expressed as percent of maximum activation induced by a saturating concentration of the indicated agonist, which was set to 100%. n indicates the number of independent experiments, and each data point represents a triplicate.

**Label-free impedance measurements**

Label-free impedance assays were performed as described earlier.

**Single-cell Ca2+ imaging experiments**

Single-cell Ca2+ imaging experiments were performed as described earlier (28). MTSMCs grown on a coverslip were loaded for 30 min with the ratiometric dye fura-2 AM (10 μM) in physiological salt solution (PSS). Then, the coverslip was transferred to a custom-made chamber of an inverted microscope and washed with PSS. Excitation light (340 nm, 380 nm) was generated by a computer-controlled monochromator (Polychrome V, TILL Photonics). The fluorescence of the cells (340 nm, 380 nm) was generated by a computer-controlled monochromator (Polychrome V, TILL Photonics). The fluorescence of the cells was imaged through a 510-nm long-pass filter and recorded by a charge-coupled device (CCD) camera (ORCA-R2, Hamamatsu). Pictures were acquired at 0.7 Hz and 30-ms exposure time using the TILLVision software (TILL Photonics).

**Reverse transcription–PCR experiments**

Reverse transcription PCR (RT-PCR) experiments were performed as described earlier (29), (30). Briefly, at least three murine tracheae were dissected from one of the lobes of the lung and placed in ice-cold Krebs-Henseleit buffer containing 2% Ficoll that had been pregassed with 95% O2/5% CO2 and stored overnight at 4°C. The following day, bronchioles (~1 mm in diameter) were dissected into 3-mm ring segments and mounted in the wire myograph containing Krebs-Henseleit buffer maintained at 37°C and constantly gassed with 95% O2/5% CO2. After a 20-min equilibration period, tension was applied to the tissue, which was allowed to relax to a final resting tension of between 0.4- and 0.7-g weight. Before each experiment, the tissues were contracted twice with 60 mM KCl. For FR dose-response curves, tissues were precontracted with the cholinergic receptor agonist carbachol to give a contraction that was around 100% of the response to the final addition of 60 mM KCl. Previous studies demonstrated that this is about 50% of the maximum response to carbachol (33) and that contractions are well maintained over time. After the carbachol-induced tone had reached a plateau, cumulative additions of FR were applied.

For the measurement of human airways, lungs were obtained from patients undergoing lobectomy through Nottingham Health Science Biobank. Segments of small bronchioles (~1 mm in diameter) were dissected out and mounted in the wire myograph in Krebs-Henseleit buffer as above. After contraction with 60 mM KCl twice, tissues were
precontracted with histamine to around 100% of the response to KCl. Cumulative concentration–response curves to FR were carried out as above. Rmax values were obtained by using a nonlinear fit log(agonist) versus response (three parameters), Hill slope = 1.

**Generating precision cut lung slices**

Precision cut lung slices were generated as described by Wenzel et al. (34). Mice were euthanized, then the trachea was cannulated, and the lungs were filled with a warm solution of 2% low–melting point agarose (Roth). The agarose was flushed into the alveoli by injecting a small volume of air using a Saf-T-Intima catheter (BD). Then, warm gelatin solution (6%) was perfused into the pulmonary vasculature via the right ventricle. The mouse was transferred to the fridges for gelling of agarose and gelatin at 4°C. Afterward, the lung was removed from the mouse, and single lobes were separated. Lung slices (200 μm thick) were cut with a vibratome (VT1200 S, Leica) and stored in serum-free medium overnight in the incubator. Experiments were performed on the next day. The slices were perfused in a custom-made chamber on the stage of an inverted microscope, and pictures of small intrapulmonary airways were taken by a CCD camera at a sampling frequency of 1 Hz. For analysis, the changes in lumen area of the airways were determined by custom-written software by pixel summing and indicated as difference from basal area as a percent.

**Animal experiments**

Animal work was performed in female 10- to 12-week-old CD1 wild-type or Balb/c mice (Charles River). Animal housing and experiments were approved by the local ethics committee and carried out according to the guidelines of the German law of protection of animal life with approval by the local government authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, NRW, Germany).

Ethical approval for the use of human tissue study was granted by the Nottingham Health Science Biobank Access Committee under their RBT approval from the North West–Greater Manchester Central Research Ethics Committee (REC reference number 15/NW/0685).

**Lung function measurements**

Experiments were performed with the flexiVent system (Scireq). Mice were anesthetized with a combination of fentanyl (50 μg/kg), medetomidine (0.5 mg/kg), and midazolam (5 mg/kg). Then, the trachea was cannulated and animals were ventilated with a tidal volume of 10 ml/kg at 150 breaths/min and a positive end-expiratory pressure of 3 cmH2O. Two deep inflation maneuvers were performed for airway recruitment before the measurements were started. Resistance was determined by “snapshot” perturbations based on the single-component model. To induce airway constriction, increasing doses of methacholine (0, 6, 12.5, 25, and 50 mg/ml, 25 μl) were applied as an aerosol via the Aeroneb Lab nebulizer (AG-AL1100, Aerogen), producing a standard volumetric flow of 350 ml/min at 180 breaths/min. Next, a small pressure catheter (1F) was inserted via the right carotid artery. Systemic pressure was recorded using the Millar Aria 1 system (Millar), whereas FR (0.1 mg/ml, 25 μl) or SNAP (100 mM, 50 μl or 200 mM, 25 μl) was applied as an aerosol directly into the trachea. Alternatively, FR (0.2 or 1 mg/ml, 12 μl) was injected into the left jugular vein. Inspection was performed with a custom-made motorized injector using a 10-μl Hamilton syringe connected to a 33-gauge needle (Terumo) via a small tube. Arterial systolic pressure 1 and 10 min after nebulization or injection was compared with basal pressure levels before injection.

**Asthma induction with OVA**

For asthma experiments, female Balb/c mice (Charles River) were used at 10 weeks of age. Sensitization was performed by applying 20 μg of OVA (Sigma-Aldrich) adsorbed in Imject Alum (2 mg/ml) (Thermo Scientific) by intraperitoneal injection of 100 μl per mouse on days 0 and 14 (34). Mice were challenged by nebulization of 1% OVA (5 ml) or NaCl (control) for 30 min per day on days 21, 22, and 23. When FR was applied before OVA challenge, FR (0.1 mg/ml in 50 μl NaCl) or the solvent control was administered 30 min before OVA nebulization. Analysis was performed on day 24 or 25.

**Asthma induction with HDM allergen**

For asthma induction with HDM (D. pteronyssinus), B82, Stellargenes Greer), female Balb/c mice at 10 weeks of age were used. Sensitization with intratracheal application of 1 μg of HDM (in 50 μl of PBS) per mouse was performed on day 0. Then, mice were challenged by intratracheal application of 10 μg of HDM (in 50 μl of PBS) on days 7 to 11. Control mice received 50 μl of PBS intratracheally on the respective days. Mice that received HDM were treated with either control solution or FR (0.1 mg/ml, 50 μl) 4 hours before each HDM application on day 0 and days 7 to 11. Analysis was done on days 13 and 14.

**Statistical analysis**

Data are indicated as means ± SEM. Statistical differences were determined using one- or two-way ANOVA and Tukey’s or Bonferroni’s post hoc test for multiple comparisons or paired or unpaired two-tailed Student’s t test for comparing two experimental groups, as indicated in the figure legends. P < 0.05 was considered significant.

Statistical analyses were performed using GraphPad Prism software.

**Supplementary materials**

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**References and notes**


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Abstracts

One-sentence summary: Local pharmacological inhibition of $G_q$ results in airway relaxation in mouse models of asthma.

Editor’s Summary: Breathing freely

Obstructive lung diseases are common disorders characterized by airway narrowing. Because some patients do not respond well to current therapies or suffer from side effects, new drugs are needed. Matthey et al. now report that the selective $G_q$ inhibitor FR900359 reduces airway tone in mouse, pig, and human airways ex vivo and decreases airway resistance in mouse models of asthma in vivo. The compound has the advantage that it can be locally applied to the lung via inhalation and shows promising properties that may prove useful for treating obstructive airway disease.