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Methacholine-induced airway hyperresponsiveness is dependent on $G\alpha_q$ signaling

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Department of Biochemistry and Molecular Biology, [1]Division of Pulmonary Medicine, [2]Division of Hematology and Oncology, Mayo Clinic Scottsdale, Scottsdale, Arizona 85259; [3]Division of Biology, California Institute of Technology, Pasadena, California 91125; and [4]Pharmacology Department, University of Texas Southwestern, Dallas, Texas 75390-9041

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Borchers, Michael T., T. Biechele, J. P. Justice, T. Ansay, S. Cormier, V. Mancino, T. M. Wilkie, M. I. Simon, N. A. Lee, and J. J. Lee. Methacholine-induced airway hyperresponsiveness is dependent on $G\alpha_q$ signaling. *Am J Physiol Lung Cell Mol Physiol* 285: L114–L120, 2003. First published February 28, 2003; 10.1152/ajplung.00322.2002.—Airway function in health and disease as well as in response to bronchospastic stimuli (i.e., irritants, allergens, and inflammatory mediators) is controlled, in part, by cholinergic muscarinic receptor regulation of smooth muscle. In particular, the dependence of airway smooth muscle contraction/relaxation on heterotrimeric G protein-coupled receptor signaling suggests that these events underlie the responses regulating airway function. $G\alpha_q$-containing G proteins are proposed to be a prominent signaling pathway, and the availability of knockout mice deficient of this subunit has allowed for an investigation of its potential role in airway function. Airway responses in $G\alpha_q$-deficient mice (activities assessed by both tracheal tension and in vivo lung function measurements) were attenuated relative to wild-type controls. Moreover, ovalbumin sensitization/aerosol challenge of $G\alpha_q$-deficient mice also failed to elicit an allergen-induced increase in airway reactivity to methacholine. These findings indicate that cholinergic receptor-mediated responses are dependent on $G\alpha_q$-mediated signaling events and identify $G\alpha_q$ as a potential target of preventative/intervening therapies for lung dysfunction.

G protein; gene knockout mice

AIRFLOW LIMITATIONS IN PATIENTS with pulmonary disease, most notably chronic obstructive pulmonary disease (COPD) (12, 13, 37) and asthma (27, 29), are controlled by receptor-ligand interactions that signal through G protein-coupled receptors. In particular, M3 and M2 muscarinic receptors on airway smooth muscle appear to regulate bronchomotor responses (8), including the demonstration that signaling through M3 receptors is capable of eliciting contraction of smooth muscle (32), whereas M2 receptors contribute to contraction by inhibiting the relaxation of smooth muscle (9). Specifically, acetylcholine released from parasympathetic cholinergic nerves bind M3 receptors on the smooth muscle, leading directly to contraction via a series of intracellular signaling events that are dependent on sustained increases in intracellular calcium and smooth muscle myosin light chain phosphorylation/activation (35). The responses elicited by cholinergic receptor binding are dependent on the activation of receptor-coupled heterotrimeric G proteins and the subsequent generation of specific effector signaling molecules such as phosphoinositol and diacylglycerol. Among the four families of G proteins (G_i, G_s, G_q, G_{12}) (39), the M3 receptor preferentially couples to the G_q family (6), whereas the M2 receptor preferentially couples to the G_i family (8).

The G_q family includes four members identified by their α-subunits as G_q, G_{11}, G_{14}, and $G_{15/16}$ (40). These proteins are functionally similar, yet multiple members of the G_q family are usually coexpressed in the same cells (40). This has led to several investigations aimed at examining whether the various members of the G_q family, most notably G_q and G_{11}, display distinct functional coupling to receptors or whether they are interchangeable with multiple receptors. For example, G_q and G_{11} have comparable abilities to activate phospholipase C in transfected cell systems (21, 28), and Xu et al. (43) have demonstrated that G_q and G_{11} promiscuously couple M3 receptors to mediate identical calcium signaling responses in pancreatic and submandibular gland cells. Furthermore, knockout mice deficient for G_q and G_{11} exhibit functional redundancy in craniofacial development and cardiomyocyte proliferation in the fetus (31) and cardiomyocyte hypertrophy in adults (38). Alternatively, distinct functions of G_q and G_{11} following activation of α_1-adrenoreceptors have been demonstrated in rat portal vein myocytes (25), and the involvement of G_q, but not G_{11}, has been shown in G protein-deficient fibroblasts in response to *Pateurella multocida* bacterial toxin (44).

Gene knockout mice deficient in the α-subunit of G_q ($G\alpha_q^{-/-}$ mice) were utilized to determine the role of this G protein in airway responsiveness. The data show that mice deficient in G_q are hyporesponsive to cholin-

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ergic receptor activation. These responses are much greater in whole animal studies than in isolated trachea, suggesting that effects of the $G\alpha_q$ deletion may not be smooth muscle autonomous but instead affect multiple cell types contributing to airway responsiveness in vivo. Furthermore, mice lacking G_q were unable to develop airway hyperresponsiveness (AHR) in an allergen-induced model of increased airway reactivity. Together, these data demonstrate an important role for G_q in the development of airway resistance and AHR.

MATERIALS AND METHODS

Mice. The generation of $G\alpha_q$ (30) knockout mice has been previously described. Mice were maintained on a 129/Sv × C57BL/6 crossbred background. All procedures were conducted on mice 10–14 wk of age that were maintained in microisolator cages housed in a specific pathogen-free animal facility. The sentinel cages within this animal colony were negative for viral antibodies and the presence of known mouse pathogens. Protocols and studies involving animals were conducted in accordance with National Institutes of Health and Mayo Clinic Foundation guidelines.

Western blot analysis. Protein was extracted from tracheal homogenates including epithelium, smooth muscle, and cartilage and subjected to SDS-PAGE (10% polyacrylamide). Rabbit antisera specific for $G\alpha_q$, $G\alpha_{11}$, and $G\alpha_{q/11}$ have been described in Hepler et al. (16). We detected proteins (~42 kDa) using the ECL Chemiluminescence Western Blotting Detection Kit (Amersham, Piscataway, NJ) with a donkey anti-rabbit IgG, horseradish peroxidase-linked secondary antibody (Amersham).

Tissue preparation and measurement of isometric tension. Measurement of mouse tracheal contractility was performed as previously described (18). Briefly, mice (20–30 g) were euthanized with 500 mg/kg pentobarbital sodium, and distal segments of trachea containing six cartilaginous rings (containing epithelium and cartilage) were rapidly removed and placed, at room temperature, in modified Krebs-Henseleit (KH) solution [(in mM) 116 NaCl, 4.7 KCl, 2.5 CaCl, 0.5 $MgCl_2$, 25 $NaHCO_3/NaH_2PO_4$, and 11 glucose] that was continuously oxygenated (95% O_2-5% CO_2) to maintain a pH of 7.4. No differences were observed in tissue wet weight between the two groups. Excess tissue was subsequently removed, and the tracheas were mounted longitudinally on stainless steel rings in a 30-ml water-jacketed organ bath (model 159920; Radnotti, Monrovia, CA) containing continuously oxygenated modified KH maintained at 37°C with a thermocirculator (model 50–1932; Harvard Apparatus, Holliston, MA). We allowed the mounted tracheas to equilibrate for 30 min before suspending them upright on glass hooks under a tension of 0.8 g. Stable tension was achieved after approximately five cycles of 15-min incubations, washings, and readjustments of tension to 0.8 g. Changes in force were measured isometrically with a force-displacement transducer (Harvard Apparatus) and collected with a BIOPAC data-acquisition unit and BIOPAC Acqknowledge software (BIOPAC, Santa Barbara, CA). Once a stable tension was established, the tracheal smooth muscle was depolarized with KCl in KH solution at a final concentration of 50 mM. Hyperosmotic KCl exposure was maintained until the isometric force generated by the tracheal contraction reached a plateau. The tracheas were then washed for two cycles to achieve a stable resting tension before methacholine (MCh) dose-response measurements. Increasing doses of MCh (10^{-9} M–10^{-4} M) were applied, allowing the isometric forces to plateau. Active tension (i.e., tension generated above resting tension) is reported as a percentage of the maximal tension obtained with 10^{-4} M MCh.

Measurements of lung mechanics. Respiratory system mechanics were assessed in mice according to the method of Gomes et al. (11). Mice were anesthetized with pentobarbital sodium (50 mg/kg ip, Nembutal; Abbott, Chicago, IL). Tracheas were surgically accessed through a ventral midline incision and connected with a small animal ventilator (SAV, FlexiVent; SCIREQ, Montreal, Quebec, Canada) via an 18-gauge needle. Mice were subsequently paralyzed with doxacurium chloride (0.5 mg/kg) (Nuromax; Catalytica, Greenville, NC) and ventilated at a frequency of 150 breaths/min and at a volume of 6 ml/kg. The mice expired passively through the expiratory valve of the ventilator against a positive end-expiratory pressure of 3 cmH_2O. MCh aerosol was generated with an in-line nebulizer (model 5500D; DeVilbiss, Somerset, PA) and administered directly through the ventilator. We determined the resistive properties of the lungs at constant volume using forced oscillations by the SAV. The oscillations consisted of applying a small-amplitude volume perturbation at 3 Hz to the airway opening. Measurements of piston volume displacement and cylinder pressure were used to calculate the impedance of the respiratory system from which respiratory system resistance values were derived.

Whole body plethysmography. We estimated total pulmonary airflow in unrestrained conscious mice with a whole body plethysmograph (Buxco Electronics, Troy, NY). Pressure differences between a chamber containing the mice and a reference chamber were used to extrapolate minute volume, tidal volume, breathing frequency, and enhanced paused (Penh). Penh is a dimensionless parameter that is a function of total pulmonary airflow in mice during the respiratory cycle. Penh is described by the equation Penh = (PEP/PIP) × Pause; where PEP is the peak expiratory pressure, PIP is the peak inspiratory pressure, and Pause is a component of expiration time. This parameter is dependent on the breathing pattern of the mice and was also shown to correlate with airway resistance as measured by traditional invasive techniques on ventilated mice (15).

Lung histology. We obtained lung tissue for histological analysis by instilling ~1 ml of 10% neutral-buffered formalin (30 cmH_2O constant pressure) through a cannula inserted into the trachea. After instillation of fixative, the trachea was ligated, and the excised lung was immersed in formalin for 24 h (at 4°C). Two nonconsecutive parasaggital sections (5 μm) were obtained from paraffin-embedded tissue, stained with hematoxylin and eosin, and analyzed by bright-field microscopy ($n = 5$ mice per group).

Antigen sensitization and challenge. Mice (6–8 wk) were sensitized by an intraperitoneal injection (100 μl) of 20 μg of chicken ovalbumin (OVA; Sigma, St. Louis, MO) emulsified in 2 mg of Imject Alum [$Al(OH)_3/Mg(OH)_2$; Pierce, Rockfield, IL] on *days 0* and *14*. Mice were subsequently challenged with an aerosol generated from 1% OVA in saline or saline alone for 20 min by ultrasonic nebulization (DeVilbiss) on *days 24*, *25*, and *26*. Assessments of airway responsiveness were made on *day 28*.

Statistical analysis. Data presented are the means ± SE. Statistical analysis was performed on parametric data using *t*-tests with differences between means considered significant when $P < 0.05$.

RESULTS

Tracheal airway smooth muscle from $G\alpha_q^{-/-}$ mice are hyporesponsive to MCh ex vivo. Isometric force generation assessments of tracheal smooth muscle in response to 50 mM KCl (i.e., depolarized maximal contraction) showed that no intrinsic differences exist between wild-type and $G\alpha_q^{-/-}$ mice regarding the capacity of these tissues to constrict (Table 1). However, MCh dose-response curves revealed that the tracheas isolated from $G\alpha_q^{-/-}$ mice were hyporeactive relative to wild type (Table 1 and Fig. 1*A*). This response was similar when plotted either as percent maximum response to MCh or as a percentage of maximum KCl response. Despite this shift in responsiveness, the tension generated in response to high doses of MCh by $G\alpha_q^{-/-}$ tracheas reached a plateau similar to wild-type tracheas (Table 1). Western blot analysis of the protein from the tracheal preparations confirmed that the knockout mice did not express $G\alpha_q$. Moreover, these data demonstrated that the expression level of another subfamily member, $G\alpha_{11}$, was significant in the trachea of wild-type and $G\alpha_q^{-/-}$ mice and was unaffected by the loss of $G\alpha_q$ (Fig. 1*B*).

$G\alpha_q^{-/-}$ mice are hyporesponsive to cholinergic stimuli in vivo. The consequences of altered smooth muscle responses were further examined in vivo by invasive measurements of airway resistance and whole body plethysmography. The lung mechanics of the $G\alpha_q^{-/-}$ mice differed significantly from wild-type littermates in response to MCh administration (Fig. 2). Lung resistance induced by MCh was minimally affected in $G\alpha_q^{-/-}$ mice by even the highest doses of MCh (50–100 mg/ml). Similar airway responses to inhaled MCh and serotonin (an agonist that acts presynaptically to induce acetylcholine release) were observed in these mice by whole body plethysmography. $G\alpha_q^{-/-}$ mice exhibited minimal reactivity to MCh regardless of the dose applied (Fig. 3*A*) and no significant reactivity to serotonin even at doses that resulted in maximum reactivity of wild-type mice (Fig. 3*B*).

Airway morphology of $G\alpha_q^{-/-}$ mice is indistinguishable from wild-type mice. Microscopic examination of the lungs from wild-type and $G\alpha_q^{-/-}$ mice revealed no obvious differences between the two groups (Fig. 4, *A* and *B*).

$G\alpha_q^{-/-}$ mice fail to exhibit airway reactivity to MCh in a model of allergen-induced AHR. Airway reactivity of OVA-sensitized/aerosol-challenged $G\alpha_q$-deficient mice following provocation with MCh was examined by whole body plethysmography to determine whether $G\alpha_q$-mediated signaling is also required for the development of allergen-mediated AHR. In contrast to OVA sensitization/aerosol challenge of wild-type mice, which leads to a significant increase in airway obstruction (i.e., Penh levels) relative to control mice challenged with saline only, $G\alpha_q^{-/-}$ mice challenged with OVA did not demonstrate an increase in Penh compared with a $G\alpha_q^{-/-}$ control group challenged with saline only (Fig. 5). It is noteworthy that in addition to this failure to develop AHR to MCh, neither group of $G\alpha_q$ mice (i.e., saline or OVA treated) had a significant dose-dependent response to MCh relative to saline-treated control wild-type animals.

Table 1. *Active tension (g) generated in response to KCl and methacholine*

	Wild Type	$G\alpha_q^{-/-}$
KCl, 50 mM	0.84 ± 0.09	0.81 ± 0.08
Methacholine, 10^{-4} M	2.12 ± 0.09	2.14 ± 0.11
EC_{50}, μM	0.31 ± 0.05	0.67 ± 0.07*

Values are means ± SE (n = 10–12 mice per group). $G\alpha_q^{-/-}$, gene knockout mice deficient in the α-subunit of G_q. *Significantly different ($P < 0.05$) from wild-type mice.

DISCUSSION

The provocative conclusion derived from the data presented is that signaling pathways mediated by the heterotrimeric G protein G_q are critical in the control of airway function in response to cholinergic receptor activation, potentially through effects on airway smooth muscle contraction. These effects appear to be complex, as tracheal tension measurements of $G\alpha_q^{-/-}$ mice differed quantitatively and qualitatively from the effects observed in whole animal experiments. Specifically, the effects of MCh on tracheal rings appear to be less pronounced compared with the in vivo assessments. The reasons for this discrepancy are unclear; however, several possible mechanisms may account for the observed differences: *1*) the resistance to airflow is inversely proportional to the fourth power of the radius of the airway (26). Therefore, small changes observed in airway smooth muscle shortening (tracheal tension) will be magnified in the in vivo measurements, because radius is the most important determinant of the resistance to airflow (e.g., decreasing the radius of an airway by half will increase the resistance to flow 16-fold). *2*) The differences observed between these two assessments of smooth muscle contraction may reflect differences in the contributions of different airway generations and differences in $G\alpha_q$-coupled receptor distribution along these airways. The airways that primarily contribute to the pressure changes measured in the in vivo experiments are thought to be the midsized and smaller-diameter intrapulmonary airways (i.e., not the trachea) (24). This hypothesis is supported by observed differences in muscarinic receptor density and distribution observed throughout the airways. Emala and colleagues (10) have shown that M2 receptors are the predominant receptor in the trachea and that they continually diminish in density in the bronchi and peripheral lung, whereas the M3 receptor is present at ~[1/10] the density of M2, but the relative density of the M3 increases continually (compared to the M2 receptor) in the bronchi and peripheral lung. *3*) It is possible that other cell types besides smooth muscle may represent a significant component of the signaling process involved in airway smooth muscle contraction. Airway responsiveness in response to a cholinergic receptor such as MCh is a complex phenotype influ-

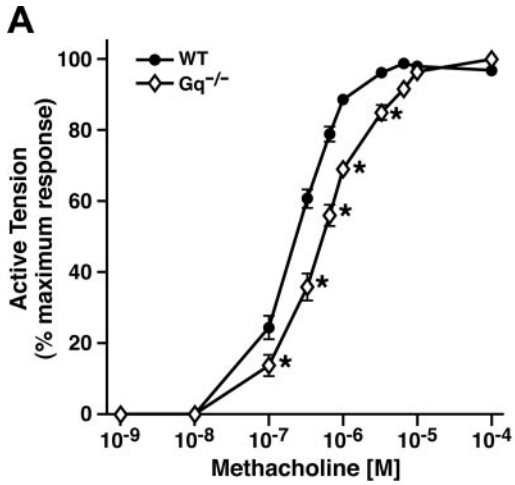

Fig. 1. Tracheas isolated from G_q-deficient mice are hyporesponsive to methacholine (MCh). *A*: active tension generated in response to increasing doses of MCh is plotted as the percentage of maximum response generated by MCh. Values presented are means ± SE (n = 10–12 mice/group). *Significantly different ($P < 0.05$) from wild-type (WT) mice. *B*: Western blot analyses of proteins isolated from the trachea of WT and $G\alpha_q$-deficient ($G\alpha_q^{-/-}$) mice. Antisera are specific for the indicated α-subunit except for the anti-$G_{q/11}$, which recognizes both proteins. Molecular mass of $G\alpha_q$ and $G\alpha_{11}$ is ~42 kDa.

enced by several cell types in the airways, including smooth muscle, neurons, epithelial cells, and endothelial cells (1, 2, 5). M1 muscarinic receptors are coupled to the $G\alpha_q$ family (6) and are located on postganglionic neurons that contribute to bronchoconstriction by positively regulating depolarization and cholinergic neurotransmission (20). However, specific inhibition of M1 receptor activation with pirenzipine does not block the effects of inhaled MCh (20), suggesting that this mechanism is not responsible for the different effects observed in the in vitro and in vivo experiments. Additionally, epithelial cells and endothelial cells respond to M3 receptor stimulation by generating nitric oxide, which may play an important role in bronchodilation (2). It is more difficult to speculate on how $G\alpha_q$ deletion in these cells would lead to a hyporesponsive phenotype in naive mice, but, without additional data, the contribution of these cells to the observed phenotype cannot be excluded.

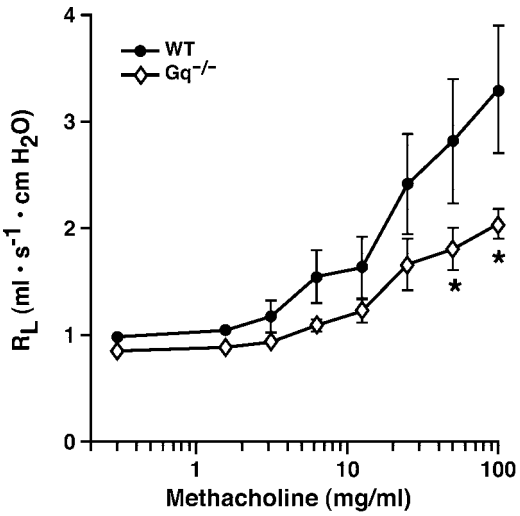

Fig. 2. Lung resistance in G_q-deficient mice is attenuated following administration of MCh. Lung resistance (R_L) values were obtained by a forced oscillation technique. Airway reactivity is plotted as a function of increasing doses of inhaled MCh. Values presented are means ± SE (n = 10–12 mice/group). *Significantly different ($P < 0.05$) from WT mice.

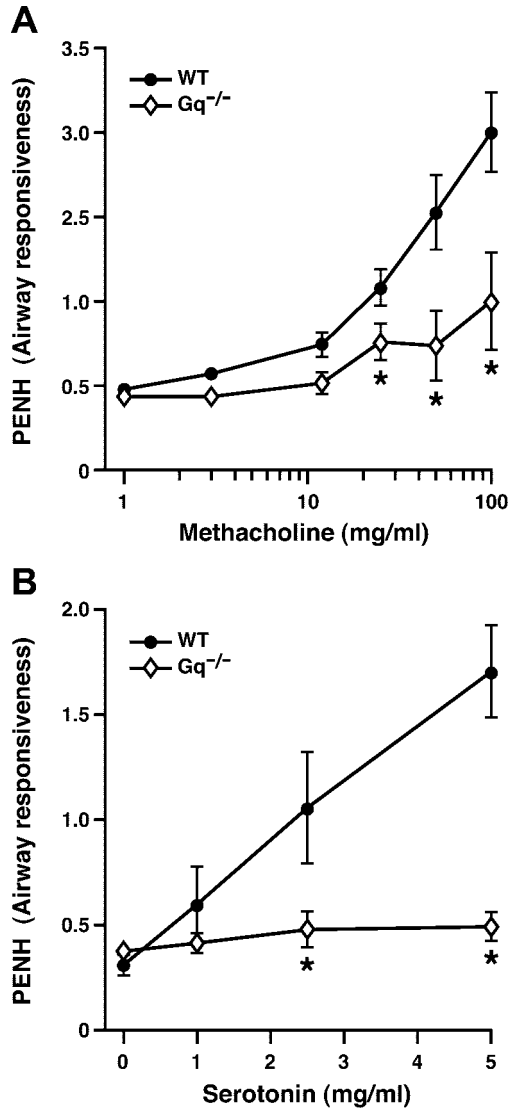

Fig. 3. G_q-deficient mice are hyporesponsive to inhaled MCh and serotonin. Airway reactivity [enhanced pause (Penh)] of each group is plotted as a function of increasing doses of inhaled MCh (*A*) or serotonin (*B*). Values presented are means ± SE (n = 9–14 mice/group). *Significantly different ($P < 0.05$) from WT mice.

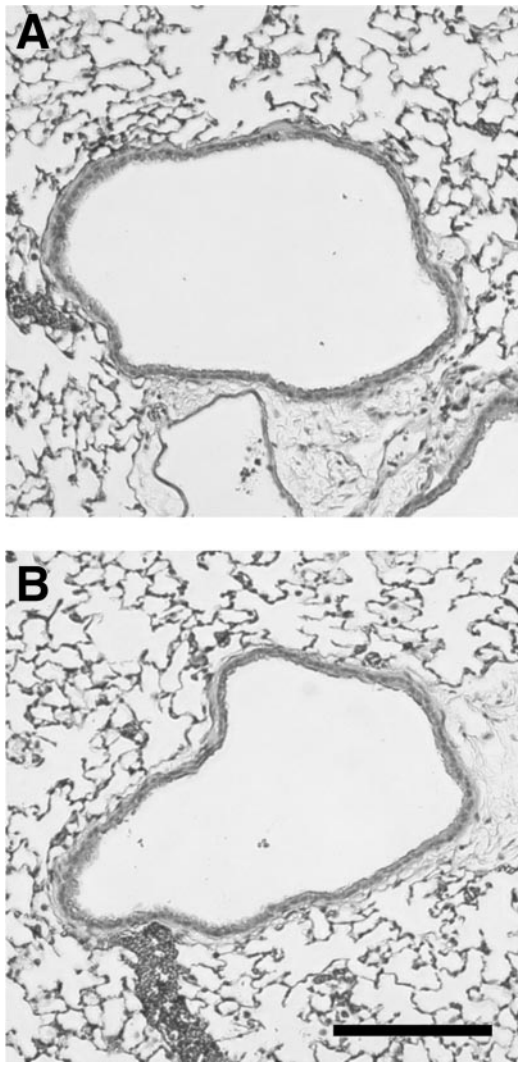

Fig. 4. Airway morphology of G_q-deficient mice is indistinguishable from WT mice. Structure of the airways and surrounding tissue within the lungs was visualized by light microscopy of hematoxylin-eosin-stained tissue sections of WT (*A*) and $G\alpha_q^{-/-}$ (*B*) mice. Photomicrographs are representative of 5 mice/group. Bar, 50 μm.

The involvement of nonairway smooth muscle cell types in the regulation of airway responsiveness may be particularly relevant to the loss of AHR in sensitized/challenged $G\alpha_q^{-/-}$ mice. AHR development in this model is incompletely understood, but there are data to support the involvement of Th2 inflammatory responses, including the production of cytokines and leukotrienes and the infiltration of the pulmonary parenchyma by activated T lymphocytes and eosinophils (22, 41). The relative importance of the recruitment and activation of these leukocytes is difficult to assess, as the magnitude of the leukocyte infiltrate is a poor prognostic indicator for the development of AHR (3, 17, 36). Nonetheless, we have previously reported that OVA-treated $G\alpha_q^{-/-}$ mice exhibit an ~67% reduction in airway inflammation and a concurrent decrease in pulmonary eosinophilia without a decrease in Th2 cytokine production or T cell activation (4), suggesting that the loss of lung eosinophils may contribute to the failure of OVA-treated $G\alpha_q^{-/-}$ mice to develop AHR. This inflammation-dependent contribution, however, is likely to be modulatory and not directly responsible for the failure of these mice to react following MCh provocation, as saline control $G\alpha_q^{-/-}$ mice (i.e., animals with little to no pulmonary eosinophil infiltrate) also failed to display significant dose-dependent reactions to this cholinergic receptor agonist.

It is noteworthy that any interpretations of these data are necessarily complicated by the fact that these signals do not function in isolation. That is, several G proteins are expressed in each of the potentially relevant cell types, and a degree of promiscuity exists regarding G protein receptor coupling. For example, signaling through M3 and thromboxane A_2 activates both G_q and G_{12} to mediate distinct effects in response to the same agonist (19, 33). Also, evidence for simultaneous coupling to G proteins with opposing effects, G_i and G_s, has been clearly demonstrated for the α_2-adrenergic receptor (7) and β_2-adrenergic receptor (42) in mammalian hearts. The β_2-adrenergic receptor has also been shown to similarly activate two members of the G_i family, G_{i2} and G_{i3} (42). Moreover, tissues are constantly exposed to and stimulated by agonists that signal through other G proteins and other signaling mechanisms (e.g., tyrosine kinases).

Seemingly conflicting data exist in the literature concerning the function of G proteins studied by various techniques and in various cell types. It has been suggested that a functional overlap exists between G_q and G_{11} on the basis of experiments in double knockout mice (31). These studies demonstrate that homozygous deficiency at both alleles results in embryonic lethality, that expression of a just single copy of the four alleles results in death within 1 h after birth, and that at least two alleles of the four contributed by these two genes are required for survival after birth. The conclusion

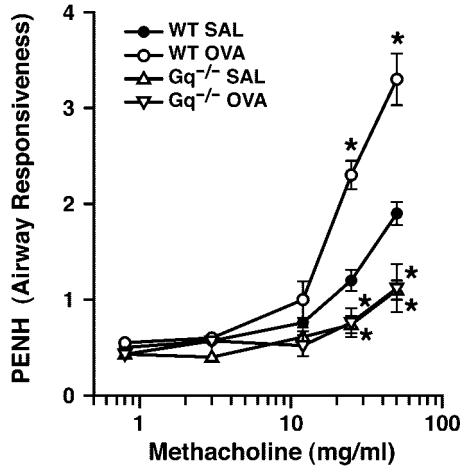

Fig. 5. Allergen-induced airway hyperreactivity does not develop in $G\alpha_q^{-/-}$ mice. WT and $G\alpha_q^{-/-}$ mice were sensitized and aerosol challenged with ovalbumin (OVA). Airway reactivity to increasing doses of MCh was determined 48 h after the last of 3 OVA challenges. Values presented are means ± SE (n = 9–12 mice/group). *Significantly different ($P < 0.05$) from WT mice. SAL, saline.

from these data was that G_q and G_{11} can functionally compensate for each other during development (31). Subsequently, mice with conditionally inactivated $G\alpha_q$ on a $G\alpha_{11}$-deficient background have been used to demonstrate that these two proteins are critical in the development of myocardial hypertrophy in adults (38). Evidence for promiscuous coupling of G_q and G_{11} to effector proteins that activate calcium release has been shown in pancreatic and submandibular gland cells in vitro (43), whereas evidence for distinct roles for G_q and G_{11} has been shown in fibroblasts (44), neurons (14), and portal vein myocytes (25). Interestingly, Western blot analyses demonstrate that a genetic deficiency in $G\alpha_q$ has no significant effect on the expression levels of the other G protein ($G\alpha_{11}$). These observations suggest that the results presented can be attributed to the loss of $G\alpha_q$ signaling and are not a simple consequence of differential G protein expression/usage in airway smooth muscle, although potential effects of G protein deletion on the complex balance of integrated signaling that occurs in any cell type cannot be ruled out.

Mouse models of human airway disease are increasingly and successfully used in laboratory investigations because of the nominal costs associated with small animals, the similarity of the genomes, and the ease of genetic manipulation. However, there are some limitations to their use and the interpretations of the data derived from experiments on mice. Although the structural components are similar between the two species, obvious differences between human and mouse airways in size and morphology may contribute to differences in airway reactivity to various agonists. Also, differences in the reactivity to certain agonists likely arise from differences in receptor expression and utilization. For example, serotonin may have direct and indirect effects on airway smooth muscle responsiveness in some species but affects only neural pathways in the mouse (23). This effect is also seen in the present study, as serotonin has potent effects on Penh (with neural component) but does not affect the response of mouse trachea ex vivo (without neural component).

The mechanisms leading to the development of airway dysfunction in pulmonary diseases are likely to be combinatorial, involving alterations in receptor function, smooth muscle electrophysiology, airway morphology/geometry, airway caliber, airway inflammation, and epithelial damage. The loss of allergen-induced AHR in $G\alpha_q^{-/-}$ mice suggests that the intracellular signaling pathways mediated by $G\alpha_q$ are required in one or more of these mechanisms. Furthermore, the demonstration of an effect on airway reactivity in naive $G\alpha_q^{-/-}$ mice implies that $G\alpha_q$ signaling directly in airway smooth muscle (e.g., muscarinic receptor signaling) is likely to be critical. The significance of these findings to human health resides in the identification of $G\alpha_q$ as a potential target that mediates bronchodilation in airway diseases by regulating both baseline responsiveness and inflammatory-mediated contraction of parasympathetic neuromuscular activity in the airways. Airway inflammation increases the level of bronchomotor tone in both asthma (34) and COPD (12) patients, each of which, as the data presented here would predict, benefits from the administration of anticholinergic agents. However, currently available anticholinergic agents inhibit all three muscarinic receptors that have a role in airway function (i.e., M1, M2, and M3). Strategies targeting $G\alpha_q$ thus represent novel therapeutic modalities to regulate airway reactivity.

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