

Transgenic techniques to delineate cell-specific effects of β_2 -adrenergic receptors in the lung

Dennis W. McGraw, MD Cincinnati, Ohio

Many, if not most, of the different cell types within the lung have been shown to express β_2 -adrenergic receptors (β_2 ARs) on the cell surface. β_2 ARs have thus been implicated in the regulation of many aspects of lung function. However, the physiologic and therapeutic relevance for many of these cell-specific responses has been difficult to establish because of confounding effects that result from the simultaneous activation of receptors on multiple cell types in an in vivo environment. It has been recognized in in vitro models that overexpression of G-protein-coupled receptors such as the β_2 ARs can increase the number of spontaneously active receptors (R^*) and thereby promote autonomous signal transduction or enhanced agonist sensitivity. With transgenic techniques, high levels of receptor expression and activation can also be attained in vivo. By further using cell-specific promoters to direct expression, it is possible to express β_2 ARs in select cell types of a heterogeneous organ such as the lung. In this manner, activation of receptor signaling is limited to the targeted cell. Promoter-directed transgenesis can therefore be a useful tool to delineate cell-specific β_2 AR-mediated effects in the lung. (*J Allergy Clin Immunol* 2002;110:S236-41.)

Key words: β_2 -adrenergic receptor, transgenic, airway smooth muscle, airway epithelial cell, alveolar type II cell

The β_2 -adrenergic receptor (β_2 AR) is a member of the G-protein-coupled receptor (GPCR) family that mediates the actions of the endogenous catecholamines epinephrine and norepinephrine. The β_2 AR is expressed by many different cell types within the lung, which give it the potential to regulate many aspects of lung function, and it is the target for β -agonists used clinically in the treatment of bronchospastic lung disease. However, confirmation of the physiologic and clinical relevance for many of these actions in an in vivo setting has been difficult due to simultaneous activation of receptors on multiple cell types. In this review, we discuss the utility of using cell-selective promoters to direct transgenic β_2 AR expression as a means to delineate the cell-specific effects of β_2 AR in a complex in vivo environment.

Abbreviations used

ASM: Airway smooth muscle
 β_2 AR: β_2 -adrenergic receptor
cAMP: Cyclic adenosine monophosphate
CCSP: Clara cell secretory protein
GPCR: G-protein-coupled receptor
NTG: Nontransgenic
Penh: Enhanced pause

β_2 AR EFFECTS IN THE LUNG

The lung is a markedly heterogeneous organ composed of more than 40 different cell types. Radioligand binding, receptor autoradiography, in situ hybridization, and various functional assays have shown that most of these cell types express the β_2 AR to some extent.¹ As a result, the β_2 AR has been implicated in the regulation of a wide variety of processes that occur within the lung. By far, the best characterized of these actions is the relaxation of airway smooth muscle (ASM). This effect, which results from the direct activation of ASM receptors, provides the primary therapeutic benefit of β -agonists when used in the treatment of asthma and other bronchospastic lung diseases.

However, β -agonists may have additional benefits that occur via the activation of receptors on other cell types. For example, stimulation of prejunctional β_2 AR on nerve terminals inhibits acetylcholine release and thereby reduces the cholinergic component of bronchoconstriction.² β_2 ARs on bronchial epithelial cells stimulate chloride transport and promote serous and mucus cell secretion.³ These actions serve to increase periciliary fluid, which, together with an increase in ciliary beat frequency,⁴ enhance mucociliary clearance. Airway epithelial cells also have synthetic function and are capable of producing a variety of mediators that can potentially modulate smooth muscle tone.⁵ In some studies, removal of the airway epithelium attenuated the relaxant effect of isoproterenol on isolated tracheal rings, suggesting that the β_2 ARs on airway epithelial cells cause release of relaxant factors.⁶ Such findings, though, have not been consistently observed, and the concept of a physiologically relevant β_2 AR-regulated epithelial-derived relaxant factor remains in question.⁷ Resident and circulating inflammatory cells also express β_2 ARs.⁸ β_2 AR activation inhibits mediator release by mast cells, lymphocytes, and neutrophils. Eosinophils and macrophages also express β_2 ARs, but the inhibitory effect is much less in these cells. β_2 AR activation causes relaxation of endothelial cells that may act to reduce vascular permeability.⁹ How-

From the Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Supported by grants from the National Institutes of Health.

Reprint requests: Stephen B. Liggett, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Room G062, Cincinnati, OH 45267-0564; E-mail: stephen.liggett@uc.edu.

© 2002 Mosby, Inc. All rights reserved.

0091-6749/2002 \$35.00 + 0 1/0/129428

doi:10.1067/mai.2002.129428

ever, increases in blood flow resulting from vasodilation of bronchial arterioles may oppose this effect.¹⁰ The overall effect of β -agonists on mucosal edema is thus unclear.⁹ Lastly, β_2 ARs are expressed by cells lining the alveoli, including type I cells, type II cells, and possibly capillary endothelial cells. Activation of these receptors by endogenous and exogenous agonists promotes ion transport and clearance of fluid from the alveolar space. β_2 ARs on alveolar lining cells may therefore serve a protective function against pulmonary edema.¹¹

Many of these potentially beneficial actions of β_2 ARs have been established in isolated cells and excised tissues. It has been difficult, however, to establish their physiologic and therapeutic relevance in an *in vivo* setting, because endogenous and exogenous agonists in the intact animal act on β_2 ARs of multiple cell types simultaneously. As a result, for example, the potential bronchodilating effect of epithelial receptors is not readily distinguished from the profound bronchodilation produced by the activation of receptors on ASM. Recent studies, however, have shown that promoter-directed transgenesis can be used to selectively activate β_2 AR signaling in specific cell populations of heterogeneous organs such as the lung.

β_2 AR ACTIVATION BY OVEREXPRESSION

As reviewed in this supplement (see article by Liggett), it is now believed that a small proportion of GPCRs, such as the β_2 AR, spontaneously toggle into a conformation (denoted as R^*) that can stimulate the receptor's associated G protein. While this conformation is not favored in the absence of agonist, the amount of R^* may be sufficient to transduce a measurable signal even in the absence of agonist if receptor expression is significantly increased (eg, overexpression by transfection).¹² Signal transduction and cellular responses in the overexpressing cell may then act as if the receptor were bound by an agonist. Increases in R^* , the conformation that favors agonist binding, may also enhance agonist sensitivity, which can be manifested as a leftward shift in the agonist dose-response curve. Receptor overexpression can therefore be used to augment both agonist-independent and agonist-dependent signaling.

Advances in tissue-targeted transgenesis, made possible by ongoing identification of cell-specific promoters, have permitted the concept of receptor activation by overexpression to be extended to the *in vivo* setting. The experimental paradigm for transgenic activation has been to overexpress the β_2 AR or other GPCR under control of a cell-specific promoter and, depending on the level of expression achieved, obtain either heightened agonist sensitivity (agonist-dependent activation) or tonic activation due to autonomous signaling (agonist-independent activation). In either scenario, the increase in receptor signaling is limited to the targeted cells, since GPCRs are not secreted products. Any gain in physiologic or pathophysiologic function can thus be attributed to the effects of receptor signaling in the targeted cell. Milano et al¹³ first used this approach to overexpress the β_2 AR in trans-

genic mice under control of the cardiac-specific α myosin heavy chain promoter. These mice displayed significant increases in both heart rate and contractility even in the absence of exogenous agonists, illustrating the gain-of-function that can be achieved with overexpression. We have recently employed this strategy to investigate cell-specific actions of β_2 AR signaling in the lung by using cell-specific promoters to selectively overexpress and activate signal transduction in ASM, the bronchial epithelium, and the alveolus.

TRANSGENIC MODELS OF β_2 AR ACTIVATION IN THE LUNG

Airway smooth muscle

To better understand the relationship between ASM β_2 AR expression and signaling, integrated smooth muscle function, and airway responsiveness in an intact organism, we overexpressed the β_2 AR in transgenic mice under the control of a smooth muscle α -actin promoter.¹⁴ With this promoter, transgene expression in the lung is primarily limited to ASM (Fig 1, A), with lower levels of expression observed in the larger pulmonary arteries. Quantitation of receptor density in primary cultures of ASM cells grown from tracheal explants showed that β_2 AR density was ~75-fold greater than that of nontransgenic (NTG) ASM cells. The increase in receptor expression in these transgenic cells was associated with marked stimulation of adenylyl cyclase activity and cyclic adenosine monophosphate (cAMP) production. Basal coupling was increased 2.5- to 3-fold over levels in NTG (Fig 1, B). In fact, it was equivalent to that of the maximal agonist-stimulated levels in NTG cells. Because basal coupling in this *in vitro* setting represents signal transduction in the absence of agonist, these findings indicate that the increase in basal activity was the result of autonomous signaling produced by a greater number of receptors in the R^* state. However, this constitutive activation of signaling was not maximal, as isoproterenol stimulated adenylyl cyclase activity to even higher levels (Fig 1, B). Previous studies have suggested that ASM may contain a substantial receptor reserve or "spare" β_2 AR pool that could serve as a mechanism to limit the effects of receptor desensitization and downregulation. In such a setting, increasing receptor density would be expected to have little effect on maximal agonist activation, although a leftward shift in the concentration that produces 50% of its maximal effect (ie, EC_{50}) could still be expected. Our observation of increased agonist responsiveness in ASM cells overexpressing β_2 ARs contrasts with the findings of these previous studies and suggests that the β_2 AR may be the limiting component in the receptor/ G_s /adenylyl cyclase cascade.

We further investigated the effect of β_2 AR overexpression and activation on integrated ASM function by contraction/relaxation studies of excised tracheal rings. Sensitivity to isoproterenol was significantly enhanced in the β_2 AR overexpressors, with the EC_{50} for relaxation being 60-fold less than that in NTG specimens (Fig 1, C). However, the maximal extent of relaxation was not altered,

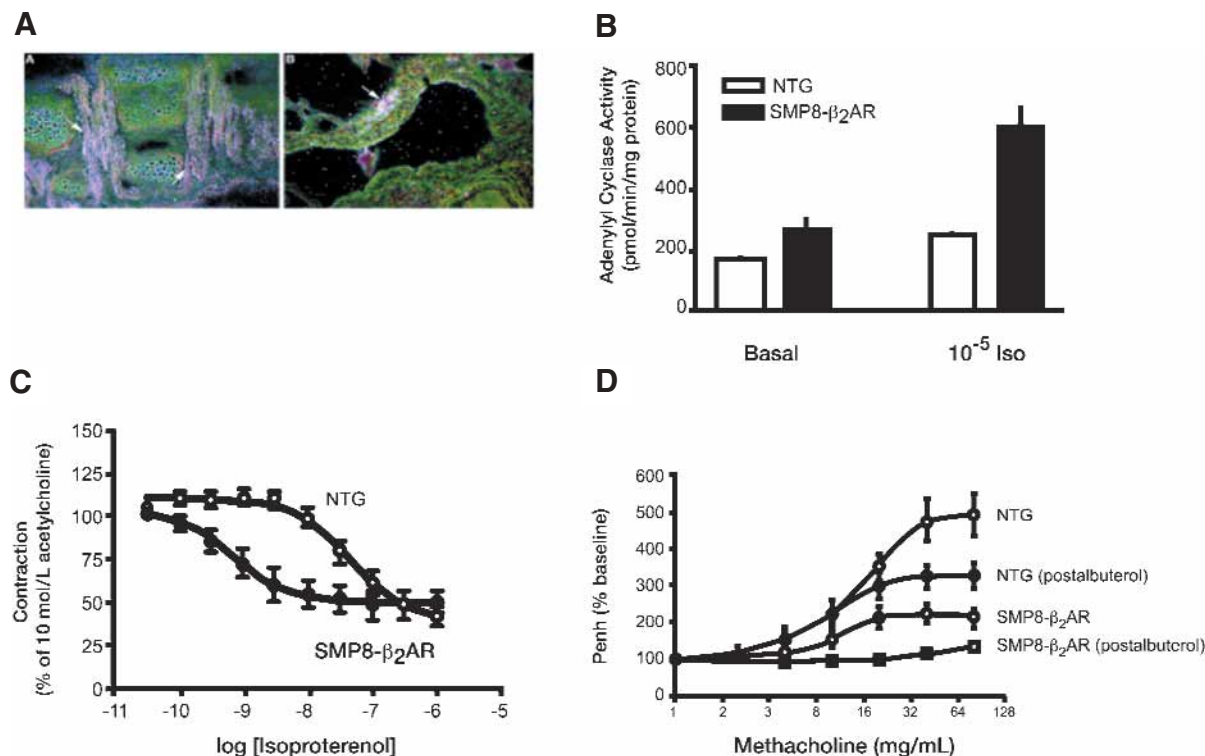


FIG 1. Smooth muscle-directed β_2 AR expression in lungs of transgenic mice. **A**, In situ hybridization shows that the smooth muscle α -actin promoter directs β_2 AR transgene expression to ASM in the trachea (*left panel*) and distal bronchi (*right panel*). **B**, Basal and agonist-stimulated adenylyl cyclase activity in transgenic and NTG ASM cells. **C**, Isoproterenol-stimulated relaxation of precontracted tracheal rings. **D**, Airway reactivity to methacholine measured by whole-body plethysmography using Penh as the index of bronchoconstriction. (From McGraw DW, Forbes SL, Kramer LA, Witte DP, Fortner CN, Paul RJ, et al. Transgenic overexpression of β_2 -adrenergic receptors in airway smooth muscle alters myocyte function and ablates bronchial hyperreactivity. *J Biol Chem* 1999;274:32241-7. With permission.)

suggesting that factors in the signaling cascade distal to cAMP were limiting in this *ex vivo* assay.

These studies showed that smooth muscle-directed expression of the β_2 AR could enhance signaling in both isolated ASM cells and intact tracheal rings. To determine whether such activation could prevent or attenuate airway reactivity to contractile agonists in the intact animal, and thus impart a bronchoprotective phenotype, bronchoconstrictor responses to methacholine were assessed *in vivo* by whole-body plethysmography, using enhanced pause (Penh) as the index of bronchoconstriction. Consistent with the cellular signaling findings, the maximal level of bronchoconstriction attained in the transgenic mice in the absence of exogenous agonist was less than that in NTG mice pretreated with albuterol (Fig 1, *D*). Moreover, treatment of transgenic mice with albuterol completely ablated the bronchoconstrictor response to methacholine (Fig 1, *D*). Selective expression of the β_2 AR in ASM thus imparted an "anti-asthmatic" phenotype to the transgenic mice. The combination of these findings indicates that the β_2 AR on ASM is a limiting element in the receptor signaling cascade and that this constraint can be alleviated by increasing receptor number.

Bronchial epithelium

Studies of transgenic mice overexpressing β_2 ARs in ASM clearly demonstrate the ability of these receptors to promote profound bronchodilation. However, several lines of evidence suggest that the airway epithelium also has the capacity to inhibit smooth muscle tone and responsiveness. The mechanical barrier formed by the epithelium, its enzymatic activity, and its regulation of epithelial lining fluid and mucociliary clearance may act indirectly to limit exposure of ASM to luminal spasmogens. The airway epithelium may also release factors such as prostaglandin E_2 that can act directly to relax ASM.

There is also evidence to suggest that the inhibitory actions of the airway epithelium are modulated by epithelial cell β_2 ARs. Such studies have usually involved *in vitro* assays of isolated cells or excised tissues. The physiologic and therapeutic relevance of epithelial β_2 ARs, however, has been difficult to discern due to the fact that endogenous or exogenous β -agonists simultaneously activate receptors on ASM. Therefore, to address the question as to whether activation of airway epithelial β_2 ARs promotes bronchodilation independently of ASM receptors, we generated trans-

genic mice that overexpressed the β_2 AR under control of the Clara cell secretory protein (CCSP) promoter.¹⁵ With this promoter, transgene expression was confined to cells of the proximal airway epithelium and was not detected in ASM (Fig 2, A). By using receptor autoradiography and semiquantitative grain counting, β_2 AR density in the airway epithelium was estimated to be increased ~3-fold. Although signaling at the cellular level could not be performed because of the difficulties associated with culturing murine epithelial cells, *in vivo* assessment of airway reactivity showed that epithelial overexpression of β_2 ARs had a markedly inhibitory effect on airway reactivity. By using whole-body plethysmography with Penh as the index of bronchoconstriction, we found that the dose of methacholine required to cause a 2-fold increase (ED_{200}) in Penh was more than twice that required for the NTG animals (Fig 2, B). Interestingly, the bronchoprotective effect afforded to transgenic mice by epithelial β_2 AR overexpression approached that of exogenous albuterol treatment in the NTG mice. However, maximal bronchoconstriction could still be achieved in transgenic mice, albeit at a much higher dose of methacholine than that needed for nontransgenic animals. This protective effect was also observed after low-dose ozone exposure, because the transgenic mice exhibited significantly less bronchoconstriction than the NTG mice (Fig 2, C). The attenuation of methacholine and ozone reactivity did not appear to be the result of changes in lung structure as morphologic and histologic features were not altered. Although we were unable to identify the mechanisms underlying the inhibitory effect on airway reactivity, these results nevertheless demonstrate that β_2 ARs on epithelial cells have the capacity to regulate airway reactivity and that this effect is distinct from the direct bronchodilator effect of ASM receptors.

Alveolar type II cells

A number of studies have demonstrated that exogenously administered β -agonists can accelerate the removal of excess fluid from the alveolar space.¹⁶ It has also been demonstrated in animal models that enhanced clearance of pulmonary edema fluid after neurologic insults, hemorrhage, and sepsis depends on release of endogenous catecholamines.^{11,17} Activation of β_2 ARs in the alveolar lining cells thus appears to serve a protective function that limits alveolar flooding and enhances its resolution. This has led to the suggestion that β -agonists may be a useful adjunct in the treatment of patients with pulmonary edema.^{18,19}

The cells responsible for β_2 AR-mediated increases in alveolar fluid clearance have not been clearly elucidated. The alveolar type II cell has received the bulk of interest because it has both the anatomic and metabolic potential to support the active transepithelial ion fluxes required for driving fluid transport. However, other cells, such as type I cells, vascular endothelial cells, and vascular smooth muscle, also have the potential to affect fluid transport and are known to express β_2 ARs. Therefore, to minimize potentially confounding effects of receptor activation from other cells, and thereby selectively assess the effects of type II cell β_2 AR signaling on alveolar fluid

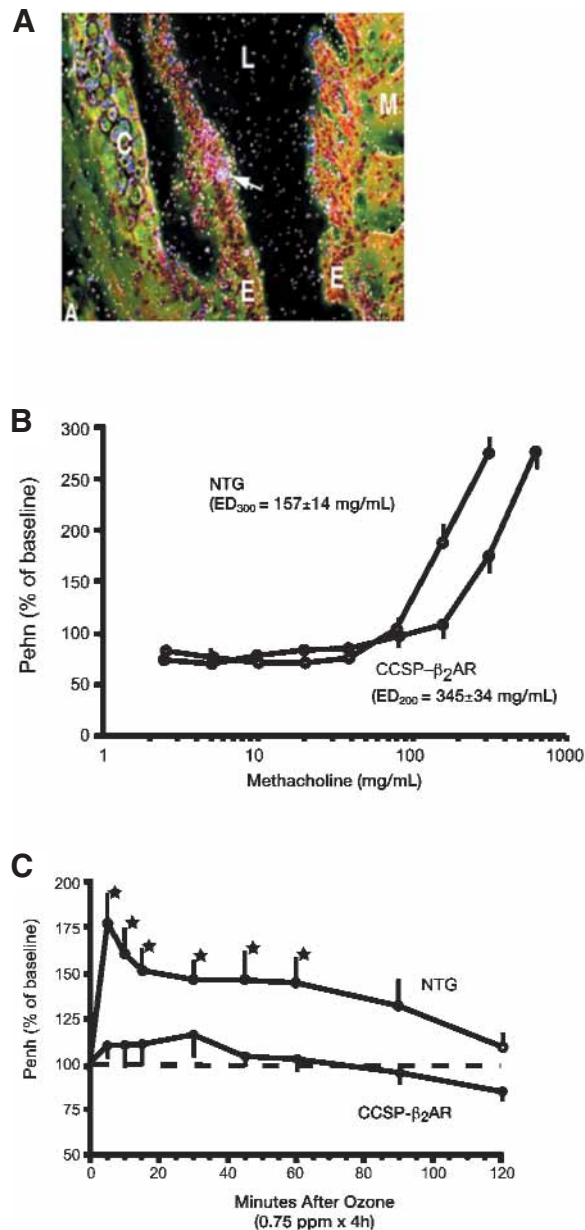


FIG 2. Transgenic expression of β_2 AR in proximal airway epithelium. **A**, In situ hybridization shows that CCSP promoter-directed β_2 AR expression in the bronchus is limited to the bronchial epithelium. **B**, Airway reactivity to methacholine measured by whole-body plethysmography using Penh as the index of bronchoconstriction. **C**, Airway response after ozone exposure (0.75 ppm $\times$ 4 h) measured by whole-body plethysmography using Penh. (From McGraw DW, Forbes SL, Mak JCW, Witte DP, Carrigan PE, Leikauf GD, et al. Transgenic overexpression of β_2 -adrenergic receptors in airway epithelial cells decreases bronchoconstriction. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L379-89. With permission.)

clearance, we transgenically expressed the β_2 AR under control of the surfactant protein C promoter.²⁰ With this promoter, β_2 AR expression was observed in distinct clusters at the corners of alveoli (Fig 3, A), consistent with the location of type II cells. Radioligand binding

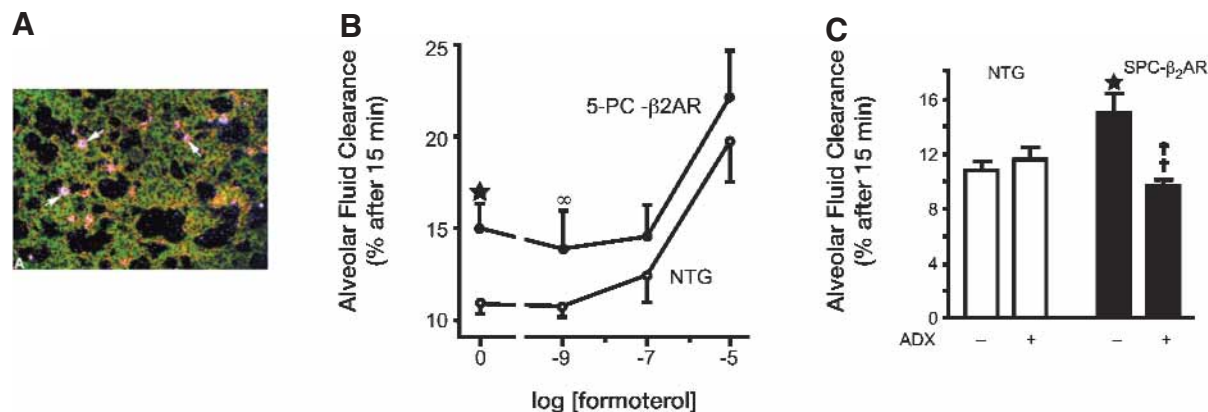


FIG 3. Transgenic expression of β_2 AR in alveolar type II cells. **A**, The surfactant protein C promoter limits β_2 AR expression to the alveolus. Expression in the corners of alveoli is consistent with the known distribution of type II cells. **B**, Basal and agonist-stimulated alveolar fluid clearance rates. **C**, Basal alveolar fluid clearance rates in mice after adrenalectomy (ADX+) or sham adrenalectomy (ADX-). (From McGraw DW, Fukuda N, James PF, Forbes SL, Woo AL, Lingrel JB, et al. Targeted transgenic expression of β_2 -adrenergic receptors to type II cells increases alveolar fluid clearance. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L895-903. With permission.)

with whole-lung homogenates showed that receptor density in transgenic mice was nearly 5-fold greater than that in NTG mice. Consistent with an acquired increase in agonist sensitivity, adenylyl cyclase studies showed that the EC_{50} for isoproterenol in transgenic mice was less (by ~50%) than that in the NTG animals. However, neither basal nor maximal agonist-stimulated adenylyl cyclase activity was increased in transgenic mice, consistent with previous studies, indicating that the lung parenchyma contains a population of spare receptors.

As might be expected, β_2 AR overexpression in the distal respiratory epithelium had no effect on airway reactivity in either the presence or absence of agonist. However, measurement of alveolar fluid clearance rates in the intact lung showed that basal fluid clearance was significantly greater in the β_2 AR overexpressors (Fig 3, B and C). The increased alveolar fluid clearance rate in these transgenic mice appeared to be the result of increased sensitivity to endogenous catecholamines rather than autonomous activation, as it was abolished by adrenalectomy (Fig 3, C). Alveolar fluid clearance was increased in NTG mice and further increased in transgenic mice by the β_2 AR-selective agonist formoterol (Fig 3, B). At each concentration, the formoterol-stimulated alveolar fluid clearance tended to be higher in the transgenic animals, but at the lowest doses the differences did not reach statistical significance. It remains unclear whether this additional agonist-stimulated increase is the result of actions on type II cells or the action of receptors on other cells. However, the finding of increased alveolar fluid clearance rates in these transgenic mice show clearly that β_2 ARs on type II cells have the capacity to regulate alveolar fluid clearance in the in vivo setting.

CONCLUSIONS

Overexpression of GPCRs, such as the β_2 AR, can promote increased signal transduction through either autonomous activity or increased sensitivity to agonist.

With transgenic techniques, receptor expression can be increased and signal transduction activated in the in vivo setting. By targeting receptor expression to selected cells, promoter-directed transgenesis can be used as a tool to delineate the cell-specific actions of β_2 ARs in heterogeneous organs such as the lung.

DISCUSSION SESSION

Question: Is there a relationship between overexpression of β_2 AR and increased nitric oxide activation?

Dr McGraw: We have not specifically blocked or tried to look at other pathways. At least in the lavage fluid, the content of PGE2 and nitric oxide did not differ. However, small amounts of local release may not be reflected in changes in lavage fluid. It is difficult to decide which pathway to study other than to focus on the ones that are traditionally thought to be important.

Question: Are there any differences in alveolar fluid clearance between short-acting, long-acting, or complete β -agonists?

Dr McGraw: There is some suggestion that isoproterenol and formoterol are better stimulators of alveolar fluid clearance than albuterol. We studied formoterol because it is a full or near-full agonist that is specific for the β_2 AR. Overexpression has a tendency essentially to transform what looks like partial agonists to full agonists, so in retrospect, it may have been better to use a weaker agonist.

Question: Did the mice with overexpression exhibit inflammatory cell accumulation or structural changes?

Dr McGraw: There was no inflammatory cell accumulation in these mice. By light microscopy, there were no structural changes. The lungs looked normal for both the epithelial and the alveolar overexpressors. Phenotypically, morphologically, and histologically, there were no differences. There did not appear to be any additional fibrosis and no increase in mucous-secreting cells. We did not look for differences after agonist treatment. The agonist treatment in our experiments involved acute exposure, so we were not looking for chronic changes in the airway. In addition, there was no inflammation following ozone exposure. Even in the nontransgenic mice, we did not see large amounts of perivascular or peribronchial edema. The ozone response probably involves bronchoconstriction because it resolves rapidly on treatment with a β -agonist.

Comment: It has never been clear to me that there actually is a

constitutively expressed epithelial-derived inhibitory factor. This may be seen in the overexpressed state but not in the wild type.
Dr McGraw: Our data do not attribute the effect to release of a relaxant factor or the decreased release of a contracting factor. We simply note a bronchoprotective effect. Other possibilities include alteration of the periciliary fluid content and mechanical barriers that affect the bioavailability of methacholine. This suggests that β_2 AR on epithelial cells have the capacity to regulate smooth muscle tone, at least in the overexpressed state.

Question: Are you certain that the up-regulation you induced only occurred in the epithelium exclusively and not just predominantly, meaning that you may have had some up-regulation in smooth muscle?

Dr McGraw: Based on the in situ hybridization as far as localization of the transgene, it was fairly clear that this occurred only in the epithelial cells.

Question: Did you analyze the structure of the smooth muscle overexpression and the protein content of the contractile apparatus?

Dr McGraw: Structurally and histologically at the light microscopy level, there were no differences. There was no apparent change in mass.

Question: Which smooth muscle showed the overexpression? Was the airway vasculature involved?

Dr McGraw: The trachea and smooth muscle areas such as the bladder, the uterus, and the gastrointestinal tract showed expression of the transgene. We did not study the airway vasculature. There are some changes in the peripheral vasculature. At present, we have not developed the methodology to assess pulmonary vascular responses.

Question: Regarding the epithelial overexpression model, does the transgene light up in the submucosal glands?

Dr McGraw: If one looks at the epithelium as a continuous strip down the airway, one sees the transgene lighting up down the airway. To determine whether the transgene is present in specific areas would probably require a colocalization study, although in situ hybridization may not be the best way to accomplish this.

Question: What is the receptor distribution in the different cell types in the non-transgenic mice studied?

Dr McGraw: The receptor distribution in these specific mice is not known. All of the cells have some responses regulated by β -agonists, though in some cases, there are binding studies to show that the receptor is expressed at least natively in those cells.

REFERENCES

1. Nijkamp FP, Engels F, Henricks PAJ, Van Oosterhout AJM. Mechanisms of β -adrenergic receptor regulation in lungs and its implications for physiological responses. *Physiol Rev* 1992;72:323-67.
2. Barnes PJ. Modulation of neurotransmission in airways. *Physiol Rev* 1992;72:699-729.
3. Basbaum CB, Finkbeiner WE. Mucous-producing cells of the airways. In Massaro D, editor. *Lung cell biology*. New York: Marcel Dekker; 1989. p. 37-79.
4. Devalia JL, Sapsford RJ, Rusznak C, Toubis MJ, Davies RJ. The effects of salmeterol and salbutamol on ciliary beat frequency of cultured human bronchial epithelial cells, in vitro. *Pulmonol Pharmacol* 1992;5:257-63.
5. Holtzman MJ. Arachidonic acid metabolism in airway epithelial cells. *Annu Rev Physiol* 1992;54:303-29.
6. Stuart-Smith K, Vanhoutte PM. Heterogeneity in the effects of epithelium removal in the canine bronchial tree. *J Appl Physiol* 1987;63:2510-5.
7. Jacoby DB. Role of the respiratory epithelium in asthma. *Res Immunol* 1997;148:48-58.
8. Barnes PJ. β -Adrenoceptors on smooth muscle, nerves and inflammatory cells. *Life Sciences* 1993;52:21019.
9. Persson CGA. The action of β -receptors on microvascular endothelium or: is airways plasma exudation inhibited by β -agonists? *Life Sciences* 1993;52:2111-21.
10. Kelly WT, Baile EM, Brancatisano A, Paré PD, Engel LA. Effects of inspiratory resistance, inhaled beta-agonists and histamine on canine tracheal blood flow. *Eur Respir J* 1992;5:1206-14.
11. Pittet JF, Wiener-Kronish JP, McElroy MC, Folkesson HG, Matthay MA. Stimulation of lung epithelial liquid clearance by endogenous release of catecholamines in septic shock in anesthetized rats. *J Clin Invest* 1994;94:663-71.
12. Green SA, Cole G, Jacinto M, Innis M, Liggett SB. A polymorphism of the human β_2 -adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. *J Biol Chem* 1993;268:23116-21.
13. Milano CA, Allen LF, Rockman HA, Dolber PC, McMinn TR, Chien KR, et al. Enhanced myocardial function in transgenic mice overexpressing the β_2 -adrenergic receptor. *Science* 1994;264:582-6.
14. McGraw DW, Forbes SL, Kramer LA, Witte DP, Fortner CN, Paul RJ, et al. Transgenic overexpression of β_2 -adrenergic receptors in airway smooth muscle alters myocyte function and ablates bronchial hyperreactivity. *J Biol Chem* 1999;274:32241-7.
15. McGraw DW, Forbes SL, Mak JCW, Witte DP, Carrigan PE, Leikauf GD, et al. Transgenic overexpression of β_2 -adrenergic receptors in airway epithelial cells decreases bronchoconstriction. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L379-89.
16. Berthiaume Y, Staub NC, Matthay MA. Beta-adrenergic agonists increase lung liquid clearance in anesthetized sheep. *J Clin Invest* 1987;79:335-43.
17. Pittet JF, Brenner TJ, Modelska K, Matthay MA. Alveolar liquid clearance is increased by endogenous catecholamines in hemorrhagic shock in rats. *J Appl Physiol* 1996;81:830-7.
18. Matthay MA, Folkesson HG, Verkman AS. Salt and water transport across alveolar and distal airway epithelia in the adult lung. *Am J Physiol Lung Cell Mol Physiol* 1996;270:L487-503.
19. Verghese GM, Ware LB, Matthay BA, Matthay MA. Alveolar epithelial fluid transport and the resolution of clinically severe hydrostatic pulmonary edema. *J Appl Physiol* 1999;87:1301-12.
20. McGraw DW, Fukuda N, James PF, Forbes SL, Woo AL, Lingrel JB, et al. Targeted transgenic expression of β_2 -adrenergic receptors to type II cells increases alveolar fluid clearance. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L895-903.