

Effects of β -agonists on airway epithelial cells

Matthias Salathe, MD *Miami, Fla*

β -Adrenergic receptor (β AR) agonists exert a variety of effects on airway epithelial cells. Among their best known actions is their ability to increase ciliary beat frequency, mediated by cyclic adenosine monophosphate (cAMP) production, stimulation of protein kinase A (PKA), and phosphorylation of an outer dynein arm light chain. Submucosal glands express β ARs, and β -agonists may stimulate secretion of mucus from airways, although human data are controversial. β -Agonists may also affect ion transport across epithelial cells by opening apical ion channels such as the cystic fibrosis transmembrane regulator. This effect, likely to occur in submucosal glands, can influence water fluxes across the airway epithelium and may have profound influences on mucus hydration. β AR activation can increase intracellular calcium in some ciliated cells, thereby stimulating ciliary beating and possibly influencing transepithelial ion transport. β AR-mediated activation of cAMP-dependent protein kinase accelerates epithelial cell migration, thereby enhancing epithelial wound repair. β -Agonists reduce the ultrastructural damage seen with infection and potentiate secretion of certain cytokines from epithelial cells while inhibiting secretion of others. Finally, β -agonists may have effects on airway epithelial cells that are mediated through β ARs but do not require cAMP production. The signaling mechanisms of some β -agonist effects are not well understood but are important to our understanding of airway epithelial cell growth, differentiation, and function. (*J Allergy Clin Immunol* 2002;110:S275-81.)

Key words: β -Agonists, signaling, cyclic adenosine monophosphate, airway epithelium, calcium

β -Adrenergic receptor (β AR) agonists elicit a variety of effects on airway epithelial cells, including an increase in ciliary beat frequency. This article briefly reviews responses of airway epithelial cells to β -agonists.

EXPRESSION OF β AR ON THE AIRWAY EPITHELIUM

The first step in understanding airway epithelial cell responses to β -agonists was to evaluate which cells in the superficial airway epithelium and in submucosal glands express β ARs. Several approaches were used for this purpose. Application of radiolabeled β -agonists and antago-

Abbreviations used

ATP: Adenosine triphosphate
 β AR: β -Adrenergic receptor
cAMP: Cyclic adenosine monophosphate
CBF: Ciliary beat frequency
CFTR: Cystic fibrosis transmembrane regulator
EC₅₀: Median effective concentration
G_i: Adenylyl cyclase inhibitory G-protein
G_s: Adenylyl cyclase stimulatory G-protein
ICAM-1: Intercellular cell adhesion molecule-1
PKA: cAMP-dependent protein kinase

nists to detect β ARs by autoradiography in human lung revealed that the superficial epithelium expresses mainly β ARs and that β_1 ARs and β_2 ARs coexist on submucosal gland cells.^{1,2} Another approach, the use of in situ hybridization techniques to detect β AR mRNA in lung tissues, confirmed the findings obtained with radiolabeled β AR agonists or antagonists.³ The last approach, immunodetection of cyclic adenosine monophosphate (cAMP) produced after β -agonist application in cat trachea, showed that β ARs were functional on ciliated cells and that goblet cells of the surface epithelium did not respond to β -agonists (at least as assessed by cAMP production).⁴ This study also showed that, in submucosal glands, both serous and mucous cells expressed functional β ARs. Further studies in the ferret showed that mucous cells expressed relatively more β ARs than serous cells.⁵ Thus, these results suggest that ciliated cells in the superficial epithelium, as well as both mucous and serous cells in submucosal gland cells, can respond to β -agonist stimulation by producing cAMP.

Acutely dissociated human airway epithelial cells also express functional β_3 AR (approximately 8900 receptors/cell, 80% of the β_2 subtype) with an estimated ligand dissociation constant of 45 pM and a median effective concentration (EC₅₀) of 131 nM.⁶ However, β AR expression and its functional coupling to adenylyl cyclase varies considerably between persons. For instance, the EC₅₀ can vary up to 39-fold.⁶ Finally, experiments that use specific agonists for β ARs suggested that these receptors might be expressed on canine ciliated cells⁷; however, molecular approaches did not reveal any evidence for β_3 AR expression in human lung tissues.⁸

β ARs signal mainly by activating the α subunit of an adenylyl cyclase stimulatory G-protein (G_s) to stimulate adenylyl cyclase, which produces cAMP.⁹ More recently it has become clear, however, that β ARs can signal through mechanisms not involving cAMP.¹⁰ Whereas

From the Division of Pulmonary and Critical Care Medicine, University of Miami School of Medicine, Miami, Fla.

Supported by grants from the National Institutes of Health.

Reprint requests: Matthias Salathe, MD, Division of Pulmonary and Critical Care Medicine (R-47), University of Miami School of Medicine, 1600 NW 10th Ave, RMSB 7063, Miami, FL 33136.

© 2002 Mosby, Inc. All rights reserved.

0091-6749/2002 \$35.00 + 0 1/0/129412

doi:10.1067/mai.2002.129412

direct effects of the $\beta\gamma$ subunits that associate with G_s , and even G_i effects, have been implicated in some of these responses, the exact signal transduction mechanisms have not been clearly elucidated.

β -AGONISTS AND CILIARY BEAT FREQUENCY

Ciliary beating is an integral part of the mucociliary transport apparatus, and it has been well established that β -agonists stimulate ciliary beat frequency (CBF) in a variety of mammalian airway epithelial cells. β -Agonists used in these studies included isoproterenol,¹¹⁻¹⁹ fenoterol,²⁰⁻²² albuterol,^{7,23,24} BRL 37344 (a β_3 -agonist),⁷ and salmeterol.²³

The main mechanism by which these β -agonists increase ciliary beating is through stimulation of adenylyl cyclase and the subsequent rises in intracellular cAMP levels.^{13-15,18,25,26} Increasing the cellular cAMP concentration, independent of β AR stimulation, has also been shown to increase human, bovine, ovine, and lupine CBF, and this increase was blockable by a broad protein kinase inhibitor implicating cAMP-dependent protein kinase (PKA).^{18,27} By analogy, stimulation of PKA seemed to be the next step in the β AR-signaling cascade to stimulate CBF. This hypothesis is supported by the ability of PKA inhibitors to block the effects of β -agonists on CBF.^{18,24,28}

The molecular mechanism by which PKA increases mammalian CBF is likely analogous to the stimulation of ciliary activity by PKA in *Paramecium*. There, Hamasaki et al²⁹ showed that PKA phosphorylated specific axonemal targets both in vitro and in permeabilized cells. One with an apparent molecular weight of 29 kDa was extractable by procedures used for the isolation of outer arm dynein and was identified to be a dynein light chain.³⁰ The phosphorylation of this light chain has been shown to increase the velocity of microtubule gliding across dynein-coated surfaces in vitro and to increase the swimming speed (ie, CBF) of *Paramecium* in vivo.³⁰ Axonemal targets of PKA with similar molecular weights have also been identified by virtue of their cAMP-dependent phosphorylation in mammalian respiratory cilia,³¹⁻³³ and in an ovine study the target was present on outer dynein arms.³⁴ The experiments that used mammalian tissues also revealed that PKA itself must be localized to cilia,^{32,33} a fact supported by the presence of an immunopositive protein in bovine axonemes stained for PKA³¹ and the presence of a specific A-kinase anchoring protein in human airway cilia.³³

In addition to this classic pathway, a more complex β -adrenergic signaling to stimulate CBF has been delineated, first in rabbit tracheal explants and then in ovine airway epithelial cells, where CBF increased through both an initial calcium-coupled mechanism and a prolonged calcium-independent mechanism.^{14,24} It seems that PKA, this time likely a cytosolic form, was mediating the initial calcium rise in ovine cells because an inhibitor of PKA prevented it.²⁴ These data support previous findings in canine airway epithelia, where β -agonists caused cAMP-dependent release of calcium from intracellular

stores.³⁵ The cAMP-dependent calcium increase did not seem to be mediated by stimulated adenosine triphosphate (ATP) secretion and subsequent activation of purinergic receptors as isoproterenol did not alter cellular accumulation of inositol phosphates.³⁹

In any cell that responds with an initial cAMP-dependent calcium increase, the production of nitric oxide can be activated to contribute to ciliary stimulation, as shown in canine and bovine cells.^{19,36} However, nitric oxide production has not been detected in human nasal cells upon stimulation with β -agonists.²⁸ This could be because human airway epithelial cells do not respond with a significant transient calcium increase upon β -agonist stimulation.^{37,38}

In summary, β AR activation in mammalian ciliated cells increases CBF through two mechanisms: an initial calcium-coupled mechanism in cells from some species and a prolonged, calcium-independent mechanism in all cells. These mechanisms seem to depend on the activation of different PKAs through a signaling cascade involving the β AR, adenylyl cyclase, and cAMP (Fig 1).

β -AGONISTS AND MUCUS SECRETION

The majority of data on mucus secretion in response to β -agonists stems from the 1980s, and whether human airways secrete mucus upon β -agonist stimulation is controversial. In keeping with the data on receptor expression⁴ and the data on morphometric evaluation after stimulation,^{39,40} β -agonists cause degranulation of mainly mucous cells in the submucosal glands in the cat and ferret. These findings may explain why relatively thick secretions are produced in cat trachea after β -agonist stimulation⁴¹ and why β -agonists cause a relatively high increase of secreted glycoproteins compared with the secreted volume in ferret trachea.⁴² The molecular mechanism by which β -agonists cause mucus secretion in these preparations has not been studied in detail. From early work in the cat trachea, it is clear that both cAMP and PKA are involved.⁴³ Whether PKA activation alone is sufficient for mucus secretion or whether PKA also needs to cause calcium increases in these cells remains unknown.

Human tissues mounted in Ussing chambers revealed increased glycoprotein release in one study upon salbutamol stimulation.⁴⁴ Isoproterenol, however, did not elicit radiolabeled glycoconjugate release in other human preparations.^{45,46} In addition, SPOC1 cells, which are derived from rat airway epithelium and are used to study mucus secretion, expressed functional β ARs⁴⁷ but did not secrete mucus upon stimulation with β -agonists, forskolin, or membrane-permeable cAMP analogs.⁴⁸ Human bronchial epithelial cell xenograft cultures were also nonresponsive to membrane-permeable cAMP analogues, whereas ATP stimulated mucus secretion (C. William Davis, University of North Carolina at Chapel Hill, personal communication). Although the final word on mucus secretion in human airways upon β AR stimulation is not out, it is likely that β -agonists do not cause mucin secretion in significant amounts.

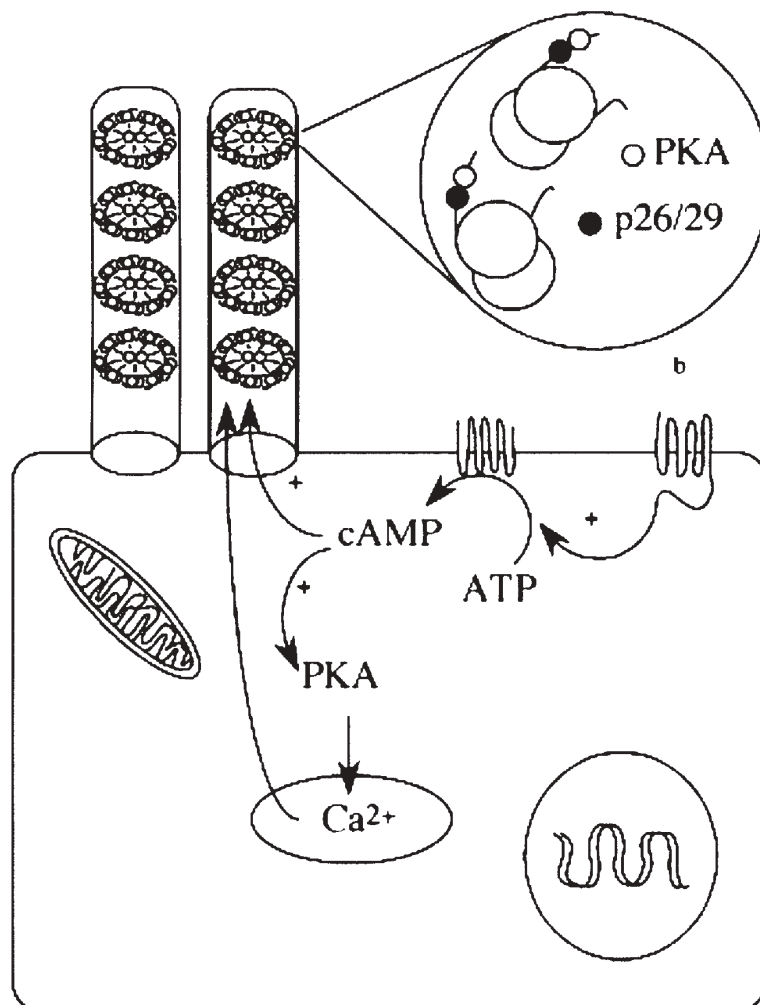


FIG 1. Proposed signal transduction mechanisms by which β -agonists increase CBF. Stimulation of β ARs activates adenylyl cyclase to produce cAMP, which activates an axonemal PKA on outer dynein arms. This PKA phosphorylates a dynein light chain, which mediates an increase in CBF. In some ciliated cells, cytosolic PKA activation also releases calcium from intracellular stores, which increases CBF through an additional mechanism.

β -AGONISTS AND ION TRANSPORT

Proper water and ion transport across the airway epithelium are critically important to maintain an adequate airway surface liquid layer and effective mucociliary clearance. Despite this fact and current intense investigations, no definite consensus has been reached on the physiology of the airway surface liquid layer with respect to production, regulation, and composition. Two competing theories have been developed, the isotonic model and the hypotonic model, with many arguments seeming to favor the isotonic model. Details about these competing theories are beyond the scope of this discussion but have been extensively reviewed elsewhere (eg, Boucher et al,⁴⁹ Widdicombe et al⁵⁰).

There is consensus, however, that the superficial human airway epithelium is mainly sodium-absorbing

(and thus also water-absorbing) and that β -agonists have a relatively small effect on overall ion and water transport, as originally evaluated in excised human bronchi.⁵¹ This may not reflect the whole truth, though. One of the best known effects on ion transport of increasing intracellular cAMP concentrations is the opening of the cystic fibrosis transmembrane regulator (CFTR). Because CFTR is highly expressed in submucosal glands,⁵² effects of β -agonists on ion transport may be found there and may contrast with the effects on the mainly water-absorbing superficial epithelium.⁴⁹ In submucosal gland cells, it is therefore possible that β -agonists actually stimulate chloride secretion and water flux toward the glandular lumen. Water secretion in submucosal glands is, in fact, highly relevant for proper mucus hydration and secretion from the glandular ducts. In a porcine model, for instance, inhibition of anion (and presumably liquid)

secretion into the lumen resulted in substantial ductal mucus accumulation, leading to mucus obstruction of submucosal gland ducts, similar to lesions seen in early cystic fibrosis.⁵³

β-AGONISTS AND EPITHELIAL PROTECTION

Bacteria, including *Pseudomonas aeruginosa*, have been shown to release proteases that cause human nasal epithelial disruption as well as CBF slowing in vitro.⁵⁴ The release of bacterial products such as hydroxyphenazine, pyocyanin, and a rhamnolipid has also been shown to decrease CBF,⁵⁵⁻⁵⁸ as well as to decrease mucus transport velocity.^{59,60} The mechanism of pyocyanin-induced ciliary inhibition has been studied in detail, and two mechanisms have been implicated: direct action of pyocyanin on airway cells to cause a decrease in cAMP and ATP and subsequent decrease in CBF,⁵⁸ and stimulation of hydrogen peroxide release from inflammatory cells to cause a protein kinase C-mediated decrease in CBF.⁵⁵

β-Agonists have been shown to counteract some of the adverse effects of bacteria and bacterial products on the airway epithelium. For instance, salmeterol has been shown to reduce *Pseudomonas aeruginosa*-induced (including pyocyanin-induced) and *Haemophilus influenzae*-induced epithelial damage.^{61,62} Salmeterol also reduced pyocyanin-induced cytoplasmic blebbing and reduced mitochondrial damage in ciliated cells.⁶¹ The number of bacteria adhering to the epithelium in the *Pseudomonas* study decreased on treatment with salmeterol⁶² (consistent with the notion that these bacteria preferentially adhere to damaged cells), but there was no change in the number of *Haemophilus* adhering to the cell cultures.⁶² Note that in one study using Calu-3 cells (a carcinoma cell line with features of serous cells), short-acting β-agonists were found to decrease the antibacterial activity of recovered surface liquid.⁶³ Thus, the exact mechanism by which salmeterol and potentially other β-agonists protect airway epithelial cells from damage remains unknown but is likely related to maintaining structural integrity rather than to an increase in the antibacterial activity of surface fluid.

In addition, salmeterol and isoprenaline have been shown to reduce pyocyanin-induced slowing of CBF by preventing the fall in intracellular cAMP and ATP.⁶⁴ The ability of salmeterol to prevent toxin-mediated decreases in CBF was sustained at concentrations that did not have an effect on baseline CBF.⁶⁵

β-AGONISTS AND EPITHELIAL REACTION TO INFLAMMATORY STIMULI

Airway epithelial cells are capable of releasing a variety of inflammatory mediators, including prostaglandins, 15-lipoxygenase products, cytokines (eg, IL-6, IL-8, GM-CSF, and TNF-α), and nitric oxide. The cells are also capable of up-regulating the expression of adhesion molecules, including intercellular cell adhesion mole-

cule-1 (ICAM-1), in response to inflammatory stimuli. Recent investigations have examined the influence of β-agonists on stimulated cytokine release and ICAM-1 expression. When human bronchial epithelial cells were exposed to IFN-γ a significant increase in ICAM-1 expression could be detected and fenoterol inhibited this response in a dose-dependent manner.⁶⁶ Another study investigated the effect of formoterol on TNF-α-stimulated human bronchial epithelial cells grown in submerged culture. Formoterol reduced GM-CSF levels in culture supernatants by 40% to 50% but increased IL-8 levels by 50%,⁶⁷ confirming older data obtained with a transformed human bronchial epithelial cell line that used salbutamol and isoprenaline.⁶⁸ How these studies translate to the in vivo situation in inflammatory diseases remains to be determined, especially because one study looking at bronchial biopsies from asthmatic subjects did not find any change in epithelial GM-CSF or IL-8 immunoreactivity upon treatment with formoterol.⁶⁹

β-AGONISTS AND EPITHELIAL WOUND REPAIR

cAMP and PKA have been implicated in cell migration and wound repair. In bovine bronchial epithelial cells, isoproterenol increased the migration of cells to speed up the closure of mechanically and enzymatically induced wounds of submerged monolayers.⁷⁰ The signaling pathway responsible for this response was found to go through cAMP and subsequently PKA, which in turn inhibited Rho. These findings, although not confirmed in clinical trials, may have important implications for patients with airway diseases, where β₂-agonists may help to accelerate airway epithelial repair after insults.

More recent work indicates that isoproterenol can also induce apoptosis in bovine bronchial epithelial cells.⁷¹ This effect was mediated through βARs but did not seem to involve cAMP production. These data are reminiscent of results obtained in cardiac myocytes, where β₂ARs have been shown to couple to G_i to activate a variety of intracellular signaling pathways, including extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. Thus, it seems likely that the classical signal transduction by βARs in airway epithelial cells is complemented by signaling that does not involve G_s and adenylyl cyclase. Whether the differentiation state of these cells plays a role in the determination of signaling by these receptors remains to be investigated.

CONCLUSIONS

β-Agonists exert a variety of effects on airway epithelial cells. They clearly increase CBF, and the mechanism of this increase has been studied in detail. The increase in CBF is likely the main mechanism by which these agonists increase mucociliary clearance in vivo. Effects on mucin secretion are likely subtle in human airways and possibly clinically irrelevant. Other effects of β-agonists on airway epithelial cells are less well studied and some

remain controversial. Thus, we are left with several important questions about the effects of β -agonists on airway epithelial cells: what are the effects of β -agonists on cytokine release from airway epithelial cells and are these effects clinically relevant; what role do β -agonists play in epithelial cell migration, wound repair, and apoptosis; what mechanisms of action of β -agonists other than cAMP production and stimulation of PKA are relevant in airway epithelial cells (and at which differentiation state are they active); and which mechanisms of action could be clinically useful? These issues will, we hope, be clarified in the near future.

I thank Drs Richard J. Bookman, Gregory E. Conner, Rosanna Forteza, and Adam Wanner for their valuable comments and support over many years.

DISCUSSION SESSION

Question: Regarding the effect of *Pseudomonas* on the cilia, are the cilia lost or does the whole cell desquamate?

Dr Salathe: They are clearly damaged cells, and it seems that they initially lose the cilia. Whether they can be recovered is a difficult question to answer.

Question: Does *Pseudomonas* activate PKC or inhibit PKC- ϵ ? We tried to determine whether we could block PKC action, but the cells kept coming off the dish.

Comment: When *Pseudomonas* comes into contact with the cells, they produce secretory cytotoxins. Some of those factors may need to be controlled to avoid release of the cells from the dish.

Comment: There is evidence that the entire ciliated cell lifts off the dish rather than just the cilia. Treatment of epithelial cells for 24 hours with salmeterol causes up-regulation of junctional proteins. It looks as if the long-acting β -agonist is increasing the integrity of the epithelium and preventing the toxins from getting in.

Dr Salathe: There are two mechanisms involved by *Pseudomonas*. One depends on the presence of inflammatory cells involving PKC activation and the other seems to be a more direct effect of high concentrations of bacteria and pyocyanin. One is probably reversible and the other is probably not.

Question: Would you comment on β -agonist stimulation of nitric oxide production in the ciliated cell. There is a great deal of nitric oxide-generating machinery, probably linked to motility.

Dr Salathe: Nitric oxide is stimulated upon β -agonist stimulation, but this does not occur in all cells from all species. However, if that pathway operates, it will end up being a PKA-mediated phosphorylation event. In some species, this may be more important than in others.

Question: Are there any ciliary beat data for long-acting β -agonists?

Dr Salathe: I personally do not have any experience with long-acting β -agonists. We tried to conduct experiments with formoterol, but we encountered toxicity problems with the cells. There are data on salmeterol. Even when you give a short-acting β -agonist and then wash it away, the CBF response will remain for at least a half-hour. This is probably mediated by the phosphorylation event in the cilia rather than activation of the signaling transduction pathway.

Question: Could some of the association you observed between effects of β -adrenergic agonists on the cell relative to the amount of agonists be due to the secretion of cAMP from one cell type that then enters the next cell?

Dr Salathe: If one uses human cells and applies cAMP to the outside in doses similar to what you would give for the β -agonist,

nothing happens. Thus, there seems to be no receptor, and I doubt that cAMP could cross the membrane.

Question: Is the protective effect of salmeterol dependent upon either inhibiting the adhesion of bacteria or promoting clearance of the bacteria, or is there a dissociation between those two?

Dr Salathe: That seems to depend on the bacteria you study. With *Pseudomonas*, for example, there is a clear decrease in adherence with the protective effect. Whether that is a direct effect or because cilia are still moving and therefore there is a problem of this bacteria to adhere is not clear. However, if you look at *Haemophilus*, for example, there is no difference in cell adherence.

Question: Did you see any response to a β -agonist in the smooth muscle cells? What cell is the target of inhaled β -agonist bronchodilators? Is it the epithelial cell, which in turn is producing a secondary bronchodilator? Do epithelial cells metabolize the β -agonist?

Comment: Smooth muscle cells usually need a lower concentration of cAMP to respond in some way. Airway epithelial cells seem to have a requirement at least for ciliary beat frequency, to have a fairly high increase in cAMP. That may explain some of the signal differences seen between these different cells. They still may produce a lot of cAMP, but not enough that you see the biological effect.

Comment: Well, I think that is an intriguing question. Is the inhaled β -agonist metabolized locally, or is it all absorbed and metabolized elsewhere in the body? The question is, are the enzymes necessary to inactivate catecholamines? Are they expressed in airway epithelial cells and to what extent? The airway epithelium may have a very important role to play regarding the inhaled β -agonists.

Dr Salathe: There is another mechanism of inactivating them—oxidation. Many of these beta agonists are very sensitive to oxidation, and the airway itself is a very oxidative environment. So you could think about inactivation of some of these β -agonists easily in the airway by oxidation itself.

Question: You commented that in most of the human cell lines you could not see secretion of mucus in response to a β -agonist. Yet in the in vivo situation one of the major symptoms observed with β -agonists was increased cough and sputum production. Could there be major differences in the in vitro and in vivo situations?

Dr Salathe: Yes, there are discrepancies between the in vitro and in vivo effects. If mucociliary clearance is increased, this might bring more mucus to the central airways that can then be coughed out. So maybe the effect of coughing and being able to produce sputum does not necessarily reflect only mucus secretion.

REFERENCES

1. Carstairs JR, Nimmo AJ, Barnes PJ. Autoradiographic visualization of beta-adrenoceptor subtypes in human lung. *Am Rev Respir Dis* 1985;132:541-7.
2. Mak JC, Grandordy B, Barnes PJ. High affinity [3H]formoterol binding sites in lung: characterization and autoradiographic mapping. *Eur J Pharmacol* 1994;269:35-41.
3. Hamid QA, Mak JC, Sheppard MN, Corrin B, Venter JC, Barnes PJ. Localization of β_2 -adrenoceptor messenger RNA in human and rat lung using in situ hybridization: correlation with receptor autoradiography. *Eur J Pharmacol* 1991;206:133-8.
4. Lazarus SC, Basbaum CB, Gold WM. Localization of cAMP in dog and cat trachea: effects of β -adrenergic agonists. *Am J Physiol* 1984;247:C327-34.
5. Barne PJ, Basbaum CB. Mapping of adrenergic receptors in the trachea by autoradiography. *Exp Lung Res* 1983;5:183-92.
6. Kelsen SG, Higgins NC, Zhou S, Mardini IA, Benovic JL. Expression and function of the β -adrenergic receptor coupled-adenylyl cyclase system on human airway epithelial cells. *Am J Respir Crit Care Med* 1995;152:1774-83.

7. Tamaoki J, Chiyotani A, Sakai N, Konno K. Stimulation of ciliary motility mediated by atypical β -adrenoceptor in canine bronchial epithelium. *Life Sci* 1993;53:1509-15.
8. Thomas RF, Liggett SB. Lack of β_3 -adrenergic receptor mRNA expression in adipose and other metabolic tissues in the adult human. *Mol Pharmacol* 1993;43:343-8.
9. Barnes PJ. Beta-adrenergic receptors and their regulation. *Am J Respir Crit Care Med* 1995;152:838-60.
10. Johnson M. Mechanism of action of β_2 -adrenergic agonists. In: Busse WW, Holgate ST, eds. *Asthma and rhinitis*. 2nd ed. Oxford: Blackwell Science Ltd; 2000. p. 1541-57.
11. Yanaura S, Imamura N, Misawa M. Effects of β -adrenoceptor stimulants on the canine tracheal ciliated cells. *Jpn J Pharmacol* 1981;31:951-6.
12. Lopez-Vidriero MT, Jacobs M, Clarke SW. The effect of isoprenaline on the ciliary activity of an in vitro preparation of rat trachea. *Eur J Pharmacol* 1985;112:429-32.
13. Sanderson MJ, Dirksen ER. Mechanosensitive and β -adrenergic control of the ciliary beat frequency of mammalian respiratory tract cells in culture. *Am Rev Respir Dis* 1989;139:432-40.
14. Lansley AB, Sanderson MJ, Dirksen ER. Control of the beat cycle of respiratory tract cilia by Ca^{2+} and cAMP. *Am J Physiol* 1992;263:L232-42.
15. Verdugo P, Johnson NT, Tam PY. Beta-adrenergic stimulation of respiratory ciliary activity. *J Appl Physiol* 1980;48:868-71.
16. Ingels KJ, Meeuwse F, Graamans K, Huizing EH. Influence of sympathetic and parasympathetic substances in clinical concentrations on human nasal ciliary beat. *Rhinology* 1992;30:149-59.
17. Agu RU, Jorissen M, Willems T, Van den Moortel G, Kinget R, Augustijns P. Effects of pharmaceutical compounds on ciliary beating in human nasal epithelial cells: a comparative study of cell culture models. *Pharm Res* 1999;16:1380-5.
18. Wyatt TA, Spurzem JR, May K, Sisson JH. Regulation of ciliary beat frequency by both PKA and PKG in bovine airway epithelial cells. *Am J Physiol* 1998;275:L827-35.
19. Jain B, Rubinstein I, Robbins RA, Leise KL, Sisson JH. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. *Biochem Biophys Res Commun* 1993;191:83-8.
20. Wong LB, Miller IF, Yeates DB. Regulation of ciliary beat frequency by autonomic mechanisms: in vitro. *J Appl Physiol* 1988;65:1895-901.
21. Wong LB, Miller IF, Yeates DB. Stimulation of ciliary beat frequency by autonomic agents: in vivo. *J Appl Physiol* 1988;65:971-81.
22. Chandra T, Yeates DB, Miller IF, Wong LB. Stationary and nonstationary correlation-frequency analysis of heterodyne mode laser light scattering: magnitude and periodicity of canine tracheal ciliary beat frequency in vivo. *Biophys J* 1994;66:878-90.
23. Devalia JL, Sapsford RJ, Rusznak C, Toumbis MJ, Davies RJ. The effects of salmeterol and salbutamol on ciliary beat frequency of cultured human bronchial epithelial cells, in vitro. *Pulm Pharmacol* 1992;5:257-63.
24. Frohock JJ, Wijkstrom-Frei C, Salathe M. Effects of albuterol enantiomers on ciliary beat frequency in ovine tracheal epithelial cells. *J Appl Physiol* 2002;10:1152.
25. Tamaoki J, Kondo M, Takizawa T. Effect of cyclic AMP on ciliary function in rabbit tracheal epithelial cells. *J Appl Physiol* 1989;66:1035-9.
26. Di Benedetto G, Manara-Shediac FS, Mehta A. Effect of cyclic AMP on ciliary activity of human respiratory epithelium. *Eur Respir J* 1991;4:789-95.
27. Salathe M, Lieb T, Bookman RJ. Lack of nitric oxide involvement in cholinergic modulation of ovine ciliary beat frequency. *J Aerosol Med* 2000;13:219-29.
28. Yang B, Schlosser RJ, McCaffrey TV. Dual signal transduction mechanisms modulate ciliary beat frequency in upper airway epithelium. *Am J Physiol* 1996;14:L745-51.
29. Hamasaki T, Murtaugh TJ, Satir BH, Satir P. In vitro phosphorylation of *Paramecium* axonemes and permeabilized cells. *Cell Motil Cytoskeleton* 1989;12:1-11.
30. Hamasaki T, Barkalow K, Richmond J, Satir P. cAMP-stimulated phosphorylation of an axonemal polypeptide that copurifies with the 22S dynein arm regulates microtubule translocation velocity and swimming speed in *Paramecium*. *Proc Natl Acad Sci U S A* 1991;88:7918-22.
31. Sisson JH, Mommsen J, Spurzem JR, Wyatt TA. Localization of PKA and PKG in bovine bronchial epithelial cells and axonemes. *Am J Respir Crit Care Med* 2000;161:A449.
32. Salathe M, Pratt MM, Wanner A. Cyclic AMP-dependent phosphorylation of a 26 kDa axonemal protein in ovine cilia isolated from small tissue pieces. *Am J Respir Cell Mol Biol* 1993;9:306-14.
33. Kultgen PL, Byrd SK, Ostrowski LE, Milgram SL. Protein kinase A is anchored in human ciliary axonemes by a novel A-kinase anchoring protein. *Mol Biol Cell* 2000;11:539a.
34. Salathe M, Lieb T, Bookman RJ. Do cAMP and Ca^{2+} signals in cilia converge on outer dynein arms? *Am J Respir Crit Care Med* 2000;161:A147.
35. McCann JD, Bhalla RC, Welsh MJ. Release of intracellular calcium by two different second messengers in airway epithelium. *Am J Physiol* 1989;257:L116-24.
36. Tamaoki J, Chiyotani A, Kondo M, Konno K. Role of NO generation in β -adrenoceptor-mediated stimulation of rabbit airway ciliary motility. *Am J Physiol* 1995;37:C1342-7.
37. Paradiso AM, Cheng EHC, Boucher RC. Effects of bradykinin on intracellular calcium regulation in human ciliated airway epithelium. *Am J Physiol* 1991;261:L63-9.
38. Davis PB, Silski CL, Perez A. cAMP does not regulate $[\text{Ca}^{2+}]_i$ in human tracheal epithelial cells in primary culture. *J Cell Sci* 1994;107:2899-907.
39. Basbaum CB, Ueki I, Brezina L, Nadel JA. Tracheal submucosal gland serous cells stimulated in vitro with adrenergic and cholinergic agonists. A morphometric study. *Cell Tissue Res* 1981;220:481-98.
40. Gashi AA, Nadel JA, Basbaum CB. Tracheal gland mucous cells stimulated in vitro with adrenergic and cholinergic drugs. *Tissue Cell* 1989;21:59-67.
41. Leikauf GD, Ueki IF, Nadel JA. Autonomic regulation of viscoelasticity of cat tracheal gland secretions. *J Appl Physiol* 1984;56:426-30.
42. Kyle H, Widdicombe JG, Wilfert B. Comparison of mucus flow rate, radiolabelled glycoprotein output and smooth muscle contraction in the ferret trachea in vitro. *Br J Pharmacol* 1988;94:293-8.
43. Liedtke CM, Rudolph SA, Boat TF. Beta-adrenergic modulation of mucin secretion in cat trachea. *Am J Physiol* 1983;244:C391-8.
44. Phipps RJ, Williams IP, Richardson PS, Pell J, Pack RJ, Wright N. Sympathomimetic drugs stimulate the output of secretory glycoproteins from human bronchi in vitro. *Clin Sci (Lond)* 1982;63:23-8.
45. Boat TF, Kleinerman JI. Human respiratory tract secretions. 2. Effect of cholinergic and adrenergic agents on in vitro release of protein and mucous glycoprotein. *Chest* 1975;67:32S-4S.
46. Shelhamer JH, Marom Z, Kaliner M. Immunologic and neuropharmacologic stimulation of mucous glycoprotein release from human airways in vitro. *J Clin Invest* 1980;66:1400-8.
47. Cao W, McGraw DW, Lee TT, Dicker-Brown A, Hiller FC, Cornett LE, et al. Expression of functional β_2 -adrenergic receptors in a rat airway epithelial cell line (SPOC1) and cell density-dependent induction by glucocorticoids. *Exp Lung Res* 2000;26:421-35.
48. Abdullah LH, Conway JD, Cohn JA, Davis CW. Protein kinase C and Ca^{2+} activation of mucin secretion in airway goblet cells. *Am J Physiol* 1997;273:L201-10.
49. Boucher RC. Molecular insights into the physiology of the 'thin film' of airway surface liquid. *J Physiol* 1999;516:631-8.
50. Widdicombe JH. Structure, composition and function of airway surface liquid. In: Busse WW, Holgate ST, eds. *Asthma and rhinitis*. 2nd ed. Oxford: Blackwell Sciences Ltd; 2000. p. 761-70.
51. Knowles M, Murray G, Shallal J, Ashkin F, Ranga V, Gatzky J, et al. Bioelectrical properties and ion flow across excised human bronchi. *J Appl Physiol* 1984;56:868-77.
52. Engelhardt JF, Yankaskas JR, Ernst SA, Yang Y, Marino CR, Boucher RC, et al. Submucosal glands are the predominant site of CFTR expression in the human bronchus. *Nat Genet* 1992;2:240-8.
53. Inglis SK, Corboz MR, Taylor AE, Ballard ST. Effect of anion transport inhibition on mucus secretion by airway submucosal glands. *Am J Physiol* 1997;272:L372-7.
54. Amitani R, Wilson R, Rutman A, Read R, Ward C, Burnett D, et al. Effects of human neutrophil elastase and *Pseudomonas aeruginosa* proteinases on human respiratory epithelium. *Am J Respir Cell Mol Biol* 1991;4:26-32.
55. Jackowski JT, Szeplafusi ZS, Wanner DA, Seybold ZV, Szelczak MW, Lauro IT, et al. Effects of *P. aeruginosa*-derived bacterial products on tracheal ciliary function: role of O_2 radicals. *Am J Physiol* 1991;260:L61-7.
56. Wilson R, Pitt T, Taylor G, MacDermot J, Sykes D, Roberts D, et al. Pyocyanin and 1-hydroxyphenazine produced by *Pseudomonas aeruginosa* inhibit the beating of human respiratory cilia in vitro. *J Clin Invest* 1987;79:221-9.

57. Hingley ST, Hastie AT, Kueppers F, Higgins ML, Weinbaum G, Shryock T. Effect of ciliostatic factors from *Pseudomonas aeruginosa* on rabbit respiratory cilia. *Infect Immun* 1986;51:254-62.
58. Kanthakumar K, Taylor G, Tsang KW, Cundell DR, Rutman A, Smith S, et al. Mechanisms of action of *Pseudomonas aeruginosa* pyocyanin on human ciliary beat in vitro. *Infect Immun* 1993;61:2848-53.
59. Munro NC, Barker A, Rutman A, Taylor G, Watson D, McDonald-Gibson WJ, et al. Effect of pyocyanin and 1-hydroxyphenazine on in vivo tracheal mucus velocity. *J Appl Physiol* 1989;67:316-23.
60. Seybold ZV, Abraham WM, Gazeroglu H, Wanner A. Impairment of airway mucociliary transport by *Pseudomonas aeruginosa* products. Role of oxygen radicals. *Am Rev Respir Dis* 1992;146:1173-6.
61. Dowling RB, Rayner CF, Rutman A, Jackson AD, Kanthakumar K, Dewar A, et al. Effect of salmeterol on *Pseudomonas aeruginosa* infection of respiratory mucosa. *Am J Respir Crit Care Med* 1997;155:327-36.
62. Dowling RB, Johnson M, Cole PJ, Wilson R. Effect of salmeterol on *Haemophilus influenzae* infection of respiratory mucosa in vitro. *Eur Respir J* 1998;11:86-90.
63. Zhang Y, Reenstra WW, Chidekel A. Antibacterial activity of apical surface fluid from the human airway cell line Calu-3: pharmacologic alteration by corticosteroids and β_2 -agonists. *Am J Respir Cell Mol Biol* 2001;25:196-202.
64. Kanthakumar K, Cundell DR, Johnson M, Wills PJ, Taylor GW, Cole PJ, et al. Effect of salmeterol on human nasal epithelial cell ciliary beating: inhibition of the ciliotoxin, pyocyanin. *Br J Pharmacol* 1994;112:493-8.
65. Kanthakumar K, Taylor GW, Cundell DR, Dowling RB, Johnson M, Cole PJ, et al. The effect of bacterial toxins on levels of intracellular adenosine nucleotides and human ciliary beat frequency. *Pulmonol Pharmacol* 1996;9:223-30.
66. Oddera S, Silvestri M, Lantero S, Sacco O, Rossi GA. Downregulation of the expression of intercellular adhesion molecule (ICAM)-1 on bronchial epithelial cells by fenoterol, a β_2 -adrenoceptor agonist. *J Asthma* 1998;35:401-8.
67. Korn SH, Jerre A, Brattsand R. Effects of formoterol and budesonide on GM-CSF and IL-8 secretion by triggered human bronchial epithelial cells. *Eur Respir J* 2001;17:1070-7.
68. Linden A. Increased interleukin-8 release by β -adrenoceptor activation in human transformed bronchial epithelial cells. *Br J Pharmacol* 1996;119:402-6.
69. Wilson SJ, Wallin A, Della-Cioppa G, Sandstrom T, Holgate ST. Effects of budesonide and formoterol on NF-kappaB, adhesion molecules, and cytokines in asthma. *Am J Respir Crit Care Med* 2001;164:1047-52.
70. Spurzem JR, Gupta J, Veys T, Kneifl KR, Rennard SI, Wyatt TA. Activation of protein kinase A accelerates bovine bronchial epithelial cell migration. *Am J Physiol* 2001;10:1152.
71. Merogi AJ, Kneifl K, Veys T, Rennard SI, Spurzem JR. Apoptosis induced in bronchial epithelial cells by isoproterenol is mediated through β -adrenoceptors [abstract]. *Am J Respir Crit Care Med* 2002;165 (suppl):A60.