

## Alveolar Epithelial $\beta_2$ -Adrenergic Receptors

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$\beta_2$ -adrenergic receptors are present throughout the lung, including the alveolar airspace, where they play an important role for regulation of the active  $\text{Na}^+$  transport needed for clearance of excess fluid out of alveolar airspace.  $\beta_2$ -adrenergic receptor signaling is required for up-regulation of alveolar epithelial active ion transport in the setting of excess alveolar edema. The positive, protective effects of  $\beta_2$ -adrenergic receptor signaling on alveolar active  $\text{Na}^+$  transport in normal and injured lungs provide substantial support for the use of  $\beta$ -adrenergic agonists to accelerate alveolar fluid clearance in patients with cardiogenic and noncardiogenic pulmonary edema. In this review, we summarize the role of  $\beta_2$ -adrenergic receptors in the alveolar epithelium with emphasis on their role in the regulation of alveolar active  $\text{Na}^+$  transport in normal and injured lungs.

**Keywords:** pulmonary edema; acute respiratory distress syndrome; acute lung injury; alveoli; albuterol

$\beta_2$ -adrenergic receptors ( $\beta_2\text{AR}$ ) are present throughout the lung. In the alveolar airspace they are important for regulation of the active  $\text{Na}^+$  transport needed for clearance of excess fluid out of alveolar airspace (1). Both experimental and limited clinical data suggest that  $\beta$ -adrenergic agonists working via the  $\beta_2\text{AR}$  accelerate clearance of excess fluid from the alveolar airspace, creating the possibility of their use for treatment of pulmonary edema and acute lung injury (ALI).

In this review, we summarize the role of  $\beta_2$ -adrenergic receptors in the alveolar epithelium with emphasis on their role in regulation of alveolar active  $\text{Na}^+$  transport in normal and injured lungs. We also overview data regarding  $\beta_2$ -agonist therapy for pulmonary edema and lung injury.

### $\beta$ -ADRENERGIC RECEPTORS

#### Subtypes and Distribution in the Lung

$\beta$ -adrenergic receptors are ubiquitous throughout the human body and are classified into three distinct subtypes ( $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ ) on the basis of their function, agonist-binding patterns, and genetics (Table 1). There is 65 to 70% homology among the  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ -receptors. The  $\beta_3$ -receptor is found primarily in adipocytes but is also present in pulmonary endothelial cells. The proposed  $\beta_4$ -receptor appears to be a conformational state of  $\beta_1$ -receptor in myocardial cells (2). Neither the  $\beta_3$  or  $\beta_4$

#### CLINICAL RELEVANCE

This review will help clinicians understand the mechanisms by which  $\beta_2$ -adrenergic therapy may be useful in the management of acute lung injury.

receptors have been linked with regulation of ion transport in epithelial cells.

In the lung,  $\beta_2$ -adrenergic receptor ( $\beta_2\text{AR}$ ) expression increases with each airway generation, with the greatest total amounts in the distal airways and alveoli (3). Greater than 90% of all  $\beta$ -adrenergic receptors in human lung are located in the alveoli (4). Although both  $\beta_1$  and  $\beta_2$  subtypes coexist and are distributed uniformly in the alveolar walls, the  $\beta_2$ -subtype predominates (70%) (4). Isolated rat alveolar type II cells possess  $\beta_2\text{AR}$  and data from autoradiographic and immunohistochemical studies support their presence in the alveolar type 1 cells (4, 5).

#### $\beta_2$ -Adrenergic Receptor Structure and Function

The  $\beta_2$ -adrenergic receptor is a 1,200-base pair, single-copy, intronless gene located on the long arm of human chromosome 5 that encodes a 413-amino acid protein with a molecular mass of approximately 46.5 kD (1). The  $\beta_2$ -receptor is a prototypical G protein-coupled receptor (GPCR) with seven-transmembrane domains, an extracellular amino terminus, an intracellular carboxyl terminus, three interconnecting extracellular loops, and three intracellular loops.

$\beta_2$ -adrenergic receptors exist in the plasma membrane in an equilibrium between at least two structural conformations; inactive and active forms that are defined based on their ability to associate with the stimulatory guanosine triphosphate (GTP)-binding protein, Gs. Like many GPCRs,  $\beta_2$ -adrenergic receptors spontaneously oscillate between inactive and active conformations. In the absence of agonist, the basal equilibrium favors the inactive conformation (6, 7). Spontaneous receptor activation explains the presence of basal  $\beta_2\text{AR}$ -driven adenylyl cyclase activity in cells and observations of increased receptor function in the absence of agonist in models of  $\beta_2\text{AR}$  overexpression (8–10). Engagement of the  $\beta_2\text{AR}$  by a  $\beta_2$ -agonist produces a conformational change shifting the equilibrium between receptor conformations toward the active form causing exchange of guanosine diphosphate (GDP) on  $\text{Gs}\alpha$  for GTP and dissociation of  $\text{Gs}\alpha$  from  $\text{Gs}\beta\gamma$ . The inactive  $\beta_2\text{AR}$  conformation is stabilized by inverse agonists and does not activate  $\text{Gs}\alpha$ ; likewise, replacement of GTP by GDP on Gs uncouples it from the receptor promoting a switch to the inactivate conformation of the  $\beta_2\text{AR}$ .

The conformational state and hence activity of the  $\beta_2\text{AR}$  changes with phosphorylation of the receptor. Phosphorylation of ligand occupied receptors by GPCR kinase 2 (GRK2) and protein kinase A (PKA) produces conformational changes that reduce receptor interactions with  $\text{Gs}\alpha$  and diminish the affinity

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TABLE 1. SUBTYPES OF  $\beta$ -ADRENERGIC RECEPTORS

Subtype	Location	Physiologic effects	Agonist	Antagonist
$\beta_1$	Heart	$\uparrow$ Myocardial contractility	(-) Ro-363	Metoprolol
	Brain			
	Kidney	Renin release		Atenolol
$\beta_2$	Vessels			CGP 20712
	Brain and coronary	Vasodilation		
	Lung			
	Alveolar epithelium	$\uparrow$ Alveolar fluid clearance	Albuterol	Butoxamine
	Bronchial epithelium		Formoterol	ICI 118551
	Smooth muscle	Bronchodilation	Procaterol	
	Skeletal muscle		Salmeterol	
	Cerebellum		Terbutaline	
	Uterine		S1319	
$\beta_3$	Vessels (peripheral)	Vasodilation		
	Adipose tissue	$\uparrow$ Lipolysis	L 755,507	SR 59230
	Ureteral muscle	Relaxation of ureter	CL 316,243	L 748337
	Heart	$\uparrow$ Myocardial contractility	LY 368842	L 747328
	Vasculature	Vasodilation	Ro 40-2148	
			SR 58611	
			BRL 37344	

Nonspecific agonists include isoproterenol, norepinephrine, and epinephrine. Nonselective antagonists include propranolol.

of receptors for ligand, thereby shifting the  $\beta_2$ AR toward an inactive state.

Activation of  $\beta_2$ AR results in a variety of distinct signaling events besides activation of adenylyl cyclase. GRK2 phosphorylation of  $\beta_2$ AR allows for binding of  $\beta$ -arrestins to the receptor, which uncouples the receptor from Gs, and promotes receptor endocytosis via clathrin-coated vesicles (11). Phosphorylation of  $\beta_2$ AR also promotes signaling via  $G_i\gamma$  with subsequent down-regulation of adenylyl cyclase activity and activation of mitogen-activated protein kinase (p44/p42). Both of these inhibitory pathways are important for regulation of Na,K-ATPase trafficking and function in alveolar epithelial cells (12–14). Table 2 summarizes protein–protein interactions and signal transduction systems in which the  $\beta_2$ AR participates (15–35).

Traditionally,  $\beta_2$ -agonists have been classified simply as full, near-full, or partial agonists based on their ability to promote cAMP production. A recent study by Swift and coworkers suggested that the possibility of Gs/cAMP-independent signaling by the  $\beta_2$ AR creates much pleiotropy among  $\beta_2$ AR ligands and as such quantification of agonist activity in terms of cAMP production may no longer be relevant (7).

## ROLE OF ALVEOLAR EPITHELIAL $\beta_2$ -ADRENERGIC RECEPTORS

### Regulation of Alveolar Active $Na^+$ Transport

$\beta_2$ -adrenergic receptors, via cAMP-dependent and -independent pathways, regulate several of the key proteins needed for alveolar epithelial ion and fluid transport including amiloride-sensitive epithelial  $Na^+$  channels, the cystic fibrosis transmembrane conductance regulator (CFTR), and the Na, K-ATPase (36). The initial observation by Goodman and colleagues showing  $\beta$ -agonist-induced active transcellular ion flux in confluent monolayers of isolated rat alveolar epithelial cells (37) was followed by many studies from isolated rat lungs (38) and anesthetized sheep (39) demonstrating that  $\beta$ -adrenergic agonists might be useful for the treatment of pulmonary edema.

Endogenous and exogenous catecholamines stimulate alveolar fluid clearance in newborn and adult animals via activation

TABLE 2. PROTEINS/PATHWAYS INVOLVED IN  $\beta_2$ -ADRENERGIC RECEPTOR SIGNALING

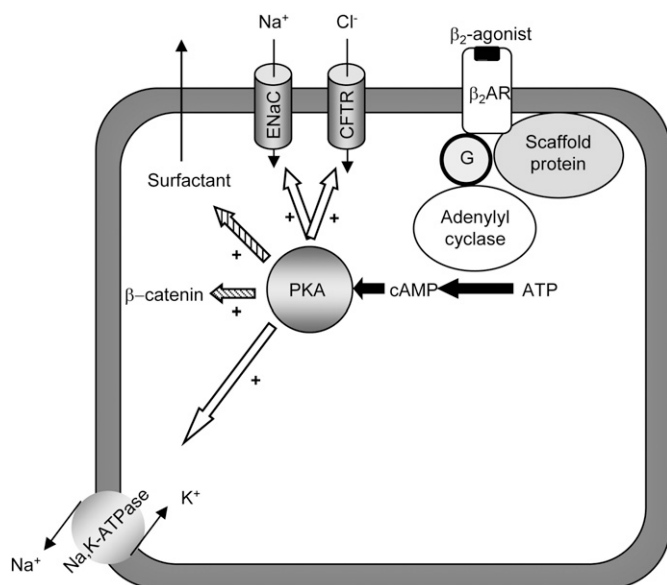
	Protein Kinases
Protein kinase A (15, 16)	Receptor phosphorylation $\downarrow$ Interaction with Gs $\uparrow$ Interaction with $G_i \rightarrow$ Activation of MAPK (ERK)
Protein kinase C	Receptor phosphorylation $\downarrow$ Interaction with Gs
Tyrosine kinases	
Insulin receptor (17, 18)	Receptor phosphorylation (Tyrosine residues) $\downarrow$ (Tyr 141) or $\uparrow$ (Tyr 350/354) cAMP production $\uparrow$ Src binding and GRK2 activation (Tyr 350/354) $\uparrow$ Internalization
Insulin-like growth factor-1	Receptor tyrosine phosphorylation
EGFR (19)	EGFR transactivation ERK1/2 activation
G protein-coupled receptor kinases (GRK)	
GRK2 (20–22)	Receptor phosphorylation (agonist occupied) $\uparrow$ Arrestin binding $\rightarrow$ $\downarrow$ Interaction with Gs
GRK5 (23, 24)	Receptor phosphorylation (agonist occupied) $\downarrow$ Interaction with PDZ-domain-containing proteins $\uparrow$ Arrestin binding $\rightarrow$ $\downarrow$ Interaction with Gs Scaffold Proteins
A-kinase anchoring proteins (AKAP) (25–27)	
AKAP250/AKAP79/150 (Gravin)	Receptor phosphorylation, and internalization Bind receptor to apical cytoskeleton Bring receptor in proximity to PKA
Arrestins (28–31)	Bind to GRK-phosphorylated receptor $\downarrow$ Interaction with Gs Promote ubiquitination of receptor $\uparrow$ Internalization (via interaction with Src) Facilitate receptor interaction with MAPK
PDZ-domain-containing proteins (24, 32, 33)	
EBP50	$\downarrow$ Attenuation of activity of $Na^+/H^+$ -exchanger 3 $\uparrow$ Receptor cycling
N-ethylmaleimide-sensitive factor	Other Proteins (34, 35) $\uparrow$ Internalization and receptor cycling
Eukaryotic initiation factor 2B $\alpha$ -subunit	$\downarrow$ Agonist-promoted adenylyl cyclase activity

*Definition of abbreviations:* cAMP, cyclic-adenosine monophosphate; EBP50, ezrin-radixin-moesin-binding phosphoprotein 50; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; PKA, protein kinase A.

Adapted from Ref. 1.

of  $\beta$ -receptors. Nonspecific  $\beta$ AR (isoproterenol, epinephrine, dobutamine) and  $\beta_2$ AR-specific agonists (procaterol, salmeterol, terbutaline) increase alveolar fluid clearance in normal rat (40), dog (41), sheep (42), guinea pig (43), and mouse lungs (44, 45), and in human lung tissue (46).  $\beta_2$ -receptors appear to be responsible for the bulk of the  $\beta$ -receptor-sensitive alveolar active  $Na^+$  transport (9). Activation of the  $\beta_1$ -adrenergic receptor can accelerate alveolar active  $Na^+$  transport (47); however, data from studies in  $\beta_2$ AR knockout mice suggest that the  $\beta_2$ AR is responsible for most of the  $\beta$ -adrenergic-mediated up-regulation of AFC in fluid-filled lungs (9).

$\beta$ -receptor-mediated increases in alveolar active  $Na^+$  transport are likely due to direct and indirect up-regulation of the epithelial  $Na^+$  channel, CFTR, and Na,K-ATPase (38, 43, 48–51) (Figure 1). *In vitro*,  $\beta$ -agonists stimulate both highly selective  $Na^+$  channels and amiloride-sensitive,  $Na^+$ -permeable, nonselective cation channels (52). Yue and coworkers have demonstrated that stimulation of  $\beta$ AR with terbutaline increases the number of epithelial  $Na^+$  channels and their open



**Figure 1.** Effects of  $\beta_2$ -adrenergic receptor activation in alveolar epithelial cells. Activation of  $\beta_2$ -adrenergic receptor ( $\beta_2$ AR) increases alveolar active  $\text{Na}^+$  transport via upregulation of epithelial  $\text{Na}^+$  channel (ENaC) and cystic fibrosis transmembrane conductance regulator (CFTR) as well as basolaterally located Na,K-ATPase (open arrows). Activation of the receptor also increases  $\beta$ -catenin and surfactant release, which might be important in the pathogenesis/resolution of acute lung injury. ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A.

time in alveolar type II cells (51). These effects of  $\beta_2$ -agonists are mediated via PKA, which phosphorylates cytoskeleton proteins and promotes trafficking of  $\text{Na}^+$  channels to the cell membrane (53) and direct phosphorylation of epithelial  $\text{Na}^+$  channel  $\beta$  and  $\gamma$  subunits. In addition to translocation from intracellular pools to the plasma membrane,  $\beta_2$ -receptors increase the expression of epithelial  $\text{Na}^+$  channel  $\alpha$ -subunit mRNA and protein (54).  $\beta$ -agonists and cAMP analogs increase the open probability and open time of amiloride-sensitive  $\text{Na}^+$  channels in confluent rat alveolar type II cells *in vitro* (55). Thus,  $\beta_2$ AR agonists increase  $\text{Na}^+$  flux across the apical cell membrane by increasing both membrane-bound channel abundance and  $\text{Na}^+$  flux through the channels. Data supporting this conclusion come primarily from rat alveolar type II cells; however, the observation that alveolar type I cells have functional ion transporters and  $\beta_2$ AR suggests similar regulation of ion transport in alveolar type I cells (5, 56, 57).

Activation of  $\beta_2$ -adrenergic receptor increases cellular Na,K-ATPase activity in alveolar epithelial cells *in vitro* and lung tissue (8, 39, 49, 58).  $\beta_2$ -adrenergic receptor-mediated short-term regulation of  $\text{Na}^+$  pumps occurs within minutes of receptor engagement via highly regulated recruitment of assembled Na,K-ATPases from intracellular compartments through phosphorylation of intermediary proteins and RhoA-kinase (49, 59). Long-term regulation is carried out via transcription (54) and translation of  $\alpha_1$ -subunit of Na,K-ATPase through PKA-induced phosphorylation of cAMP-responsive elements and post-transcriptional regulation (via mitogen-activated protein kinase/extracellular signal-regulated kinase and rapamycin-sensitive pathways) (60). There are no clear data to support a role for  $\beta$ -agonist-mediated up-regulation of the activity of individual Na,K-ATPases. These mechanistic findings suggest that up-regulation of Na,K-ATPase activity by  $\beta_2$ -receptor signaling is a complex process that occurs primarily through

increased number of  $\text{Na}^+$  pumps in the cell membrane rather than increased activity of individual pumps.

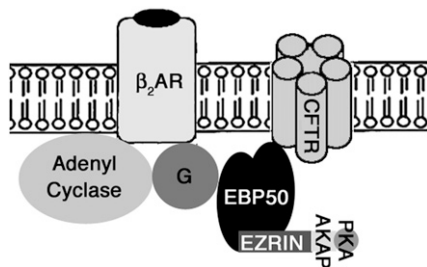
$\beta_2$ AR-mediated up-regulation of fluid transport also involves  $\text{Cl}^-$  transport via CFTR (61–63). Data from alveolar epithelial cells convincingly indicate that  $\beta_2$ -receptor signaling increases  $\text{Cl}^-$  flux through the CFTR (61, 64, 65), similar to that seen in proximal airway epithelial cells (32, 66). Data from CFTR-deficient mice ( $\Delta\phi 508$  transgenics) indicate that CFTR is not required for alveolar fluid homeostasis in the uninjured lung but is essential in the presence of excess airspace fluid and  $\beta_2$ AR-mediated enhancement of AFC (61, 67). *In vitro* studies reveal that cAMP produces an initial and rapid increase in  $\text{Cl}^-$  current, which precedes increases in amiloride-sensitive  $\text{Na}^+$  current offering the possibility that CFTR and/or  $\text{Cl}^-$  flux may influence ENaC function and  $\text{Na}^+$  flux into the cell (68). Both  $\beta$ -agonists or adenoviral overexpression of  $\beta_2$ AR do not increase alveolar fluid clearance in  $\Delta\phi 508$  transgenics to the same degree as in wild-type mice (61, 67); likewise, overexpression of CFTR in mice that lack the  $\beta_2$ AR does not up-regulate alveolar fluid clearance rate compared to control mice infected with adenovirus that encodes CFTR (61). These data suggest an interdependency between  $\beta_2$ AR and CFTR and that both are essential in up-regulation of active  $\text{Na}^+$  transport and fluid clearance in the alveolus (61). They also support a model in which CFTR may be the principal effector of  $\beta_2$ AR-mediated up-regulation of alveolar ion transport.

An intriguing question is why engagement of  $\beta_2$ -receptors produces highly compartmentalized activation of cAMP-sensitive pathways. Recent data indicate that the  $\beta_2$ AR interacts with scaffold and adaptor proteins via its carboxy-terminal end (reviewed in Refs. 69 and 70). These interactions link the  $\beta_2$ -receptor directly or indirectly via ezrin-radixin-moesin-binding phosphoprotein 50 to the cytoskeleton at the apical domain of the cell membrane, forming a macromolecular complex composed of protein kinase A, GPCR kinases, ion channels (e.g., CFTR) and phosphodiesterases, which hydrolyze cAMP (66) (Figure 2). This regulatory complex brings the receptor in close proximity to its principal effector molecule (protein kinase A) and to its downstream targets (CFTR) as well as proteins that turn receptor signaling off (GPCR kinase) and prevent diffusion of cAMP (phosphodiesterases). Studies by Sun and colleagues suggested that ezrin-radixin-moesin-binding phosphoprotein 50-mediated interactions between the receptor and CFTR are important for regulation of  $\text{Cl}^-$  transport in airway epithelial cells (Calu-3) (71, 72). Whether similar interactions occur in alveolar epithelial cells is not yet known.

The concentration of cAMP in the cell is determined by the activities of adenylyl cyclases and phosphodiesterases, which inactivate cAMP (73). There are nine types of adenylyl cyclases, which are all transmembrane proteins, synthesizing cAMP at the plasma membrane (73, 74). This results in a gradient in which higher concentrations of cAMP are found at the membrane and lower concentrations in the cytosol (73, 75–78). This gradient may allow localization of the physiologic effect of  $\beta$ AR and resultant production of cAMP to microenvironments, which in turn activate specific target proteins.

### Maintenance of Alveolar Fluid Homeostasis

Recent data provide some clues regarding whether  $\beta_2$ AR are required for maintenance of alveolar fluid balance in the normal lung (39, 41, 44–46, 79, 80). While initial studies of adrenalectomized animals (8, 45) or desensitization of  $\beta$ -receptors (81, 82) note no net effect on lung water content or basal alveolar fluid clearance, a more recent study showed reduced basal alveolar fluid clearance in adrenalectomized mice (83). The differences between these studies may be due to inability to



**Figure 2.** Proposed interaction of  $\beta_2$ -adrenergic receptor with CFTR and other proteins. In the cell membrane, the  $\beta_2$ AR is in close proximity to transport molecules such as CFTR and possibly ENaC. This macromolecular complex is maintained through interactions of the  $\beta_2$ AR with scaffold and adaptor proteins including EBP50 and ezrin, which also link the receptor with submembrane cytoskeletal elements. ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; EBP50, ezrin-radixin-moesin-binding phosphoprotein 50; PKA, protein kinase A. (Adapted from Reference 32.)

fully desensitize alveolar  $\beta_2$ -receptors or completely eliminate serum catecholamines and methods used to quantify alveolar fluid clearance (*in vivo* live model versus *ex vivo*). Data from mice with no functional  $\beta_2$ -receptor ( $\beta_2$ -knockout) or  $\beta_1$ -receptor and  $\beta_2$ -receptor ( $\beta_1\beta_2$ -knockout) (9) similarly reveal normal water content when uninjured but decreased ability to clear excess water from the airspace and more pulmonary edema and decreased survival from acute lung injury (hyperoxia). These findings suggest that the  $\beta_2$ AR is functionally required in the presence of excess airspace fluid, but may not be required to maintain lung fluid balance in the uninjured lung or when fluid shifts are modest. Importantly, these data indicate that other regulatory pathways are not sufficient to accelerate alveolar active  $\text{Na}^+$  transport mechanisms in the injured lung.

### Other Functions

The effects of  $\beta$ -adrenergic receptor in the alveolar epithelium are not limited to up-regulation of active  $\text{Na}^+$  transport.  $\beta_2$ -adrenergic receptor may also increase levels of the Wnt pathway member  $\beta$ -catenin, which regulates cell-to-cell adhesions via cadherin (84, 85). Phosphorylation of  $\beta$ -catenin by PKA stabilizes  $\beta$ -catenin post-translationally through inhibition of its ubiquitination (86). Experimental data also suggest that  $\beta$ -agonists can improve endothelial barrier function (87–89) by inhibition of endothelial cell contraction and reduced intracellular gap formation (90, 91).  $\beta_2$ AR also regulate surfactant secretion by alveolar type II cells (92, 93). These additional effects may be responsible for some of the protective effects of  $\beta_2$ -agonists in the acutely injured lung.

In contrast to the beneficial effects of  $\beta_2$ -agonists on the alveolar epithelium, activation of  $\beta_2$ AR (94) has an inhibitory effect on the function of alveolar macrophages where they diminish motility (95) via increased levels of cAMP, which has also been reported to result in diminished fluid phase endocytosis (96) and phagocytosis function (97).

## $\beta_2$ -AGONIST THERAPY FOR TREATMENT OF PULMONARY EDEMA

### Experimental Studies

There is a great body of experimental data indicating that specific and nonspecific  $\beta$ -agonists enhance AFC in experimental models of cardiogenic and noncardiogenic pulmonary edema (1, 9, 61). Recent data suggest that preservation of  $\beta$ -adrenergic receptor signaling attenuates loss of endothelial cell barrier

function in mice with severe bacterial pneumonia (98). Stimulation of  $\beta$ -adrenergic receptors is also capable of preventing hypoxia-induced reduction in alveolar active  $\text{Na}^+$  transport and fluid clearance in rats (99). Physiologic concentrations of  $\beta$ -agonists do not alter neutrophil chemotaxis, death/apoptosis *in vitro*, or affect alveolar recruitment and activation of neutrophils *in vivo* (100); thus, the beneficial effects of  $\beta$ -agonists in these models are unlikely to be a reflection of their putative anti-inflammatory effects.

McAuley and colleagues investigated the effect of clinically relevant doses of  $\beta_2$ -agonists (in the alveolar epithelial lining fluid) on alveolar fluid clearance in an acid aspiration model of acute lung injury in rats (89). Racemic albuterol ( $10^{-5}$  M), salmeterol ( $10^{-6}$  M), and isoproterenol ( $10^{-6}$  M) each stimulated basal alveolar fluid clearance to levels comparable to maximal cAMP-dependent alveolar fluid clearance using a stable analog of cAMP (dibutyl cAMP  $10^{-3}$  M) (89). This improvement in alveolar fluid clearance correlated with attenuation of acid aspiration lung injury.

Both transgenic and adenoviral-mediated overexpression of  $\beta_2$ -receptor in the alveolar epithelium increase alveolar active  $\text{Na}^+$  transport (8, 9, 101), probably by increasing the number of receptors in active conformation. Transgenic overexpression of  $\beta_2$ -receptor in alveolar type II cells increases alveolar fluid clearance in mice by approximately 40% (101). Adenoviral-mediated transfer of a human  $\beta_2$ -receptor to the alveolar epithelium increases alveolar fluid clearance in normal rats and mice by up-regulating the expression and/or function of amiloride-sensitive epithelial  $\text{Na}^+$  channels and  $\text{Na,K-ATPases}$  in the distal lung (8, 9). These effects were attributed, in part, to improved responsiveness to endogenous catecholamines. Importantly, overexpression of the  $\beta_2$ -receptor in mouse lungs markedly improved survival of mice exposed to 100% oxygen.

### Clinical Studies

Pulmonary edema clearance is impaired in animal models of hydrostatic and noncardiogenic pulmonary edema (102, 103). Loss of the ability to increase clearance of pulmonary edema is associated with increased risk of pulmonary edema and mortality from acute lung injury in humans (104–106) and animals (9). In a study of 79 patients with ALI, greater than half had impaired pulmonary edema clearance and only 13% had maximal expected clearance rate (106). Hospital mortality was 20% in patients with maximal clearance, compared with 62% in patients with impaired or submaximal clearance. These data raise the possibility that reduced alveolar fluid clearance may contribute to mortality in acute lung injury. Recent human studies of fluid management in ALI/ARDS (107) do not clearly link total body fluid balance with clinical outcome; thus, it is not yet possible to implicate reduced alveolar fluid clearance as a contributor to, or cause of, respiratory failure.

Limited clinical data regarding the use of  $\beta$ -agonists for pulmonary edema has expanded in the last few years. Salmeterol, a long-acting  $\beta_2$ -agonist, has been shown to reduce the incidence of high-altitude pulmonary edema in mountain climbers when used as preventive therapy (104). Aerosol delivery of albuterol at clinically approved doses to mechanically ventilated patients with respiratory failure yields clinically significant levels of this  $\beta$ -agonist in lung edema fluid (108). Furthermore,  $\beta$ -agonist use correlates with improved outcome in patients with acute lung injury (109). In a single-center, double-blind, randomized controlled trial (BALTI, The  $\beta$ -Agonist Lung Injury Trial), treatment with intravenous salbutamol ( $15 \mu\text{g/kg/h}$ ) in patients with ARDS significantly lowered extravascular lung water content measured by thermodilution at Day 7 compared

with placebo (110). Patients who received salbutamol had improved respiratory system compliance and a trend toward lower lung injury scores at Day 7. In contrast to experimental data (46, 89), the effect of  $\beta$ -agonist therapy on lung water content was not evident until 48 hours after initiation of therapy. The mechanism responsible for this delay in  $\beta$ -agonist response might be linked to alveolar epithelial damage during early ARDS (110).

### Limitations

The question of whether the alveolar epithelium may be critically injured and even denuded, interfering with and offsetting the beneficial effects of  $\beta$ -agonists on the alveolar epithelium, remains unanswered (36). As such, it is unclear whether  $\beta_2$ AR-mediated up-regulation of active  $\text{Na}^+$  transport is possible during severe lung injury. Some experimental ALI models (i.e., prolonged hemorrhagic shock, hyperoxia, ischemia reperfusion after lung transplantation, and ventilator-induced lung injury) have been linked with diminished  $\beta$ -receptor function (111–116). Recently, Davis and coworkers have reported decreased sensitivity to  $\beta$ -agonists in a murine model of respiratory syncytial virus infection (83). The inhibitory effect of viral infection was attributed to impaired  $\beta_2$ -AR signaling as a consequence of GRK-2-mediated uncoupling of the receptor from adenylyl cyclase (83). Restoration of  $\beta$ -agonist-sensitive active  $\text{Na}^+$  transport with inhibition of inducible nitric oxide synthase (111) and N-acetylcysteine (114) in some of these models implicates oxidation-dependent impairment of  $\beta_2$ -adrenergic receptor signaling. NF- $\kappa$ B-dependent activation of inducible nitric oxide synthase impairs the function of membrane proteins (i.e., adenylyl cyclase) involved in the  $\beta_2$ -receptor signaling pathway (111). These effects may be due to alterations in  $\beta_2$ AR signaling but could also be attributed to diminished alveolar barrier function, loss of epithelial cells, or down-regulation of transport protein function.

Theoretically, a potential limitation of  $\beta_2$ -agonist therapy for treatment of pulmonary edema is receptor desensitization (a regulated process that leads to attenuation of the biologic effect of receptor during prolonged agonist exposure) and down-regulation (a form of desensitization during which density/number of receptors decreases), both of which will diminish the efficacy of  $\beta$ -agonist therapy (82, 117, 118).

Regulation of  $\beta_2$ -receptors occurs primarily through phosphorylation-dependent loss of sensitivity to agonist. These processes have been extensively studied in cardiac cells and airway smooth muscle cells. Proclivity for desensitization varies among tissues; for example, cardiac myocytes are readily desensitized, whereas airway smooth muscle cells may not have the necessary GPCR kinases to affect receptor phosphorylation.

Continuous stimulation with isoproterenol causes impairment in the ability of  $\beta$ -agonists to increase alveolar fluid clearance only when nonspecific  $\beta$ -agonists (isoproterenol) or high doses of a  $\beta_2$ -agonist are used (119, 120). Prolonged stimulation with a  $\beta$ -agonist impairs its ability to continue to up-regulate alveolar fluid clearance, likely due to reduction in receptor density (120) and impaired of post-receptor signaling (82). A broad base of data supports  $\beta$ -agonist-induced attenuation of  $\beta_2$ AR-mediated airway relaxation. Whether similar agonist-dependent (homologous) or -independent (heterologous) desensitization occurs in alveolar epithelial cells in humans is not known, and thus the implications of prolonged  $\beta_2$ AR engagement on the protective effects of  $\beta$ -agonists are not known.

Another potentially limiting factor for use of  $\beta_2$ -agonists is the  $\beta_2$ -adrenergic receptor polymorphism, which might influence the response to the agonists and  $\beta_2$ AR regulation (121,

122). While the effect of  $\beta_2$ -adrenergic receptor polymorphism in asthma has been studied and has been shown to affect clinical response, it has not been evaluated in the alveolar epithelium of humans (123–124).

Finally, it is important to recognize the detrimental effects of  $\beta_2$ -agonist therapy such as induction of tachycardia and increased oxygen consumption, which may cause adverse effects particularly in patients with underlying cardiovascular disease. Another concern is the worsening of ventilation–perfusion mismatch that results from  $\beta$ -agonist-mediated vasodilation, which precedes bronchodilation and thereby causes deterioration of oxygenation.

### CONCLUSIONS

$\beta_2$ -adrenergic receptor signaling is required for up-regulation of alveolar epithelial active ion transport in the setting of excess alveolar edema fluid. The positive, protective effects of  $\beta_2$ AR signaling on alveolar active  $\text{Na}^+$  transport in normal and injured lungs provide substantial support for the use of  $\beta$ -adrenergic agonists to accelerate alveolar fluid clearance in patients with cardiogenic and noncardiogenic pulmonary edema.

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