Translational Review

Alveolar Epithelial β_2-Adrenergic Receptors

Gökhan M. Mutlu[1] and Phillip Factor[2]

[1]Pulmonary and Critical Care Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois; and [2]Pulmonary, Allergy and Critical Care Medicine, Columbia University College of Physicians and Surgeons, New York, New York

β_2-adrenergic receptors are present throughout the lung, including the alveolar airspace, where they play an important role for regulation of the active Na^+ transport needed for clearance of excess fluid out of alveolar airspace. β_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 β_2-adrenergic receptor signaling on alveolar active Na^+ transport in normal and injured lungs provide substantial support for the use of β-adrenergic agonists to accelerate alveolar fluid clearance in patients with cardiogenic and noncardiogenic pulmonary edema. In this review, we summarize the role of β_2-adrenergic receptors in the alveolar epithelium with emphasis on their role in the regulation of alveolar active Na^+ transport in normal and injured lungs.

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

β_2-adrenergic receptors (β_2AR) are present throughout the lung. In the alveolar airspace they are important for regulation of the active Na^+ transport needed for clearance of excess fluid out of alveolar airspace (1). Both experimental and limited clinical data suggest that β-adrenergic agonists working via the β_2AR 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 β_2-adrenergic receptors in the alveolar epithelium with emphasis on their role in regulation of alveolar active Na^+ transport in normal and injured lungs. We also overview data regarding β_2-agonist therapy for pulmonary edema and lung injury.

CLINICAL RELEVANCE

This review will help clinicians understand the mechanisms by which β_2-adrenergic therapy may be useful in the management of acute lung injury.

β-ADRENERGIC RECEPTORS

Subtypes and Distribution in the Lung

β-adrenergic receptors are ubiquitous throughout the human body and are classified into three distinct subtypes (β_1, β_2, and β_3) on the basis of their function, agonist-binding patterns, and genetics (Table 1). There is 65 to 70% homology among the β_1-, β_2-, and β_3-receptors. The β_3-receptor is found primarily in adipocytes but is also present in pulmonary endothelial cells. The proposed β_4-receptor appears to be a conformational state of β_1-receptor in myocardial cells (2). Neither the β_3 or β_4 receptors have been linked with regulation of ion transport in epithelial cells.

In the lung, β2-adrenergic receptor (β_2AR) expression increases with each airway generation, with the greatest total amounts in the distal airways and alveoli (3). Greater than 90% of all β-adrenergic receptors in human lung are located in the alveoli (4). Although both β_1 and β_2 subtypes coexist and are distributed uniformly in the alveolar walls, the β_2-subtype predominates (70%) (4). Isolated rat alveolar type II cells possess β_2AR and data from autoradiographic and immunohistochemical studies support their presence in the alveolar type 1 cells (4, 5).

β_2-Adrenergic Receptor Structure and Function

The β_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 β_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.

β_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, β_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 β_2AR-driven adenylyl cyclase activity in cells and observations of increased receptor function in the absence of agonist in models of β_2AR overexpression (8–10). Engagement of the β_2AR by a β_2-agonist produces a conformational change shifting the equilibrium between receptor conformations toward the active form causing exchange of guanosine diphosphate (GDP) on Gsα for GTP and dissociation of Gsα from Gsβγ. The inactive β_2AR conformation is stabilized by inverse agonists and does not activate Gsα; likewise, replacement of GTP by GDP on Gs uncouples it from the receptor promoting a switch to the inactivate conformation of the β_2AR.

The conformational state and hence activity of the β_2AR 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 Gsα and diminish the affinity

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Correspondence and requests for reprints should be addressed to Gökhan M. Mutlu, M.D., Northwestern University Feinberg School of Medicine, Pulmonary and Critical Care Medicine, 240 E. Huron Street, McGaw M-300, Chicago, IL 60611. Email: g-mutlu@northwestern.edu

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TABLE 1. SUBTYPES OF β-ADRENERGIC RECEPTORS

Subtype	Location	Physiologic effects	Agonist	Antagonist
β_1	Heart	↑ Myocardial contractility	(−) Ro-363	Metoprolol
	Brain			Atenolol
	Kidney	Renin release		CGP 20712
	Vessels			
	Brain and coronary	Vasodilation		
β_2	Lung			
	Alveolar epithelium	↑ Alveolar fluid clearance	Albuterol	Butoxamine
	Bronchial epithelium		Formoterol	ICI 118551
	Smooth muscle	Bronchodilation	Procaterol	
	Skeletal muscle		Salmeterol	
	Cerebellum		Terbutaline	
	Uterine		S1319	
	Vessels (peripheral)	Vasodilation		
β_3	Adipose tissue	↑ Lipolysis	L 755,507	SR 59230
	Ureteral muscle	Relaxation of ureter	CL 316,243	L 748337
	Heart	↑ 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 propranol.

of receptors for ligand, thereby shifting the β_2AR toward an inactive state.

Activation of β_2AR results in a variety of distinct signaling events besides activation of adenylyl cyclase. GRK2 phosphorylation of β_2AR allows for binding of β-arrestins to the receptor, which uncouples the receptor from Gs, and promotes receptor endocytosis via clathrin-coated vesicles (11). Phosphorylation of β_2AR also promotes signaling via Giβγ 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 β_2AR participates (15–35).

Traditionally, β_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 β_2AR creates much pleiotropy among β_2AR ligands and as such quantification of agonist activity in terms of cAMP production may no longer be relevant (7).

TABLE 2. PROTEINS/PATHWAYS INVOLVED IN β_2-ADRENERGIC RECEPTOR SIGNALING

	Protein Kinases
Protein kinase A (15, 16)	Receptor phosphorylation ↓ Interaction with Gs ↑ Interaction with Gi → Activation of MAPK (ERK)
Protein kinase C	Receptor phosphorylation ↓ Interaction with Gs
Tyrosine kinases	
Insulin receptor (17, 18)	Receptor phosphorylation (Tyrosine residues) ↓ (Tyr 141) or ↑ (Tyr 350/354) cAMP production ↑ Src binding and GRK2 activation (Tyr 350/354) ↑ 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) ↑ Arrestin binding → ↓ Interaction with Gs
GRK5 (23, 24)	Receptor phosphorylation (agonist occupied) ↓ Interaction with PDZ-domain-containing proteins ↑ Arrestin binding → ↓ 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 ↓ Interaction with Gs Promote ubiquitination of receptor ↑ Internalization (via interaction with Src) Facilitate receptor interaction with MAPK
PDZ-domain-containing proteins (24, 32, 33)	
EBP50	↓ Attenuation of activity of Na^+/H^+-exchanger 3 ↑ Receptor cycling
	Other Proteins (34, 35)
N-ethylmaleimide–sensitive factor	↑ Internalization and receptor cycling
Eukaryotic initiation factor 2B α-subunit	↓ Agonist-promoted adenylyl cyclase activity

Definition of abbreviations: cAMP, cyclic-adenosine monophosphate; EBP50, ezrin-radixin-moesin–binding phoshoprotein 50; EGFR, epidermal growth factor receptor; ERK, extracellular signal–regulated kinase; MAPK, mitogen-activated protein kinase; PKA, protein kinase A.
Adapted from Ref. 1.

ROLE OF ALVEOLAR EPITHELIAL β_2-ADRENERGIC RECEPTORS

Regulation of Alveolar Active Na^+ Transport

β_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 β-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 β-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 of β-receptors. Nonspecific βAR (isoproterenol, epinephrine, dobutamine) and β_2AR-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). β_2-receptors appear to be responsible for the bulk of the β-receptor–sensitive alveolar active Na^+ transport (9). Activation of the β_1-adrenergic receptor can accelerate alveolar active Na^+ transport (47); however, data from studies in β_2AR knockout mice suggest that the β_2AR is responsible for most of the β-adrenergic–mediated up-regulation of AFC in fluid-filled lungs (9).

β-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*, β-agonists stimulate both highly selective Na^+ channels and amiloride-sensitive, Na^+-permeable, nonselective cation channels (52). Yue and coworkers have demonstrated that stimulation of βAR with terbutaline increases the number of epithelial Na^+ channels and their open

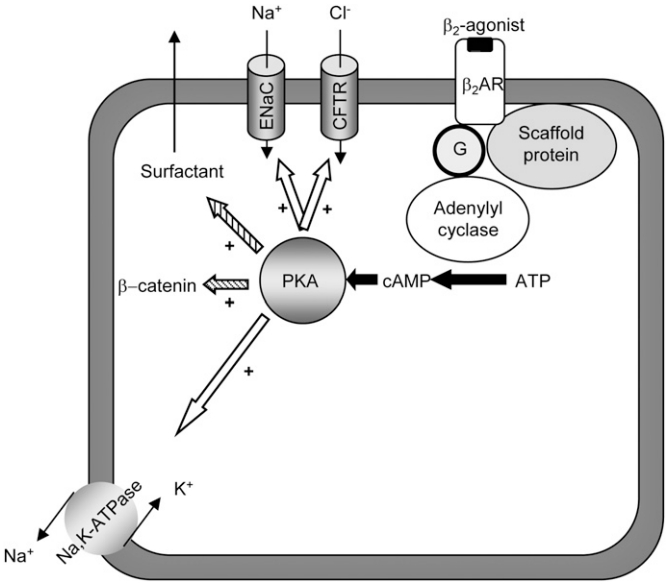

Figure 1. Effects of β_2-adrenergic receptor activation in alveolar epithelial cells. Activation of β_2-adrenergic receptor (β_2AR) increases alveolar active Na^+ transport via upregulation of epithelial Na^+ channel (ENaC) and cystic fibrosis transmembrane conductance regulator (CFTR) as well as basolaterally located Na,K-ATPase (*open arrows*). Activation of the receptor also increases β-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 β_2-agonists are mediated via PKA, which phosphorylates cytoskeleton proteins and promotes trafficking of Na^+ channels to the cell membrane (53) and direct phosphorylation of epithelial Na^+ channel β and γ subunits. In addition to translocation from intracellular pools to the plasma membrane, β_2-receptors increase the expression of epithelial Na^+ channel α-subunit mRNA and protein (54). β-agonists and cAMP analogs increase the open probability and open time of amiloride-sensitive Na^+ channels in confluent rat alveolar type II cells *in vitro* (55). Thus, β_2AR agonists increase Na^+ flux across the apical cell membrane by increasing both membrane-bound channel abundance and 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 β_2AR suggests similar regulation of ion transport in alveolar type I cells (5, 56, 57).

Activation of β_2-adrenergic receptor increases cellular Na,K-ATPase activity in alveolar epithelial cells *in vitro* and lung tissue (8, 39, 49, 58). β_2-adrenergic receptor–mediated short-term regulation of 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 α_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 β-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 β_2-receptor signaling is a complex process that occurs primarily through increased number of Na^+ pumps in the cell membrane rather than increased activity of individual pumps.

β_2AR-mediated up-regulation of fluid transport also involves Cl^- transport via CFTR (61–63). Data from alveolar epithelial cells convincingly indicate that β_2-receptor signaling increases Cl^- flux through the CFTR (61, 64, 65), similar to that seen in proximal airway epithelial cells (32, 66). Data from CFTR-deficient mice (Δϕ508 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 β_2AR-mediated enhancement of AFC (61, 67). *In vitro* studies reveal that cAMP produces an initial and rapid increase in Cl^- current, which precedes increases in amiloride-sensitive Na^+ current offering the possibility that CFTR and/or Cl^- flux may influence ENaC function and Na^+ flux into the cell (68). Both β-agonists or adenoviral overexpression of β_2AR do not increase alveolar fluid clearance in Δϕ508 transgenics to the same degree as in wild-type mice (61, 67); likewise, overexpression of CFTR in mice that lack the β_2AR 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 β_2AR and CFTR and that both are essential in up-regulation of active Na^+ transport and fluid clearance in the alveolus (61). They also support a model in which CFTR may the principal effector of β_2AR-mediated up-regulation of alveolar ion transport.

An intriguing question is why engagement of β_2-receptors produces highly compartmentalized activation of cAMP-sensitive pathways. Recent data indicate that the β_2AR interacts with scaffold and adaptor proteins via its carboxy-terminal end (reviewed in Refs. 69 and 70). These interactions link the β_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 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 phophodiesterases, 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 βAR 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 β_2AR 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 β-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 adrenelectomized mice (83). The differences between these studies may be due to inability to

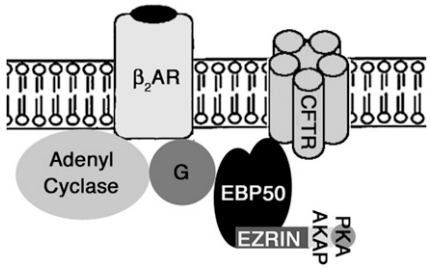

Figure 2. Proposed interaction of β_2-adrenergic receptor with CFTR and other proteins. In the cell membrane, the β_2AR is in close proximity to transport molecules such as CFTR and possibly ENaC. This macromolecular complex is maintained through interactions of the β_2AR with scaffold and adaptor proteins 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 β_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 β_2-receptor (β_2-knockout) or β_1-receptor and β_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 β_2AR 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 Na^+ transport mechanisms in the injured lung.

Other Functions

The effects of β-adrenergic receptor in the alveolar epithelium are not limited to up-regulation of active Na^+ transport. β_2-adrenergic receptor may also increase levels of the Wnt pathway member β-catenin, which regulates cell-to-cell adhesions via cadherin (84, 85). Phosphorylation of β-catenin by PKA stabilizes β-catenin post-translationally through inhibition of its ubiquitination (86). Experimental data also suggest that β-agonists can improve endothelial barrier function (87–89) by inhibition of endothelial cell contraction and reduced intracellular gap formation (90, 91). β_2AR also regulate surfactant secretion by alveolar type II cells (92, 93). These additional effects may be responsible for some of the protective effects of β_2-agonists in the acutely injured lung.

In contrast to the beneficial effects of β_2-agonists on the alveolar epithelium, activation of β_2AR (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).

β_2-AGONIST THERAPY FOR TREATMENT OF PULMONARY EDEMA

Experimental Studies

There is a great body of experimental data indicating that specific and nonspecific β-agonists enhance AFC in experimental models of cardiogenic and noncardiogenic pulmonary edema (1, 9, 61). Recent data suggest that preservation of β-adrenergic receptor signaling attenuates loss of endothelial cell barrier function in mice with severe bacterial pneumonia (98). Stimulation of β-adrenergic receptors is also capable of preventing hypoxia-induced reduction in alveolar active Na^+ transport and fluid clearance in rats (99). Physiologic concentrations of β-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 β-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 β_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 (dibutyryl 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 β_2-receptor in the alveolar epithelium increase alveolar active Na^+ transport (8, 9, 101), probably by increasing the number of receptors in active conformation. Transgenic overexpression of β_2-receptor in alveolar type II cells increases alveolar fluid clearance in mice by approximately 40% (101). Adenoviral-mediated transfer of a human β_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 Na^+ channels and 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 β_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 β-agonists for pulmonary edema has expanded in the last few years. Salmeterol, a long-acting β_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 β-agonist in lung edema fluid (108). Furthermore, β-agonist use correlates with improved outcome in patients with acute lung injury (109). In a single-center, double-blind, randomized controlled trial (BALTI, The β-Agonist Lung Injury Trial), treatment with intravenous salbutamol (15 μg/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 β-agonist therapy on lung water content was not evident until 48 hours after initiation of therapy. The mechanism responsible for this delay in β-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 β-agonists on the alveolar epithelium, remains unanswered (36). As such, it is unclear whether β_2AR-mediated up-regulation of active 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 β-receptor function (111–116). Recently, Davis and coworkers have reported decreased sensitivity to β-agonists in a murine model of respiratory syncytial virus infection (83). The inhibitory effect of viral infection was attributed to impaired β_2-AR signaling as a consequence of GRK-2–mediated uncoupling of the receptor from adenylyl cyclase (83). Restoration of β-agonist–sensitive active Na^+ transport with inhibition of inducible nitric oxide synthase (111) and N-acetylcysteine (114) in some of these models implicates oxidation-dependent impairment of β_2-adrenergic receptor signaling. NF-κB–dependent activation of inducible nitric oxide synthase impairs the function of membrane proteins (i.e., adenylyl cyclase) involved in the β_2-receptor signaling pathway (111). These effects may be due to alterations in β_2AR 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 β_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 β-agonist therapy (82, 117, 118).

Regulation of β_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 β-agonists to increase alveolar fluid clearance only when nonspecific β-agonists (isoproterenol) or high doses of a β_2-agonist are used (119, 120). Prolonged stimulation with a β-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 β-agonist–induced attenuation of β_2AR-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 β_2AR engagement on the protective effects of β-agonists are not known.

Another potentially limiting factor for use of β_2-agonists is the β_2-adrenergic receptor polymorphism, which might influence the response to the agonists and β_2AR regulation (121, 122). While the effect of β_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 β_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 β-agonist–mediated vasodilation, which precedes bronchodilation and thereby causes deterioration of oxygenation.

CONCLUSIONS

β_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 β_2AR signaling on alveolar active Na^+ transport in normal and injured lungs provide substantial support for the use of β-adrenergic agonists to accelerate alveolar fluid clearance in patients with cardiogenic and noncardiogenic pulmonary edema.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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