

## E-NTPDases in human airways: Regulation and relevance for chronic lung diseases

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**Abstract** Chronic obstructive lung diseases are characterized by the inability to prevent bacterial infection and a gradual loss of lung function caused by recurrent inflammatory responses. In the past decade, numerous studies have demonstrated the importance of nucleotide-mediated bacterial clearance. Their interaction with P2 receptors on airway epithelia provides a rapid ‘on-and-off’ signal stimulating mucus secretion, cilia beating activity and surface hydration. On the other hand, abnormally high ATP levels resulting from damaged epithelia and bacterial lysis may cause lung edema and exacerbate inflammatory responses. Airway ATP concentrations are regulated by ecto nucleoside triphosphate diphosphohydrolases (E-NTPDases) which are expressed on the mucosal surface and catalyze the sequential dephosphorylation of nucleoside triphosphates to nucleoside monophosphates (ATP → ADP → AMP). The common bacterial product, *Pseudomonas aeruginosa* lipopolysaccharide (LPS), induces an acute reduction in azide-sensitive E-NTPDase activities, followed by a sustained increase in activity as well as NTPDase 1 and NTPDase 3 expression. Accordingly, chronic lung diseases, including cystic fibrosis (CF) and primary ciliary dyskinesia,

are characterized by higher rates of nucleotide elimination, azide-sensitive E-NTPDase activities and expression. This review integrates the biphasic regulation of airway E-NTPDases with the function of purine signaling in lung diseases. During acute insults, a transient reduction in E-NTPDase activities may be beneficial to stimulate ATP-mediated bacterial clearance. In chronic lung diseases, elevating E-NTPDase activities may represent an attempt to prevent P2 receptor desensitization and nucleotide-mediated lung damage.

**Keywords** apyrase · bacterial clearance · CD39 · chronic obstructive lung diseases · diphosphohydrolase · endotoxin · purinergic signaling · oxidative stress

### Abbreviations

ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
ASL	airway surface liquid
MCC	mucociliary clearance
CCS	cyclic compressive stress
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane regulator
ENAC	epithelial sodium channel
IL-1 $\beta$	interleukin-1beta
IL-8	interleukin-8
LPS	lipopolysaccharide
MCC	mucociliary clearance
NS AP	non-specific alkaline phosphatase
PCL	periciliary liquid
PCR	polymerase chain reaction
TNF $\alpha$	tumor necrosis factor alpha
UTP	uridine 5'-triphosphate

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## Introduction

Airway epithelia constitute an essential protective barrier against lung infection, coordinating luminal and interstitial responses to inhaled pathogens through signals (growth factors, cytokines and nucleotides) provided by epithelial, inflammatory and immune cells [1]. It is now widely accepted that extracellular nucleotides provide an elaborated cell communication system in mammalian tissues [2, 3] including the airways [4, 5]. Each signaling event constitutes a brief ‘on-and-off’ switch mechanism allowing the target cells to perceive the subsequent signal. The major source of extracellular nucleotides in normal airways is the epithelium, releasing ATP under resting conditions and in response to mechanical or osmotic stress [6]. While basal ATP concentrations maintained under resting conditions are insufficient to activate surface receptors, the levels reached near the site of stimulated release initiate a variety of P2 receptor-mediated responses [7]. Two P2 receptor families have been identified: Fast-acting (ionotropic) P2X ligand-gated cation channels and slow-acting (metabotropic) G protein-coupled P2Y receptors [8]. Although ATP activates members of both families, P2X receptors generally respond to higher concentrations ( $EC_{50} = 1\text{--}10\ \mu\text{M}$ ) than P2Y receptors ( $EC_{50} = 0.1\text{--}1.0\ \mu\text{M}$ ). Each signal is promptly terminated by surface conversion of ATP to adenosine [9, 10] and dispersal into the interstitial fluid. Adenosine also initiates cellular responses through P1 ( $A_1$ ,  $A_{2A}$ ,  $A_{2B}$  or  $A_3$ ) surface receptors [11]. In the airways, nucleotide- and adenosine-mediated communications are involved in wound healing [4], bacterial clearance [12] and inflammatory responses [1, 3, 13, 14].

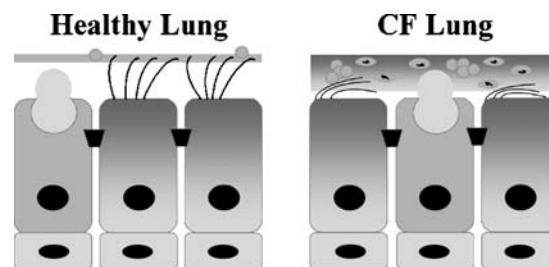
Cell surface nucleotide concentrations are regulated by three families of ectonucleotidases in mammalian tissues: Ecto-nucleotide pyrophosphatase/phosphodiesterases (E-NPPs:  $\text{ATP} \rightarrow \text{AMP}$ ), alkaline phosphatases (APs:  $\text{ATP} \rightarrow \text{AMP} \rightarrow \text{AMP} \rightarrow \text{adenosine}$ ) and ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases:  $\text{ATP} \rightarrow \text{AMP} \rightarrow \text{AMP}$ ) [15]. This review describes the importance of regulating extracellular nucleotide concentrations for airway homeostasis and the impact of chronic lung diseases on E-NTPDases. Few studies have addressed the activity or expression of airway E-NTPDases. In this review, the available information is complemented by original data.

## Nucleotide-mediated bacterial clearance

The importance of purine signaling in the airways is well illustrated in nucleotide-mediated mucociliary

clearance (MCC), which constitutes the first line of defense against bacterial infection [16]. Mucosal epithelial surfaces are protected by an airway surface liquid (ASL) layer containing a thin mucin sheath kept above the cilia by a periciliary liquid (PCL) layer (Fig. 1). Inhaled bacteria adhere to the mucin and are continuously displaced upward by coordinated cilia beating activity [17, 18]. Comparative analysis of the bioelectric properties of airway epithelia from normal donors and CF patients revealed that PCL height is regulated by a delicate balance between the activities of the epithelial sodium channel (ENAC) and the cystic fibrosis transmembrane regulator (CFTR) [19]. Cystic fibrosis is characterized by mutations in the gene encoding CFTR [16]. The lack of ion secretion through CFTR is associated with  $\text{Na}^+$  hyperabsorption by ENAC and water influx, resulting in the depletion of the PCL layer essential for cilia beating activity. The cilia become collapsed under a thick layer of mucus filled with bacteria and leukocytes, leading to severe inflammatory responses and progressive loss of lung function.

Extracellular nucleotides and nucleosides regulate CFTR and ENAC [20]. The basal activity of CFTR is maintained by adenosine ( $A_{2B}$ ) receptors, which induce G protein-coupled adenylate cyclase, followed by cAMP-dependent activation of type II protein kinase A [5]. In contrast, ATP stimulates  $\text{Cl}^-$  secretion through  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels. This signaling pathway involves P2Y<sub>2</sub> receptors, leading to G protein-coupled phospholipase C activation and cytosolic  $\text{Ca}^{2+}$  mobilization. Water efflux is also stimulated by P2Y<sub>2</sub> receptor-mediated inhibition of  $\text{Na}^+$  absorption by ENAC using  $\text{Ca}^{2+}$ -dependent signaling pathways [21]. Members of the P2X receptor family also support luminal  $\text{Cl}^-$ /water efflux in human airway



**Fig. 1** Impact of chronic obstructive lung diseases on mucociliary clearance. The polarized epithelium is composed of columnar (ciliated or mucin-secreting) cells facing the lumen and basal cells facing the serosal compartment. Bacterial clearance in healthy lungs is maintained by coordinated cilia beating activity within the PCL layer, moving upward the overlying mucus and pathogens. In chronic obstructive lung diseases, cilia are collapsed under a thick layer of mucus containing bacteria and leukocytes.

epithelia, as demonstrated in primary cultures and cell lines (Beas2B, 16HBE14o<sup>-</sup>, Calu-3, (ΣCFTE-29o<sup>-</sup>) by Ussing chamber and patch-clamp experiments [22]. The receptors identified in airway epithelia by RT-PCR were P2X<sub>2</sub>, P2X<sub>4</sub> and P2X<sub>5</sub>. These non-selective cation channels allow Ca<sup>2+</sup> influx, which then stimulates Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels. Interestingly, P2X<sub>7</sub> receptors are only detected in CF airway epithelia, primarily in nasal polyps [22]. Because they are known to induce apoptosis in response to high ATP concentrations [23], P2X<sub>7</sub> receptors may contribute to disease-associated epithelial damage.

The central role of ASL nucleotides for MCC was further substantiated by the observation that P2Y<sub>2</sub> receptor activation constitutes the most potent stimulus for cilia beating activity [24–26] and mucin secretion [27, 28]. In cultured CF airway epithelia, P2Y<sub>2</sub> receptor activation restores normal PCL height and rotational mucus transport [29]. Based on these findings, aerosolized nucleotides were proposed for the treatment of chronic obstructive lung diseases [16]. However, clinical studies showed that patients with asthma or chronic obstructive pulmonary disease exposed to aerosolized metabolites of ATP (AMP and adenosine) experience coughing and bronchoconstriction [30, 31]. Furthermore, aerosolized ATP (not adenosine) induced similar reactions from healthy subjects [32, 33]. In developing nucleotide-mediated MCC therapies, the equally potent P2Y<sub>2</sub> receptor agonist, UTP, was evaluated [6]. Airway clearance in sheep, measured with nebulized technetium-labeled albumin, was transiently increased by UTP [34]. Aerosolized UTP also improved MCC in patients with mild chronic bronchitis [35], CF [36, 37] and primary ciliary dyskinesia [38].

A major obstacle to the treatment of chronic lung diseases with aerosolized UTP is the rapid disappearance of therapeutic concentrations (0.1 mM) from the ASL layer (<5 min) [10, 29]. Incidentally, the metabolically stable UTP analogs INS365 (diquafosol) and INS37217 (denufosol) developed by Inspire Pharmaceuticals Inc. [39] were reported to improve cough induced-sputum clearance in CF patients [40, 41]. However, long-term treatments with these drugs may require dose optimization to minimize P2Y<sub>2</sub> receptor desensitization [42].

## P2 receptors and airway inflammation

*In vivo* studies conducted on the injurious effects of mechanical ventilation demonstrated that endogenous

ATP may reach ASL concentrations sufficiently high to trigger inflammatory responses and lung damage in the absence of infection [43]. The bronchoalveolar lavage fluid of rats subjected to positive-pressure mechanical ventilation contains significantly higher protein, ATP and cytokine (IL-6, TNFα) levels than control animals. Instillation of an equivalent ATP concentration increased lung fluid volume, supporting the existence of P2 and/or P1 receptor-mediated lung edema [43]. Analysis of bronchoalveolar lavage fluid nucleotide content by etheno-derivatization revealed an increase in AMP + adenosine/ADP + ATP ratio [44], suggesting that mechanical ventilation up-regulates airway ectonucleotidases. Real-time PCR on total lung tissue indicated that mechanical ventilation increases A<sub>2B</sub>, but decreases P2X<sub>7</sub> receptor expression. Rats subjected to both mechanical ventilation and positive end-expiratory pressure exhibited normal bronchoalveolar lavage composition and receptor expression. These findings suggest that patients subjected to positive end-expiratory pressure during large-volume ventilation may avoid ATP-mediated lung injuries. On the other hand, the fact that adenosine levels and A<sub>2B</sub> receptor expression were raised, whereas ATP levels and P2X<sub>7</sub> receptor expression were decreased, by mechanical ventilation supports a dynamic role for ectonucleotidases in the regulation of purine signaling in the airways.

The role of P2 receptors in innate defense against bacterial infection was investigated using mice deficient in P2Y<sub>1</sub>, P2Y<sub>2</sub> or both receptors [45]. All these mice exhibited lower survival and lower cytokine levels in lung homogenates than wild-type animals measured 24 h after intranasal instillation of *P. aeruginosa*. These results suggest a protective role for ATP-induced inflammation [45]. In human airways, P2Y<sub>2</sub> receptor activation amplified inflammatory responses to bacterial infection. Primary cultures of bronchial epithelial cells exposed to UTP and sputum from CF patients exhibited an eight-fold increase in IL-8 secretion, compared to two-fold with UTP alone [46]. Furthermore, P2X<sub>7</sub> receptors are known to support several processes relevant to inflammation, including LPS neutralization [47], pathogen killing, nitric oxide production [1] and cytokine release from mast cells, leukocytes, dendritic cells and neurons [3, 14]. In human blood, LPS-induced IL-1β release is increased 5-fold by P2X<sub>7</sub> receptor activation [48]. Altogether, these studies entail that P2 receptor activation may not only induce innate immunity but also amplify pathogen-induced inflammatory responses in chronic infectious lung diseases.

### Too much of a good thing..

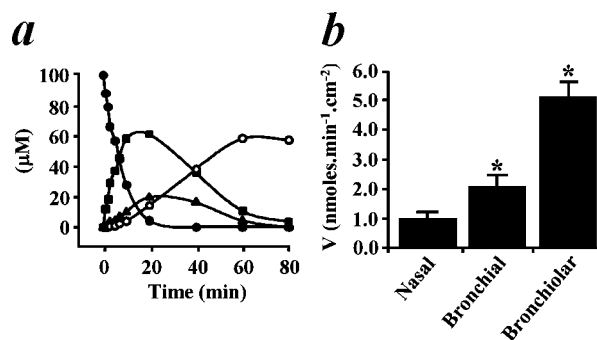
Excessive inflammatory response to recurrent bacterial infection is a major component in the pathogenesis of chronic lung diseases, irreversibly damaging the airways and leading to bronchiectasis and respiratory failure [49–51]. With respect to purine signaling, nucleotide-mediated cell communications may be overwhelmed by massive ATP release from cell lysis of bacteria and damaged epithelia, as well as stimulated leukocytes and epithelia [6]. The sensitivity of certain P2 receptors (P2Y<sub>2</sub> [42], P2X<sub>1</sub> and P2X<sub>3</sub> [52]) to agonist-induced desensitization may reduce the efficiency of nucleotide-mediated MCC [53]. On the other hand, chronically elevated ATP may recruit signaling pathways normally dormant in healthy lungs. For instance, P2X<sub>7</sub> receptor activation only induces cytokine release and cell death in response to high ATP concentrations encountered in damaged tissues [23]. On astrocytes, LPS-induced TNF $\alpha$  secretion is increased by P2Y receptor activation at low micromolar ATP, but reduced by P2X<sub>7</sub> receptor activation at high ATP concentrations [54]. The authors propose a beneficial effect for the potentiation of TNF $\alpha$  secretion by P2Y receptors during mild or acute inflammation and a protective role for P2X<sub>7</sub> receptors in damaged tissues or chronic infectious diseases. These studies suggest that P2X<sub>7</sub> receptor-mediated responses may be tissue-specific and/or influenced by bacterial infections. In the respiratory system, P2Y<sub>2</sub> receptors are expressed throughout airway epithelia [39] while P2X<sub>7</sub> receptors have only been reported in nasal polyps of CF patients [22]. The impact of P2X and P2Y receptor activation on inflammatory responses mediated by airway epithelia under normal and pathological conditions remains to be investigated.

### E-NTPDases regulate airway ATP

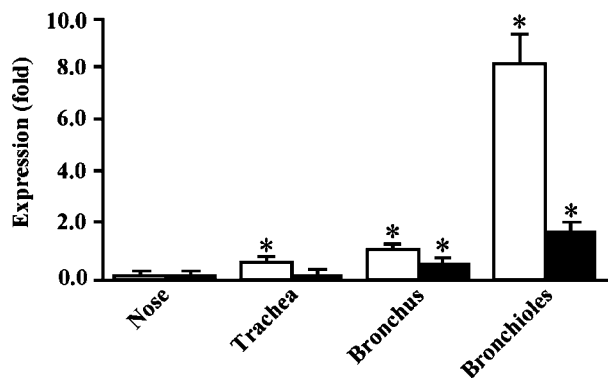
Several studies demonstrate the presence of ectonucleotidase activities on human airway epithelia [9, 10, 55–58]. Time-course experiments conducted on polarized primary cultures of human bronchial epithelial cells showed that mucosal ATP is dephosphorylated into ADP, AMP and adenosine (Fig. 2a). Experiments repeated with nasal, bronchial and bronchiolar epithelial cultures indicated that ATP metabolism accelerates toward the alveolar region (Fig. 2b). Such gradient distribution was also reported for non-specific alkaline phosphatase (NS AP) in human airways [9], an ectonucleotidase capable of sequentially dephos-

phorylating ATP to adenosine [59]. However, complete inhibition of NS AP with 10 mM levamisole [9] only reduced by 25% total ectoATPase activities [10]. Other ATP-regulating ectonucleotidases were identified in mammalian tissues as members of the E-NTPDase family [15, 60]. They catalyze the hydrolysis of  $\gamma$ - and/or  $\beta$ -phosphate residues, resulting in the formation of nucleoside monophosphates. Their activities are Ca<sup>2+</sup> and Mg<sup>2+</sup>-dependent and insensitive to inhibitors of P-, F- and V-type ATPases or APs [61]. Four E-NTPDases are localized to cell surfaces: NTPDase 1, 2, 3 and 8. Functional studies showed that NTPDase 1 [62] and NTPDase 3 [63] are inhibited by azide, whereas NTPDase 2 [64] and NTPDase 8 [65] are insensitive. The mRNA expression of NTPDase 1, 2 and 3 in human airways was reported in total lung RNA [66] and cultured bronchial epithelial cells [67]. We now provide evidence that NTPDase 1 and NTPDase 3 are both expressed throughout human airways by real-time PCR on freshly excised epithelial cells (Fig. 3). Their expression distribution followed surface ectoATPase activities (Fig. 2b), exhibiting higher mRNA levels in smaller airways (Fig. 3). The fact that 20 mM azide reduced total ectoATPase activities by 45% on human bronchial epithelial cells [67] suggests that the high affinity ( $K_m < 15 \mu\text{M}$ ) [68] NTPDases 1 and/or 3 play major roles in the regulation of physiological ASL nucleotide concentrations.

The physiological importance of azide-sensitive E-NTPDases for nucleotide-mediated MCC was recently established using an *in vitro* model of rhythmic



**Fig. 2** Ectonucleotidases regulate nucleotide concentrations on airway epithelial surfaces. **a** Polarized primary cultures of human bronchial epithelial cells were assayed with 100  $\mu\text{M}$  ATP added to the mucosal surface, as we previously described [10]. Buffer sample analysis by high-pressure liquid chromatography (10) shows that exogenous ATP ( $\bullet$ ) is dephosphorylated into ADP ( $\blacksquare$ ), AMP ( $\blacktriangle$ ) and adenosine ( $\circ$ ). **b** Similar experiments repeated on primary cultures of human nasal, bronchial and bronchiolar epithelial cells show that the elimination rate of ATP increases toward alveoli ( $N = 3$ , \*,  $P < 0.05$ ; Mann–Whitney test).



**Fig. 3** Expression level of NTPDase 1 and NTPDase 3 along human airways. Total RNA from excised epithelia was analyzed by real-time PCR using SYBR green assays and normalized to the expression level of the house-keeping gene, 18S, as we previously described [89]. The mRNA level of E-NTPDase 1 (□) and NTPDase 3 (■) increases toward alveoli ( $N = 4$ , \*,  $P < 0.05$ ; Mann–Whitney test).

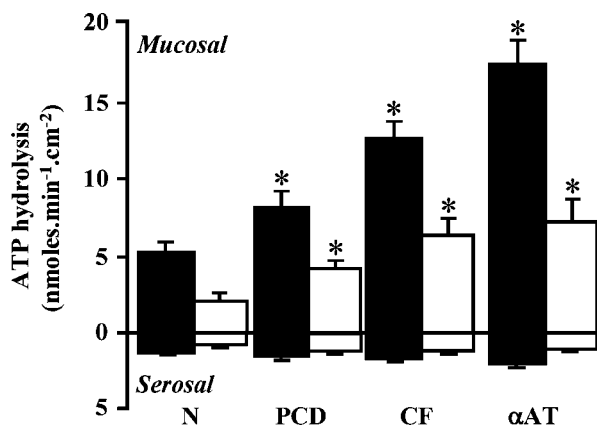
breathing. Airway epithelia are continuously subjected to mechanical stress generated by breathing, coughing or chest movement. Since mechanical stress induces epithelial ATP release [6], static culture conditions may underestimate the lungs capacity to regulate MCC *in vivo*. Rhythmic pressure changes mimicking normal tidal breathing were reproduced *in vitro* by a system applying cyclic compressive stress (CCS) to the mucosal surface of primary bronchial epithelial cultures [69]. Whereas CF cultures under static conditions exhibit a depleted PCL layer and mucostasis, CCS mimicking normal tidal breathing (20 cmH<sub>2</sub>O; 15 cycles/min) restored normal PCL height and mucus transport through ATP release and P2Y<sub>2</sub> receptor activation. These results also suggest that purine signaling may provide an explanation for the beneficial effects of oscillatory therapeutic devices clinically used to stimulate sputum clearance [70].

Cyclic compressive stress also enhances MCC through a reduction in ASL nucleotide metabolism. We recently demonstrated that CCS decreases the rate of ATP hydrolysis on the mucosal surface of normal and CF bronchial epithelial cultures [67]. More importantly, CCS restored normal ectoATPase activities on CF epithelial surfaces. The inhibitory effect of CCS on ATP metabolism was abrogated by 20 mM azide, indicating that E-NTPDase 1 and/or E-NTPDase 3 are key components in the regulation P2 receptor-mediated MCC. Perhaps the ability of CF airway epithelia to provide adequate nucleotide-mediated MCC under conditions mimicking normal breathing may contribute to the relatively healthy state of young patients before the establishment of chronic infection [71].

### Chronic lung diseases shorten the signals

Recent studies demonstrate that chronic lung diseases accelerate all metabolic steps supporting the mucosal conversion of ATP to adenosine [10, 72]. We identified the ectonucleotidases involved using primary bronchial epithelial cultures from healthy donors and patients with primary ciliary dyskinesia, CF or  $\alpha$ 1-antitrypsin deficiency. All airway diseases were characterized by a two- to four-fold increase in the elimination rate of ATP on the mucosal surface, without significant effect on the serosal surface (Fig. 4). Selective inhibitors of NS AP (10 mM levamisole), NTPDase 1 and NTPDase 3 (20 mM azide) partially restored normal activity levels. However, the lower substrate affinities of airway NS AP (36  $\mu$ M and 717  $\mu$ M) [9] suggest that NTPDase 1 and/or NTPDase 3 are responsible for the accelerated clearance of P2 receptor agonists in diseased airways.

*Pseudomonas aeruginosa* is among the most frequently isolated pathogens from the airways of patients with gram-negative infections [51]. In patients with damaged airways from mechanical ventilation, trauma or viral infection, airway colonization by *P. aeruginosa* is often followed by pneumonia, sepsis and death. Much of the airway inflammation induced by these organisms is caused by released bacterial products adhering to the epithelial surface, such as LPS. The contribution of bacterial infection to the deregulation of E-NTPDase activities in lung diseases was



**Fig. 4** Chronic lung diseases enhance azide-sensitive E-NTPDases. Primary bronchial epithelial cultures from healthy donors (N) and patients diagnosed with primary ciliary dyskinesia (PCD), CF or  $\alpha$ 1-antitrypsin deficiency ( $\alpha$ AT) were assayed with bilateral 1 mM ATP in the absence (■) or presence (□) of 20 mM azide, as we previously described [10]. Analysis of buffer samples by high-pressure liquid chromatography showed that azide-sensitive E-NTPDases are concentrated on the mucosal surface under normal and pathological conditions ( $N = 4$ , \*,  $P < 0.05$ ; Mann–Whitney test).

examined using *P. aeruginosa* LPS. Primary cultures of human bronchial epithelial cells exposed 24 h on the mucosal surface to an optimum LPS concentration (100 ng/ml) [73] exhibited a biphasic response over time (Fig. 5A). Azide-sensitive E-NTPDases were transiently reduced over 8 h, without affecting mRNA expression. In contrast, prolonged exposures (24 h) raised the activities two-fold above control levels and increased NTPDase 1 and NTPDase 3 expression by 4 and 10-fold (Fig. 5B). These opposite effects suggest that two regulatory mechanisms may be involved in the acute and chronic effects of LPS on airway E-NTPDases.

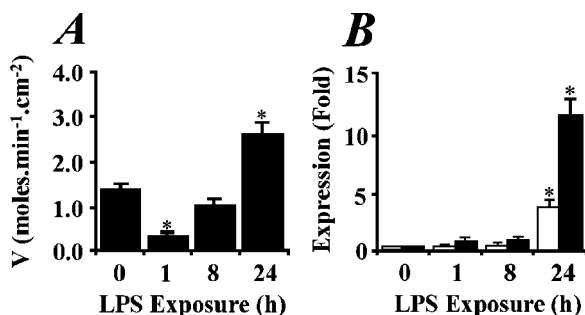
Oxidative stress is one of the major causes of epithelial injury in chronic lung diseases [74]. Reactive oxygen and nitrogen species released from airway epithelia or activated leukocytes damage cell membranes by peroxidation of lipids, amino acids and carbohydrates. *P. aeruginosa* LPS was reported to induce their release from airway epithelia [75]. Incidentally, intrahepatic cholestasis induced by intraperitoneal injection of LPS reduced total liver NTPDase 1 activity within 2 h [76]. In a rat model of glomerulopathy, short-term exposure (1 h) to LPS inactivated endothelial NTPDase 1, which was prevented by pre-treatment with the anti-oxidant superoxide dismutase [77]. Oxidative stress induced by membrane depolarization [78] or ischemia/ reperfusion [79] also inhibited endothelial NTPDase 1. Furthermore, short exposures (4 h) to the pro-inflammatory cytokine, TNF $\alpha$ , inhibited endothelial NTPDase 1, which was mimicked by hydrogen peroxide but prevented by superoxide dismutase [80]. Altogether, these studies suggest that oxidative stress may be responsible for the early and transient reduc-

tion in azide-sensitive E-NTPDase activities detected in LPS-treated airway epithelia. Since the mRNA expression of E-NTPDases is unaffected by acute oxidative stress [80] or LPS (Fig. 5B), and the inactivation of NTPDase 1 by ischemia/reperfusion was prevented by inhibitors of lipid peroxidation [79], the early loss of surface activity may involve local peroxidation of membrane lipids.

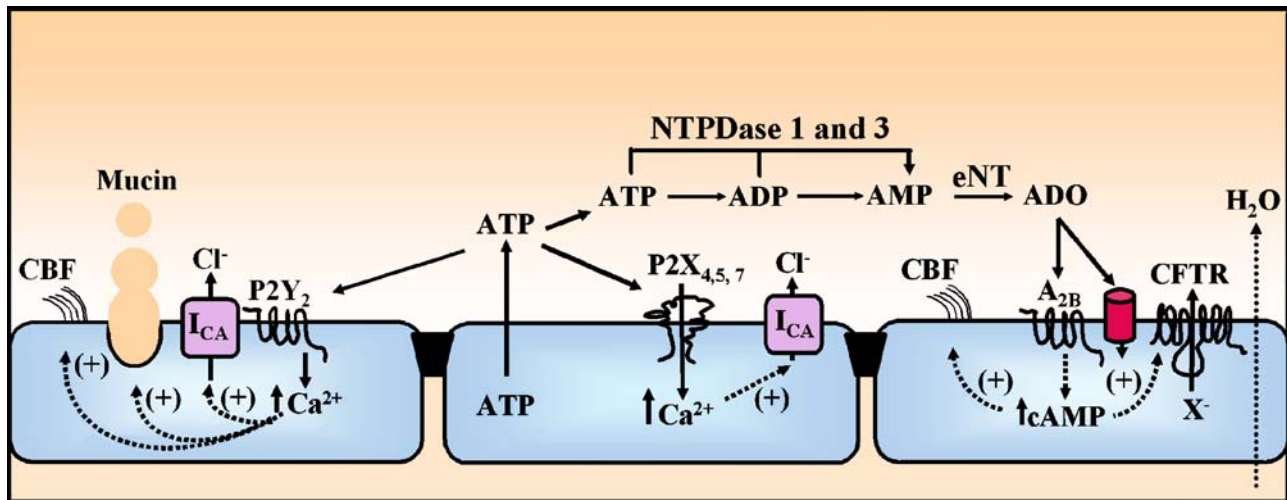
The long-term effects of LPS on the azide-sensitive E-NTPDases have not been documented in mammalian tissues. On the other hand, a 24 h exposure to endotoxin stimulated NTPDase 2 on astrocytes [81], as well as NS AP on endothelial, mesengial [82, 83] and bronchial epithelial [73] cells. Additionally, the mRNA expression of NTPDase 1 in rat forebrain was up-regulated seven days after transient ischemia [84]. *P. aeruginosa* LPS [85, 86] is well-known to influence gene expression through the activation of the transcription factors: Nuclear factor-kappa B and activator protein-1 [87]. Recent studies have shown that CFTR acts as a pattern recognition molecule for *P. aeruginosa* LPS [88]. Endocytosis of the complex triggers translocation of these transcription factors to the nucleus, which initiates innate immunity through the expression of numerous mediators recruiting and activating inflammatory cells. These signaling pathways could be involved in the up-regulation of NTPDase 1 and NTPDase 3 expression resulting from long-term exposures to *P. aeruginosa* LPS in human airways.

### Adaptation of purine signaling to airway diseases

In past decade, the therapeutic potential of aerosolized nucleotides for the treatment of chronic obstructive lung diseases has motivated numerous studies on the importance of purine signaling in the airways. This elaborated cell communication system is involved in major defense mechanisms including bacterial clearance and inflammatory responses to bacterial infection. Each signaling event constitutes a brief 'on-and-off' switch mechanism allowing the target cells to perceive the subsequent signal. Environmental changes are communicated to target cells in the form of released ATP, which binds surface receptors and triggers an appropriate response. The efficiency of this communication system depends on the cells capacity to rapidly eliminate these local bursts in extracellular ATP concentrations to prevent receptor desensitization. This review establishes the pivotal role of E-NTPDases in the regulation of airway defenses mediated by purine signaling, namely bacterial clearance (Fig. 6). Cyclic



**Fig. 5** Impact of *P. aeruginosa* LPS on NTPDase 1 and NTPDase 3. **A** The mucosal surface of primary bronchial epithelial cultures were exposed 0, 1, 8 or 24 h to 100 ng/ml LPS, then assayed for surface activity by high-pressure liquid chromatography, as we previously described [10]. The activities of azide-sensitive mucosal E-NTPDases measured with 0.03 mM ATP were transiently reduced by LPS. **B** Quantification of their mRNA expression by real-time PCR using the house-keeping gene 18S, as we previously described (89). LPS induced a delayed increase in the expression level of both E-NTPDases ( $N = 5$ , \*,  $P < 0.05$ ; Mann–Whitney test).



**Fig. 6** Purine signaling and nucleotide salvage pathways on human airway epithelia. In normal lungs, basal adenosine levels maintain adequate PCL height for mucus transport through  $A_{2B}$  receptor-mediated CFTR activity. Mechanical stimulation of the epithelial surface by an irritant, a pathogen, mechanical ventilation or cyclic compressive stress induces ATP release and elevates ASL concentrations above activation threshold level for  $P2Y_2$  receptor activation. This autocrine signal transiently enhances basal MCC through cilia beating, mucin secretion and ion/water efflux into the lumen. The signal is rapidly terminated by NTPDase 1 and/or NTPDase 3 dephosphorylating excess ATP to AMP. Ecto 5'-nucleotidase (ecto 5'-NT; CD73) produces ASL adenosine from the resulting AMP, and excess adenosine is transported back to the cytosol. Chronic lung diseases burdened by considerable tissue damage recruits additional receptors ( $P2X_{4,5,7}$ ) responding to higher ATP concentrations.

compressive stress, mimicking normal breathing or clinical oscillatory devices, stimulates P2 receptor-mediated MCC through ATP release and reduced azide-sensitive E-NTPDase activities. We also presented original data indicating that NTPDase 1 and 3 activities and expressions are up-regulated in chronic lung diseases by inflammatory mediators, including endotoxin. Interestingly, *P. aeruginosa* LPS generated a biphasic modulation of airway E-NTPDases which may reconcile previous conflicting reports based on exposure duration. Acute LPS exposures reduced E-NTPDase activities, as observed during CCS, most likely to clear a pathogen through P2 receptor-mediated MCC. The signaling pathways activated by *P. aeruginosa* LPS and the lack of concomitant reduction in E-NTPDase mRNA levels support a local inhibitory mechanism that may involve oxidative stress-mediated membrane damage. On the other hand, prolonged endotoxin exposures mimicked the impact of chronic lung diseases on NTPDases 1 and 3, characterized by an up-regulation of both activities and mRNA expression. In chronically infected lungs, an increase in E-NTPDase activities may represent an attempt to prevent lung damage, excessive inflammation and P2 receptor desensitization by chronically elevated ATP concentrations.

The information provided in this review also exposes the complexity of the signaling interactions taking place between different classes of mediators, including bacterial products, cytokines and extracellu-

lar nucleotides, as combinations of mediators may induce different responses than when tested individually. Furthermore, communication networks in the airways may evolve with time during the establishment of a chronic infection, as new target cells (bacteria, leukocytes, lymphocytes) agglomerate on either side of the epithelial barrier. It is proposed that nucleotide-mediated airway functions defined in normal tissues or aseptic culture conditions may require reassessment under conditions mimicking chronic lung diseases before their therapeutic potential may be clearly established.

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