Regulation of ciliary beat frequency in airways: shear stress, ATP action, and its modulation

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MUCOCILIARY TRANSPORT is a vital component of host defense and plays a crucial role in the removal of foreign materials within the airways. A dysfunction of ciliary beating can lead to serious respiratory problems such as airflow obstructions or Kartagener syndrome. The airway ciliary beat frequency (CBF) increases in response to a variety of stimulations, such as parasympathetic or sympathetic nervous stimulation, mechanical stimulation, hormones, etc. Winters et al., in one of the current articles in focus in this issue (8), highlighted shear stress as a physiological mechanism for CBF activation in the trachea. Since airway epithelia are continuously exposed to shear stress secondary to airflow, the focus of their report is the mechanism by which such stress can affect CBF. In their report (8), they show that repeated shear stress with caudal flow causes a large increase in CBF from 10 Hz to 25–35 Hz (37°C). These large CBF increases were induced by ATP released by shear, and this was associated with increases in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]). Moreover, adenosine, a metabolite of ATP, also stimulates tracheal CBF. However, the large CBF increase induced by shear was not completely mimicked by directly adding exogenous ATP and adenosine (in the absence of shear), leading the authors to conclude that shear appears to affect CBF increase via complex signaling pathways. It is noteworthy to consider the confounding features such as cell culture conditions, airway location (nasal, tracheal, or bronchiolar epithelium), animal species differences, and temperature that have been reported by others.

Delmette and Sanderson (1) reported that CBF of noncultured trachea or lung slice (bronchiolar) was ~20 Hz, whereas that of cultured trachea and lung slice was 12–14 Hz at 30°C. In mouse trachea, ATP increases CBF from 9 to 12 Hz in cultured cells, whereas it increased CBF from 20 to 25 Hz in noncultured cells. CBFS have been reported to vary from 6 to 15 Hz depending on the species and the anatomic location of the epithelium under study, such as differences between cultured cells from human trachea and nasal epithelia and rabbit and sheep trachea. Thus cell culture per se appears to change cellular characteristics that lead to decreased CBF. Moreover, temperature affects CBFS. CBFS in noncultured cells of mouse lung slice increase from 12 to 23 Hz as a function of increasing temperature from 20°C to 31°C (1). Similar increases in CBF with temperature were reported in cultured cells of rabbit trachea (4).

As noted above, both the anatomic source of the tissue under investigation as well as the species it is from can affect CBF. A CBF increase in response to ATP was absent in small airways in mouse and rat, although it was present in trachea (1, 2). ATP increased [Ca$^{2+}$] in noncultured cells of mouse lung slice (1), whereas it did not in those of rat lung slice (2). Moreover, the ATP response is present in guinea pig isolated single bronchiolar cells (noncultured small airway ciliary cells) (unpublished observations). Thus the regulatory mechanisms of CBF are different among animal species and airway location.

There are at least three signaling cascades to increase tracheal CBF: PKA action, PKG action, and Ca$^{2+}$ action. The activation of CBF by these are modulated by some cellular events, such as changes in cell volume, intracellular Cl$^{-}$ concentration ([Cl$^{-}$]), intracellular pH, and membrane potential. In rat bronchiolar ciliary cells, cell shrinkage and [Cl$^{-}$] decrease enhanced the CBF increase (PKA action) (6). In human tracheal cells in culture, intracellular alkalinization increases CBFS (7). In mouse trachea, high extracellular K$^{+}$ concentration ([K$^{+}$]), that depolarizes membrane potential increases CBF via activation of voltage-sensitive Ca$^{2+}$-permeable channels (unpublished observations). Many factors modulate agonist-stimulated CBF activities.

Extracellular ATP appears to be an important agonist to increase CBF because of paracrine activity in trachea. ATP is released by airway epithelial cells, and it increases [Ca$^{2+}$], via P2Y receptors. Winters et al. (8) suggest that the repeated shear causes ATP release that in turn stimulates P2Y receptors. The repeated shear increased CBFS from 10 to 25 Hz. The membrane perturbation or stretch induced by mechanical stimuli and hypoosmotic stress increases CBF via ATP release. Lansley and Sanderson (4) showed that a mechanical stimulation induces a large CBF increase (20–35 Hz at 37°C). Zhang and Sanderson (10) also showed that ATP (5 μM) alone mimicked a similar large increase in CBF in rabbit tracheal cells in culture. Moreover, Winters et al. (8) demonstrated that, in the presence of 100 μM ATP, the removal of shear further increases CBF from 25 to 35 Hz. An ATP metabolite, adenosine, has been suggested to enhance ATP actions on CBF in trachea. ATP released by shear induces various actions on CBF in tracheal ciliary cells. Thus shear is a strong stimulation for increasing CBFS, although it is not easy to compare CBF responses among the different studies as mentioned above.

ATP-stimulated CBF increases were closely related to increases in [Ca$^{2+}$]. A previous report showed that ATP evoked [Ca$^{2+}$], oscillations in rabbit tracheal ciliary cells in culture (10). The [Ca$^{2+}$] oscillation ranged from 100 to 200 nM and caused CBF oscillations that ranged from 15 to 25 Hz in cultured cells in rabbit trachea. Moreover, in human tracheal ciliary cells in culture, short-term ATP stimulation induced a prolonged increase in CBF, which is induced by PKA activation (5). In frog esophageal ciliary cells, [Ca$^{2+}$], elevation activates PKG (8). Thus CBFS during ATP stimulation are mainly regulated by [Ca$^{2+}$]; moreover, ATP also activates signaling cascades, including PKG and PKA, and these in turn enhance Ca$^{2+}$-regulated CBFS.
Some pathophysiological conditions, such as asthma, appear to increase shear stress by severe coughs, and this may enhance CBF. This leads to activation of the mucociliary clearance. Thus the shear stress highlighted (8) appears to be an important mechanism for maintaining airway clearance as the host defense mechanism of lungs. Further studies will give us an answer regarding the precise mechanism by which shear stress increases CBF.

REFERENCES