

Influence of external pH on ciliary beat frequency in human bronchi and bronchioles

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Influence of external pH on ciliary beat frequency in human bronchi and bronchioles. C. Clary-Meinesz, J. Mouroux, J. Cosson, P. Huitorel, B. Blaive. ©ERS Journals Ltd 1998.

ABSTRACT: Ciliated respiratory epithelial cells have to tolerate variations in local pH caused by the respiratory cycle and potential ventilation-perfusion mismatches. We showed previously that peripheral bronchiolar cilia beat at a lower frequency than bronchial cilia, and have now investigated whether they show differences in tolerance to changes in pH.

Using the image analysis system applied in the previous study, we compared variations in the ciliary beat frequencies (CBF) of bronchi and bronchioles sampled from human lung resections at various pH *in vitro*.

Application of nonparametric tests (the variance of samples was not similar) indicated that CBF was not significantly modified when pH was varied between 7.5 and 10.5 for bronchi, and between 5.5 and 10.5 for bronchioles. Reversible and significantly lower CBF were observed below pH 7.0 for bronchi and below pH 5.0 for bronchioles. Extreme pH values such as 11.0 or 3.0 were lethal within a few minutes.

Thus, respiratory ciliary beating is able to tolerate external pH variations between 3.5 and 10.5 without permanent impairment. In addition we found that alkaline pH values are more favourable than acidic ones and that bronchiolar ciliated cells are more tolerant to acidic pH than bronchial cells.

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Most studies concerning the importance of the pH for ciliary activity have been performed on marine invertebrates and protists [1]. In these organisms, ciliary beating appears adapted to the characteristic and stable pH of the environment. On the other hand, ciliated respiratory epithelial cells are exposed to pH variations due to the respiratory cycle [2]. In humans, normal pH values in tracheal mucus range 6.9–9.0 [3]. Moreover, infection can be accompanied by pH values as low as pH 5.8 in mucus [4]. How can ciliary movement cope with such variations in external pH? A previous study showed that nasal ciliated cells may resist pH variations between 7 and 9 [5]. The buffering capacity of bronchial secretions (periciliary fluid and mucus) is lower than in plasma, varies from day to day and individually, and pH variations may be encountered upon exposure to acidic compounds [6]. Variations in capnia induce modifications in carbonic acid concentrations of the periciliary fluid leading to pH variations [7]. Local perfusion-ventilation mismatches may enhance this phenomenon.

In a previous work [8], we showed that the ciliary beat frequencies (CBFs) of peripheral bronchioles are significantly lower than those of proximal bronchi (4.6 *versus* 7.1 Hz, respectively), which may explain the low mucociliary clearance observed in peripheral regions of the lung. Here we examine CBF modifications induced by pH variations in the medium surrounding ciliated cells and compare results from bronchi and bronchioles.

Materials and methods

Preparation of ciliated cells

Measurements were made from 17 lungs resected for tumours. Patients with preoperative treatments such as irradiation or chemotherapy were excluded. Operative procedure and general anaesthesia were similar for all patients.

Characteristics of the patients are not given as we, and others, have shown that there is no significant difference in CBF with sex, age or lung function, or with smoking status [5, 8]. Lung tissues were examined for sampling within 1 h of resection. Specimens showing purulent discharges from airways were excluded. Proximal brushings were immediately performed on normal areas of the resected cartilaginous bronchus; after dissection of a small bronchus, distal brushings were performed by brushing the surface of bronchioles less than 2 mm in diameter, longitudinally opened, and by pushing the disposable cytology brush, one millimetre in diameter, through a small bronchiole of a diameter slightly superior to that of the brush, close to the visceral pleura. Specimens were dislodged from the brush by shaking it in RPMI 1640 medium supplemented with colistine (100 IU·mL⁻¹) and penicillin (100 IU·mL⁻¹). The samples were kept at 4°C until measurements, which were performed within 24 h. Samples with

numerous normal beating ciliated cells were kept for experiments. All experiments, including CBF measurements, were performed at room temperature, constantly maintained through air-conditioning. A temperature of 22°C was chosen to minimize air drying, bacterial growth and deleterious effects. The study was approved by the local institutional ethics commission.

Experimental design

Cells were kept in RPMI 1640 supplemented with penicillin (100 IU·mL⁻¹) and colistine (100 IU·mL⁻¹) at 4°C, which allows CBFs of specimens to remain stable for 2–3 days, in our experience. All CBF measurements were made within 24 h and at room temperature (22°C). All distal samplings came from bronchioles of less than 2 mm in diameter. Measurements were made on small groups of ciliated cells, single cells being discarded as their CBFs are usually lower and variable. The CBFs of at least ten different cells were measured for each specimen. The initial pH of the medium was 7.2. A series of incubation media were made by adjusting the primary medium (RPMI 1640 which included N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid (HEPES) and glutamine) to pH ranging 3–11 with 0.1–1% v/v of 0.3 N HCl or 0.3 N NaOH. Ionic concentrations varied by not more than 1%, and osmolarity was, thus, considered stable. The sodium concentration in the medium was 100 mM, which is in the recommended range [5]. Large variations in osmolarity of the medium do not induce significant CBF changes between 300 and 450 mOs [9] or 150 and 600 mOs [10].

Fifty microlitres of cell suspension were added to 5 mL of incubation medium at the chosen pH. The pH of the resulting incubation medium was measured using a digital pH-meter (Mini 80; Tacussel, Villeurbanne, France) with a precision of 0.01 pH units, and gave the value of incubation pH. Before each measurement, the pH meter was calibrated using reference buffers. After a 5 min incubation period we sampled 100 µL to measure CBF. Reversibility was checked after a second incubation period of 5 min at pH 7, adding 0.3 N HCl or 0.3 N NaOH, less than 1% of total volume of incubation medium. Ionic strength and temperature were maintained constant.

Recording of ciliary beat frequency

A small volume of cell suspension was placed between a slide and a coverslip, and ciliated cells were examined with a negative phase contrast microscope (Optiphot 2; Nikon Corporation, Tokyo, Japan) using a 40 × 10 magnification. To minimize the influence of the microscope lamp on the temperature of the cell suspension, a caloric filter was used and CBF measurements were always made within 5 min for each experiment, so that temperature modifications were kept to a minimum and were similar in all experiments.

Images were scanned by a video camera (Panasonic CCD F15; Matsushita Electric Industrial Co., Osaka, Japan) and stored on a video tape recorder (Panasonic S-VHS 7530; Matsushita Electric Industrial Co.). Images were displayed onto a video monitor. We selected the edge of the cell where ciliary beat was observed distinctly, and at least 10

different areas were selected from each sample. Video sequences were analysed by an image analysis system, as described previously [11]. Briefly, each image was normalized by a digital time base corrector system (TBC FA-300P; For A Company Ltd, Tokyo, Japan). The video signal was digitized by an image processing card (PIP 1024B; Matrox Electronic System Ltd, Dorval, PQ, Canada) implanted in a PC AT/386 microcomputer (SOS Informatique, Nice, France). Each frame was digitized into 512 × 512 pixels with 256 grey level values. One hundred and twenty eight images were analysed over a 5 s period and statistical analyses were performed using the Stat 2005 program (Alcatel TITN, Alcatel Alsthom, Paris, France). Average optical density measurements allowed us to obtain optical density variations as a function of time for each selected area. Beat frequencies were obtained from K covariances of the optical density as a function of time.

Statistical analysis

Results are expressed as mean with one standard error of the mean (SEM). Each CBF value is the average of at least 10 different measurements, mostly 40. Each data point presented in the measurement of reversibility of pH induced CBF modifications represents the average of three or more experiments performed on different explants. Each pH group (*e.g.* 3, 3.5, 4, 4.5, *etc.*) was measured at the corresponding pH value plus or minus 0.2 units (*e.g.* "pH 7" values were between 6.8 and 7.2). CBF measurements at pH 3 and pH 11 which approached zero, were significantly different. We compared results from bronchi or bronchioles at pH values ranging 3.5–10.5, using nonparametric tests, as variance was different in some experiments. According to the Kruskal-Wallis test, all measurements were grouped in one population, ordered from the smallest to the biggest value with a serial number, taking into account similar values. Heterogeneity was significantly demonstrated ($p < 0.005$) from the calculation of the auxiliary variable of Kruskal Wallis test (H_c) [12]. The samples were compared according to a multiple comparison test [13]. Briefly the Z_{Hj} of the distribution of each sample was compared to $Z_{a'}$ of the normal law, which must lie above 3.5 for bronchi ($a' = 2 \times 10^{-4}$), and above 3.4 for bronchioles ($a' = 3 \times 10^{-4}$), to indicate a significant difference ($p < 0.05$).

Results

The influence of the pH in the medium on CBF is shown in table 1 and figures 1 and 2. CBF modifications were induced by incubating ciliated respiratory cells in culture medium at different pH values and the reversibility of those effects was subsequently monitored (fig. 2).

CBF of bronchial cells remained stable between pH 7.5 and 10.5 ($Z_{Hj} < 3.5$). When the pH value was lowered below pH 7.0, CBF decreased significantly. CBF values at pH 7.5 differed significantly from CBF values at pH 7.0, but the level of significance was not high ($Z_{Hj} = 4$, compared to 3.5). A pH value of 5.5 reduced the bronchial CBF by 50%, while ciliary activity was almost arrested at pH 3.5. At pH 3.0 or 11.0 cells were irreversibly damaged: at pH 11.0, cells were progressively damaged, allowing some initial CBF measurements within the first 5 min, whereas at pH 3.0 they showed deleterious effects too fast to allow any CBF measurement (fig. 1a).

Table 1. – Bronchial and bronchiolar mean ciliary beat frequency (CBF) obtained after incubation for 5 min at various pH of the ratio of the obtained CBF to the initial CBF (CBFi) measured at pH 7.2

pH	Bronchi			Bronchioles		
	CBF Hz	SEM	CBF/CBFi %	CBF Hz	SEM	CBF/CBFi %
3.0	0.00*	0.00	0	0.05*	0.00	0
3.5	1.54	0.23	23	1.61	0.14	57
4.0	3.69	0.23	58	2.47	0.18	72
4.5	2.58	0.11	42	2.44	0.12	64
5.0	3.13	0.12	51	2.18	0.09	61
5.5	3.33	0.53	47	2.51	0.14	70
6.0	3.73	0.19	61	2.84	0.08	86
6.5	4.69	0.11	78	2.71	0.09	84
7.0	6.29	0.09	100	3.73	0.11	100
7.5	5.96	0.79	95	3.41	0.08	96
8.0	5.37	0.17	91	3.77	0.11	108
8.5	5.20	0.27	92	3.70	0.13	107
9.0	6.33	0.25	91	3.95	0.18	109
9.5	6.06	0.09	102	4.08	0.13	111
10.0	6.79	0.26	104	4.96	0.18	107
10.5	5.01	0.22	89	3.93	0.18	106
11.0	4.11*	0.42	64	2.14*	0.30	75

*: extreme pHs 3.0 and 11.0 induced irreversible instability of CBF.

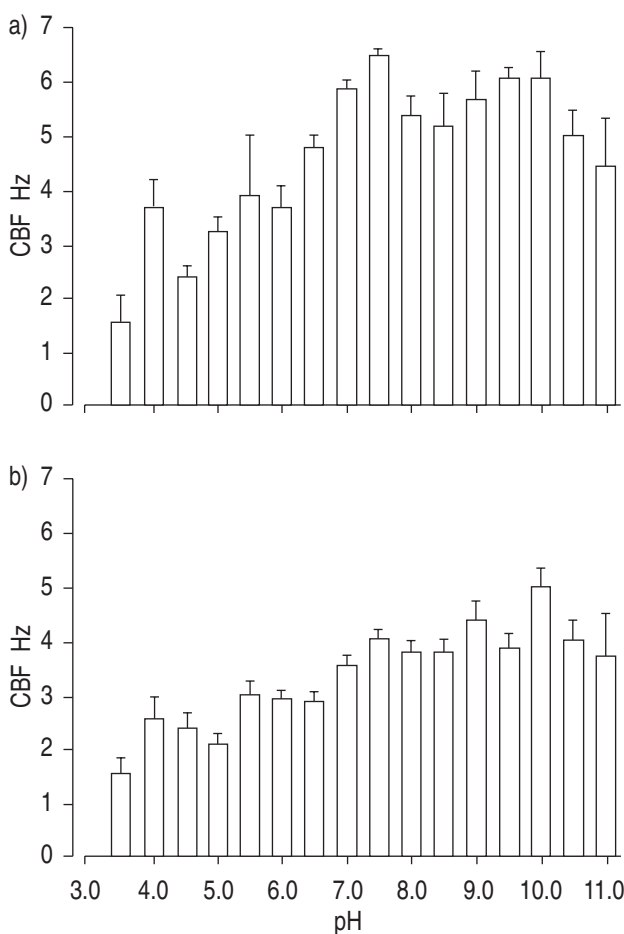


Fig. 1. – a) Bronchial and b) bronchiolar ciliary beat frequencies (CBF) according to external pH values, within 5 min of incubation (extreme pHs, 3.0 and 11.0, induce irreversible instability of CBF). Results are presented as mean and 95% confidence intervals.

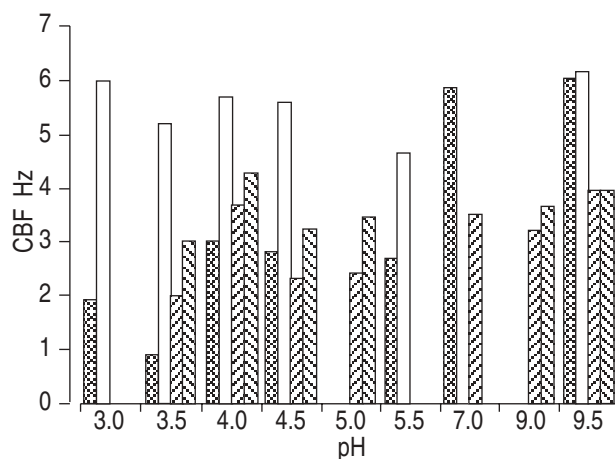


Fig. 2. – Reversibility of the pH-induced ciliary beat frequency (CBF) modifications. Pooled mean bronchial and bronchiolar CBF at pH 7.0 are given for comparison. ▨ : bronchi; □ : bronchi at pH 7; ▩ : bronchioles; ▤ : bronchioles at pH 7.

Bronchiolar ciliary beat behaviour was slightly different. Bronchiolar CBF did not differ significantly from initial values at pH 7.0 between pH 5.5 and 10.5 ($Z_{Hj1} < 3.4$). In contrast to bronchial cilia there was no difference between CBF at pH 7.0 and pH 7.5. Below pH 3.5, CBF decreased by more than 50%. As seen for bronchial cells, pH 3.0 and 11.0 were deleterious in the same way (fig. 1b).

The reversibility of the pH induced CBF modification was tested. Figure 2 shows examples of these CBF modifications which were reversible when cells were brought back to pH 7. We gave particular attention to acidic pH values, where a pH such as 3.5 induced 50% CBF decrease that was totally reversible within 5 min at pH 7.0.

Discussion

In our experimental conditions, we showed that bronchial CBF does not significantly change in response to changes in the pH of the external medium between 7.5 and 10.5. CBF values obtained at pH 7.0 compared to 7.5 did, however, show a slight but significant difference. LUK and DULFANO [5], found a stable nasal CBF between pH 7.0 and 9.0. INGELS *et al.* [9], demonstrated that nasal CBFs in humans do not change when pH varies from 6.5 to 7.5. These observations suggest that nasal and large airway ciliated cells are similar concerning their tolerance to alterations of pH.

With bronchiolar ciliated cells, we showed that they maintain their normal CBF within a limit of pH 5.5–10.5. CBFs of bronchiolar cells appear more tolerant to acidic pH than bronchial cells (5.5 *versus* 7.5).

In other animals, a variety of pH-induced modifications of CBF have been reported, but all studies confirm the high resistance of respiratory ciliated cells to large variations in pH. In rats, tracheal CBF is significantly inhibited by pH below 6 and accelerated by pH between 9 and 11 [10]. In chicken embryos, as well as in rats, VAN DE DONK *et al.* [14], found a stable tracheal CBF at pH values ranging 7–10, with a slight initial acceleration at pH 10. Cilia integrity is preserved if pH variations are restricted to values ranging 6.5–8.5 [15]. In cows, no histopathological changes or dysfunctions are observed in cilia in the pH range

6.7–9.5 [16]. These differences in threshold pH values may represent variations due to the method for measuring pH more than species characteristics.

Normal values, measured *in situ*, are around pH 6.7 in rabbit airways [2]. In rats the pH of tracheal mucus ranges 7.4–7.6 [17]. In humans, tracheal pH values of mucus range 6.9–9.0 [3]. However, in pathological conditions, acidification of the bronchial mucus has been recorded, for instance during metabolic or respiratory acidosis [7], diabetes mellitus [18] and bacterial pneumonia [3]. The pH does not, however, change significantly during simple bacterial colonization (bacterial colonization without pneumonia) [3]. Taken together, these observations suggest that the impairment of CBF at acidic pH may play a part in the development of pneumonia, together with the increased viscosity of the mucus, which impairs the mucociliary clearance even further [6].

Variations in pH values in the range 6.4–7.8 have been reported in mucus due to changes in carbon dioxide concentration with the respiratory cycle [19], or to the secretions of bicarbonate or hydrogen ions by the epithelial cells [20]. As for airway surface liquid, comprising periciliary fluid and mucus, the pH is slightly acidic compared to plasma: about 7 in ferrets [20] and 6.8 in humans [21]. It may vary from 6.85 to 6.92 following variation in carbon dioxide concentration in ferret [22]. These variations should not impair the transport of mucus, according to our experimental results. In addition bronchiolar cilia appear resistant to acidic pH values over a greater range than bronchial cilia. As far as we know, no direct pH measurement of the airway surface liquid in the distal part of the airways has been performed. GUERRIN *et al.* [2] found slightly lower *in situ* pH values in the main stem bronchus than in the trachea of rabbits. We may expect larger variations in carbon dioxide concentration at the bronchiolar level compared to the large airways according to the ventilation-perfusion ratio and, thus, larger external variations of pH. From our results, CBF is unlikely to be impaired in the pH range found along the respiratory tract. Respiratory cilia are, thus, adapted to the characteristics of pH encountered in their environment.

In experimental conditions however, pH should always be checked and specified, as ciliary modifications may be explained or even enhanced by pH modifications [23, 24]. As we show the stability of ciliary beat with alkaline external pH, we suggest the use of buffered solutions with a pH value of around 8.5 for medications to be administered by aerosol so that clearance of mucus is made easier, especially when bacterial infections may have already lowered the pH, inducing suboptimal values of CBF.

In conclusion ciliary beating of human respiratory ciliated cells tolerates external pH variations over a wide range, and bronchiolar cells, which beat slower than bronchial cells, are even more tolerant: ciliary beat frequency is stable between pH 5.5 and 10.5 for bronchiolar cells and between pH 7.5 and 10.5 for bronchial cells. This tolerance seems to be adapted to the true variations of pH in the airway surface liquid observed with respiratory cycle and level of ventilation.

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