Effect of cyclic AMP on ciliary activity of human respiratory epithelium

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ABSTRACT: In order to investigate the effect of cyclic adenosine monophosphate (cAMP) on ciliary beat frequency (CBF) in human respiratory epithelium, cells were brushed from the inferior nasal turbinates of three groups of ten subjects: awake adults (aged 20–34 yrs), anaesthetized children (2–15 yrs) and anaesthetized adults (19–61 yrs). Cells from the awake adults were also studied after storage for 24 h in tissue culture medium. CBF was measured *in vitro* with a photometric technique at room temperature ($22.0 \pm 1.5°C$). Samples were mounted in a perfusion chamber and challenged with either control solutions, dibutyryl cAMP (10^{-4} or 10^{-3} M), or the cyclic nucleotide-dependent protein kinase inhibitor 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine (H-7). Dibutyryl cAMP (10^{-3} M) caused a significant increase in CBF in all groups studied: awake adults ($+1.1$ Hz; $p<0.01$), anaesthetized children ($+1.2$ Hz; $p<0.01$), anaesthetized adults ($+1.2$ Hz; $p<0.01$), stored cells ($+1.1$ Hz; $p<0.01$). This response was inhibited by preincubation with H-7 (10^{-4} and 10^{-3} M). It is concluded that cAMP is a regulator of ciliary activity in human respiratory epithelium.
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Mucociliary clearance is one of the lung's nonspecific host defence mechanisms, effected by the beating action of cilia propelling the overlying secretions, which carry both trapped, inhaled material and locally produced biological debris toward the oropharynx [1]. Modifications in ciliary activity should lead to changes in rate of mucus transport, as predicted by theoretical models of mucociliary pumping [2]. Consequently, the cellular mechanisms that regulate ciliary beat frequency (CBF) may also be considered as mechanisms regulating mucociliary clearance.

Neurohormones and neurotransmitters associated with the autonomic nervous system have a significant influence on mucociliary activity [3]. It has been demonstrated *in vitro* that adrenergic drugs cause an increase in CBF in both animal [4–7] and human [8] tissues. Recent *in vivo* studies on dogs confirm the role of autonomic agonists in regulating CBF [9]. Beta-adrenergic agents act *via* membrane receptors and elevate the intracellular concentration of 3', 5'-cyclic adenosine monophosphate (cAMP) [10]. Cyclic AMP acts by binding to intracellular protein receptors and induces the release of the catalytic subunit of protein kinase A, which in turn phosphorylates a range of target proteins [11]. We have investigated this pathway in respiratory epithelial cells collected from the nasal turbinates of human subjects.

The aims of our study were: 1) to investigate the regulation of CBF in human respiratory epithelium by exposing the ciliated cells to an analogue of cAMP which crosses cell membranes (dibutyryl cAMP); 2) to block the effect of dibutyryl cAMP on CBF with the competitive antagonist of the adenosine triphosphate (ATP) binding site of the cAMP-dependent kinase 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine (H-7) [12]; 3) to determine whether the response to dibutyryl cAMP is affected by the age of the subject, collection of the sample during the induction of general anaesthesia, or storage of the cells in tissue culture medium for 24 h.

Materials and methods

Sample collection

Ciliated nasal epithelium was obtained using a cytology brush, as described by RUTLAND and COLE [13]. Cellular material adhering to the brush was dislodged by brisk agitation in Eppendorf tubes containing 1 ml of tissue culture medium (Medium 199). All of the experiments on fresh samples were performed within 4 h of collection. During this time the samples were kept in Medium 199 at 4°C.

Subject selection

The material for this study was obtained from three groups of subjects: awake adults, anaesthetized children, and anaesthetized adults.

The awake adults consisted of 10 healthy volunteers aged 20–34 years (mean 29.2 years). This group included 7 men and 3 women; 4 were smokers. None had a history of upper respiratory tract infection for at least one month. The samples were obtained without using local anaesthesia. Each subject was brushed repeatedly on separate occasions.

It was decided not to collect samples from conscious children due to the discomfort of the procedure. We therefore obtained Ethical Committee permission to study children who were undergoing routine surgery. This group was composed of ten children aged 2–15 yrs (mean 7.3 yrs; 5 males and 5 females). Samples were collected immediately after induction of general anaesthesia with thiopentone (4 mg·kg^{-1}) and suxamethonium (1 mg·kg^{-1}).

In order to control for the effect of general anaesthesia a group of ten anaesthetized adults were also studied. These subjects were undergoing routine surgical procedures as above and included 7 men and 3 women, aged 19–61 yrs (mean 39.7 yrs).

Signed informed consent was obtained from all subjects, and parental consent was obtained for the children.

Cell storage

In order to investigate the effect of dibutyryl cAMP on samples stored for 24 h, cells from the awake adults (n=10) were allowed to settle to the bottom of the Eppendorf tube containing Medium 199 on ice for 20 min. The supernatant was then removed and the cells were washed by resuspension in 1.5 ml of a solution of Medium 199 supplemented with gentamicin (25 µg·ml^{-1}), amphotericin B (25 µg·ml^{-1}), streptomycin (100 µg·ml^{-1}) and penicillin (100 U·ml^{-1}, followed by centrifugation at 20×g. This "wash" solution was then removed and the cells were resuspended in 3 ml of the above solution supplemented with 1% foetal calf serum, transferred to Petri dishes (diameter 5 cm) and placed in an incubator (37°C, 5% CO_2).

Measurement of CBF

Ciliated cells were transferred in Medium 199 to a glass-walled perfusion chamber (Prior modified, UK) with an internal volume of 0.35 ml. The chamber was connected to a perfusion pump delivering 0.25 ml·min^{-1}, which gave a 90% wash-out of the fluid in the chamber in 10 min. The wash-out time was calculated by filling the chamber with a coloured solution and measuring the rate of decline of absorbance during perfusion with distilled water. CBF was measured at room temperature (22.0±1.5°C) using a photometric technique [14, 15]. The sample preparation was placed on the stage of a phase-contrast microscope (Leitz Dialux 20, Germany), fitted with a photomultiplier connected to an amplifier (Leitz MPV Compact Microscope Photometer). Light directed from below and passing through the specimen was interrupted by the sweeping action of the cilia. Variations in light intensity, corresponding to the beat frequency, were detected by the photomultiplier, transduced to electric impulses and amplified. The electrical signal was fed through a low pass filter into an ultraviolet oscillograph which provided a permanent record of ciliary activity. CBF values were obtained by counting the number of ciliary beats recorded in 10 s intervals and were expressed in Hz. In addition to the "manual counting", CBF values were determined by Fast Fourier Transform analysis of the signal [16]. The two methods provided similar CBF values, with differences within 0.2 Hz (r=0.978; n=40). In order to eliminate the increase in CBF induced by perfusion [17] all recordings were taken in the absence of flow.

In this preparation the CBF may be measured in real time when the cells are exposed to a number of different agents. In order to obtain precise time-course data on the ciliary response to changes in perfusate, it was decided to study the same group of cilia in each preparation throughout the experiment *i.e.* the relative position of the photometer's field diaphragm (approximately 1.5×5 µm) and the selected epithelial border was kept constant. The choice of the ciliated area was randomized by using an eyepiece graticule and bias was avoided by choosing the group of cilia which lay closest to the crosspiece of the graticule on the equatorial line and which fulfilled the following criteria: 1) the cilia were beating perpendicularly to the light source; and 2) the ciliated cell was attached to the basement membrane [18].

All the CBF measurements were taken by the same observer, who was unaware of the nature of the perfusates. The pH of all solutions tested was between 7.2–7.4, a range known not to affect CBF [19].

Effect of dibutyryl cAMP on CBF

Following a 30 min period of equilibration on the microscope stage, the sample in the chamber was perfused for 10 min with fresh Medium 199 equilibrated with room air. Baseline values were obtained by taking the mean of CBF measurements after 1, 15 and 30 min. Following the baseline readings, the perfusate was replaced with: 1) Medium 199 alone or 2) medium supplemented with either butyric acid (2×10^{-3} M) or dibutyryl cAMP (10^{-4} or 10^{-3} M). CBF was measured after 1, 15 and 30 min following perfusion.

Inhibition of the dibutyryl cAMP-dependent effect on CBF

When the protein kinase inhibitor was used, the second perfusate was replaced with either a control solution containing the vehicle (dimethyl sulphoxide, final

concentration 1%) or a range of concentrations of H-7 (10^{-5}, 10^{-4}, 10^{-3} M). In these experiments, the samples were from the awake adult group. CBF measurements were taken every 15 minutes during the 1 h incubation period with H-7 or vehicle. At the end of the hour, the perfusate was replaced with one containing dibutyryl cAMP (10^{-3} M) and CBF was measured as above.

CBF measurements using microscope slide preparations

The effect of dibutyryl cAMP on CBF was also investigated in a separate set of experiments with an alternative method which uses sealed coverslip microscope slide preparations [20]. This measures the mean CBF of two separate aliquots of cells from the same brushing, one of which had been exposed to the active agent. Samples from the awake adults (n=10) were transferred to coded vials containing either 10^{-3} M dibutyryl cAMP dissolved in Medium 199 or Medium 199 alone. The pairs of coded vials were selected at random and the cells were transferred to sealed microscope preparations for measuring CBF. After incubation for 10 min on the microscope stage, twenty consecutive measurements were taken from different ciliated areas in each preparation and the mean CBF was calculated. The areas were randomly chosen by moving the eyepiece graticule across the specimen and the selection criteria for each group of cilia were the same as in the perfusion experiments.

Effect of phorbol myristic acid on CBF

H-7 blocks the protein kinase C as well as protein kinase A [12]. In order to examine a possible role of protein kinase C in the control of human CBF, we challenged samples from awake adults (n=10) with an activator of protein kinase C (phorbol myristic acid) using the sealed coverslip microscope slide preparation. The concentration of phorbol myristic acid (PMA) used (10^{-5} M) is known to increase protein phosphorylation in these (Mehta, unpublished data) and other cell types [21].

Chemical reagents

Medium 199 was obtained from Flow Laboratories; butyric acid, sodium salt of dibutyryl cAMP, H-7, and PMA were purchased from Sigma.

Statistical analysis

CBF values were expressed as the mean±SEM and the response as the absolute difference from the baseline CBF. Differences in CBF within groups were determined by a paired Wilcoxon's test. Differences between groups were determined by a Mann-Whitney U test. Significance was accepted when p<0.05.

Results

CBF values after perfusion

After perfusion with fresh Medium 199, butyric acid or dibutyryl cAMP there were no significant differences between CBF values recorded at times 1, 15 and 30 min. The mean CBF of the three readings was therefore taken to represent the change in CBF with respect to the baseline value due to the substance perfused.

Control experiments

In the awake adult group there was no change in CBF with respect to the baseline when the chamber was perfused with fresh Medium 199, with mean CBF of 7.8±0.3 and 7.8±0.4 Hz for baseline and Medium 199 respectively. CBF was also unchanged following perfusion with Medium 199 plus butyric acid (2×10^{-3} M), with mean CBF of 7.7±0.3 and 7.7±0.5 Hz for baseline and butyric acid, respectively. In our preparation the CBF was stable for at least 2 h, with a mean intra-cell coefficient of variation of the frequency, defined as the ratio of the standard deviation to the mean value as a percentage, of ±8.7% (n=20).

Effect of dibutyryl cAMP

Perfusion with Medium 199 plus dibutyryl cAMP (10^{-3} M) caused a significant increase (+1.1 Hz; p<0.01) in CBF in the awake adult group, with mean CBF of 7.3±0.3 and 8.4±0.4 Hz for baseline and dibutyryl cAMP respectively (fig. 1). The increment in CBF was already present at the time of the first reading following perfusion (1 min; +1.2 Hz) and did not increase further at 15 (+1.1 Hz) and 30 (+1.1 Hz) min. The increase was sustained after perfusion with fresh Medium 199 for 10 min (+1.2 Hz) (fig. 1). CBF did not change following perfusion with the lower concentration of dibutyryl cAMP (10^{-4} M), despite continuous observation for 1 h, with mean CBF of 7.3±0.3 at baseline and 7.3±0.4 Hz after dibutyryl cAMP. Cells collected from the awake adults and stored for 24 h showed the same pattern of response to dibutyryl cAMP (10^{-3} M) as fresh cells (+1.1 Hz; p<0.01), with mean CBF of 7.3±0.6 and 8.4±0.7 Hz for baseline and dibutyryl cAMP respectively (table 1).

The changes in CBF following perfusion with the higher concentration of dibutyryl cAMP (10^{-3} M) in the anaesthetized children group (+1.2 Hz; p<0.01) (table 1) and the anaesthetized adult group (+1.2 Hz; p<0.01) (table 1) were similar to those described for the awake adults. There were no significant differences in the increments in frequency between groups.

Experiments comparing the mean CBF of different aliquots of cells, using the sealed microscope slide preparation, confirmed the stimulatory effect of 10^{-3} M dibutyryl cAMP, with mean CBF of 8.4±0.5 and 9.4±0.7 Hz for control and dibutyryl cAMP respectively, (p<0.05).

Effect of H-7

Preincubation for 1 h with a control solution containing the vehicle alone did not affect the rise in CBF following exposure to 10^{-3} M dibutyryl cAMP (fig. 2).
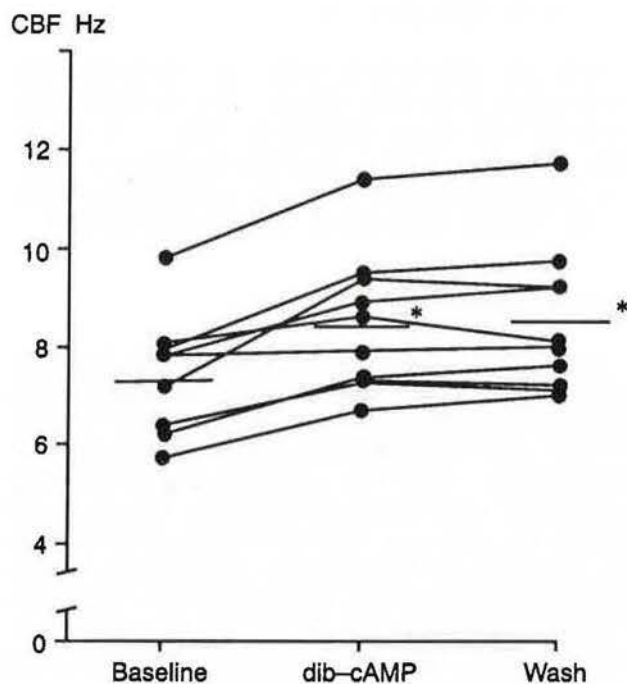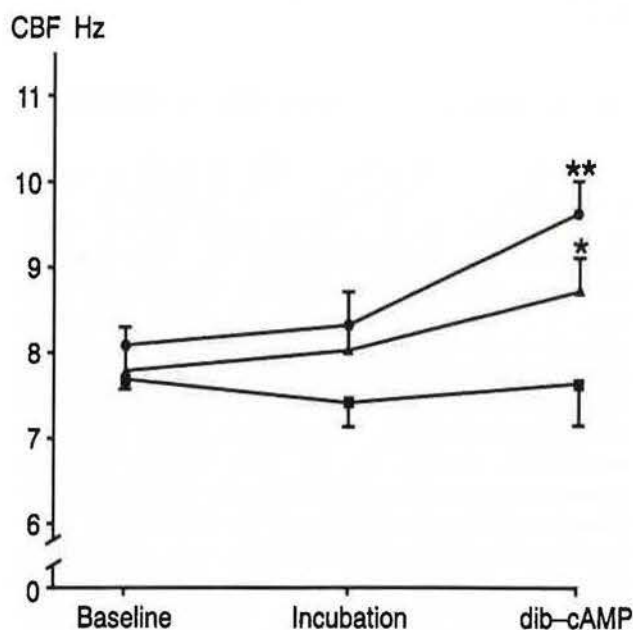

Fig. 1. – The stimulatory effect of 10^{-3} M dibutyryl cyclic adenosine monophosphate (dib-cAMP) on the ciliary beat frequency (CBF) of cells from awake adults (n=10). The bars represent the mean CBF for each period of observation. Mean CBF increased from a baseline value of 7.3 to 8.4 Hz after perfusion with dib-cAMP. When the cells were washed with control solution (Medium 199) the mean CBF did not decline, but was still significantly elevated with respect to the baseline. *: p<0.01.

Fig. 2. – The effect of 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine (H-7) on the ciliary beat frequency (CBF) response to 10^{-3} M dibutyryl cyclic adenosine monophosphate (dib-cAMP). After incubation for 1 h with control solution (circles) or 10^{-5} M H-7 (triangles), subsequent perfusion with dib-cAMP induced a rise in CBF, from 8.3 to 9.6 Hz and from 7.9 to 8.7 Hz, respectively. When the samples were incubated for 1 h with 10^{-4} M H-7 (squares) subsequent exposure to dib-cAMP did not change the CBF (7.4 to 7.6 Hz). Each point represents mean±SEM; n=10 for each group. *: p<0.05; **: p<0.01.

Table 1. – The effect of 10^{-3} M dibutyryl cyclic adenosine monophosphate (dib-cAMP) on the ciliary beat frequency (Hz) of cells stored for 24 h (collected form awake adults), cells from anaesthetized children and cells from anaesthetized adults

Sample	Stored cells		Anaesthetized children		Anaesthetized adults	
	Baseline	dib-cAMP	Baseline	dib-cAMP	Baseline	dib-cAMP
1	10.4	12.0	8.4	9.6	6.7	8.3
2	8.7	9.0	6.0	8.2	7.1	7.8
3	6.8	8.1	7.6	8.7	11.4	12.1
4	6.7	7.4	7.7	8.5	9.1	9.4
5	5.2	5.2	7.8	8.2	8.6	9.4
6	5.7	7.8	6.6	8.1	6.4	9.0
7	5.1	6.3	10.9	12.1	6.3	9.2
8	7.1	7.8	8.7	10.3	7.3	9.8
9	11.1	12.3	7.4	8.7	10.4	10.5
10	6.4	7.9	7.2	8.1	8.3	8.5
Mean (SEM)	7.3(0.6)	8.4(0.7)*	8.2(0.5)	9.4(0.3)*	7.8(0.4)	9.0(0.4)*

*: p<0.01 (dib-cAMP *versus* baseline).

Perfusion with H-7 (10^{-5}, 10^{-4}, and 10^{-3} M) did not affect the baseline CBF during the 1 h incubation period. After incubation with 10^{-5} M H-7 (fig. 2), the subsequent exposure to dibutyryl cAMP (10^{-3} M) caused an increase in CBF (+0.8 Hz, $p<0.05$). When the cells were incubated for 1 h with 10^{-4} (fig. 2) and 10^{-3} M H-7, the expected rise in CBF following perfusion with dibutyryl cAMP did not occur.

Effect of PMA

The mean CBF of aliquots of cells from single brushings (n=10) were similar, following exposure to PMA (10^{-5} M) or a control solution (8.6±0.4 and 8.5±0.4 Hz respectively).

Discussion

Experimental design

The direct action of pharmacological, chemical and physical agents on the intrinsic activity of ciliated cells cannot be conveniently investigated using preparations in which mucus secretion is present, because of the complex nature of the mucociliary interface. It has been suggested that the interaction of the cilia and mucus might lead to the mechanical stimulation of the cell and to secondary elevation of the CBF [22]. It was in order to eliminate these interactions that we used an *in vitro* method which measures CBF in a mucus depleted environment.

In our system the CBF was stable for at least 2 h, whereas some other groups working with similar perfusion preparations [22, 23] have noted a decline of CBF during their experiments. In this respect our data resemble the findings of ZAHM *et al.* [24], who recorded a constant CBF for 2 h during continuous perfusion of rat tracheal explants. It is known that ciliated cells show spontaneous fluctuations in CBF [16] and in our control experiments the mean intra-cell coefficient of variation of the frequency was ±8.7%, a value similar to that found in rat tracheal cells [24]. Perfusion with either fresh Medium 199 or butyric acid did not affect the baseline CBF.

Pathways controlling CBF

Mammalian respiratory cilia increase their CBF when exposed to sympathomimetic and parasympathomimetic agonists [25]. Animal studies have indicated that ciliated respiratory cells have at least two intracellular pathways for the control of CBF: one utilizing calcium, the other cAMP [22].

Much evidence has implicated cAMP as the second messenger in many physiological processes since the original report by SUTHERLAND *et al.* [26]. Cyclic AMP has been shown to activate or increase the beat frequency of cilia and flagella [22], but most of the studies on the

regulation of ciliary activity by cyclic nucleotides have been on unicellular eukaryotes. There have been two reports on the stimulatory effect of cAMP analogues on CBF in canine [5] and rabbit [27] trachea. There appear to be no published papers on the effects of cAMP on human respiratory tissue, except for one preliminary report on human bronchial cells in tissue culture which showed a maximal increase in CBF of 35.8% with 1 µM 8-bromo-cyclic AMP [28].

Effect of dibutyryl cAMP

Dibutyryl cAMP (10^{-3} M) caused a significant increase of CBF. This extracellular concentration of dibutyryl cAMP is similar to that known to increase CBF in rabbit tracheal epithelium [27] and to open chloride channels in canine respiratory epithelium [29]. This concentration of dibutyryl cAMP may at first sight seem to be relatively high. However, COHN [30] has found that millimolar concentrations are needed to induce changes in intracellular protein phosphorylation. Furthermore, the altered pattern of phosphorylation was the same as that seen following stimulation of cell surface receptors linked to adenyl cyclase. The explanation for this may lie in the value of the concentration necessary to produce a half-maximal response (Kd) for the butyryl analogue with respect to cAMP [31]. The Kd for protein kinase A activation for dibutyryl cAMP is 3.5×10^{-5} M relative to a value of 3×10^{-8} M for cAMP itself. If one assumes that maximal activation is seen at 10 times the Kd value, then the reason for the requirement of 10^{-3} M becomes clear, when one allows for the concentration gradient across the cell.

Although our experimental design did not allow us to determine the exact time of onset of this response, our data showed that the rise in frequency occurred within 10 minutes of exposure to dibutyryl cAMP (10^{-3} M). Furthermore, the data showed that the effect lasted for at least 30 min and was not reversed immediately after washing the chamber with fresh Medium 199 (fig. 1). This persistent effect could be due to a long lived change in the phosphorylation state of the target proteins beyond the activation of cAMP- dependent protein kinase. Exposure of cells to 10^{-4} M dibutyryl cAMP produced no change in CBF and there are two possible explanations for this in addition to those discussed above: firstly that it might have been insufficient to overcome the naturally occurring inhibitor of cAMP-dependent protein kinase [32], or secondly that the activity of the intracellular phosphodiesterase might be sufficiently high to degrade the lower dose of dibutyryl cAMP.

The magnitude of the change in CBF following a given stimulus may be affected by a number of factors including interspecies differences [25] and, possibly, site of sampling [33]. The wide range of CBF responses is illustrated by several examples in the literature using beta-adrenergic agonists in bovine (+250%) [34], rabbit (+100%) [6], rat (+50%) [7], and human (+18%) [8] ciliated epithelium. Our results showed that CBF increased by an average value of 15% after 10^{-3} M

dibutyryl cAMP. This result is similar to that seen in rabbit tracheal cells (+19%) using the same analogue of cAMP [27]. On a percentage basis, this rise in CBF appears to be small. However, this need not necessarily be taken to imply that such an increment might be physiologically insignificant. An increase of 1.2 Hz, when taken over one hour, results in an extra 4,320 beats. This, together with the persistence of the rise even after washing the chamber, suggests that the rate of mucus transport might be significantly enhanced following a rise in intracellular cAMP, especially when additional frequency-dependent factors influencing mucociliary transport (ciliary coordination, length of the metachronal wave, effective/recovery stroke time-ratio) are taken into account [25]. In a recent study, it has been shown that an increase of 16% in CBF induced by 10^{-3} M acetylcholine resulted in a simultaneous 56% acceleration of surface liquid velocity in an *in vitro* preparation of sheep trachea [35]. Furthermore, the authors demonstrated that the magnitude of the changes in one component of mucociliary interaction cannot predict those of other components. It is, therefore, not possible at present to evaluate the physiological *in vivo* significance of our observed changes.

When studying the effect of cilio-active drugs and cilio-modulating agents on CBF, the use of freshly obtained ciliated epithelium is preferable. However, these studies are time consuming and the availability of human samples cannot always be predicted. In this respect, it is reassuring to note that the response to dibutyryl cAMP was similar in fresh cells and in cells stored for 24 hours in Medium 199 supplemented with 1% foetal calf serum and broad spectrum antibiotics (table 1).

Age of subject and effect of anaesthesia

The response of CBF to dibutyryl cAMP was compared in different age groups of subjects. The cells from anaesthetized children responded to dibutyryl cAMP in a similar manner to those from awake adults. Anaesthetics generally decrease ciliary activity *in vitro* [36] and it therefore remained possible that despite our attempt to collect samples from the children immediately after the induction of anaesthesia, the anaesthetic agents might themselves have altered the capacity to respond to cAMP. Since the response of awake adults and those under anaesthetic did not differ, it appears that the *in vitro* effect of dibutyryl cAMP on CBF is not affected by the induction of general anaesthesia.

Effect of H-7 on the cAMP-response

H-7 is a complex competitive blocker of the ATP binding site on the catalytic subunit of cyclic nucleotide-dependent protein kinases [12]. H-7 (10^{-3} and 10^{-4} M) blocked the expected rise in CBF following exposure to dibutyryl cAMP. 10^{-5} M H-7 failed to prevent the subsequent rise in CBF, but the mean increment in

frequency (+0.8 Hz) was the lowest we have ever recorded in our studies on 10^{-3} M dibutyryl cAMP. Although this attenuation in response did not reach statistical significance when compared to control experiments, it is likely that H-7 begins to act near this concentration.

Effect of PMA on CBF

H-7 blocks cyclic nucleotide-dependent and calcium phospholipid-dependent protein kinases (protein kinase C) [12]. We used PMA to stimulate protein kinase C in an initial attempt to exclude a major role for this kinase in the control of CBF. The failure to change CBF with PMA suggests that protein kinase C is not involved in the control of human CBF, whereas there are data supporting a cilio-inhibitory role in rabbit tracheal cells [37].

In conclusion we have shown that *in vitro* dibutyryl cAMP increases the CBF of respiratory epithelium from adults and children and that anaesthesia does not affect this rise. This response is likely to be mediated by a cAMP dependent mechanism, because it may be blocked by a competitive antagonist of cyclic nucleotide-dependent kinases.

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Effet de l'AMP cyclique sur l'activité ciliaire de l'épithélium respiratoire humain. G. Di Benedetto, F.S. Manara-Shediac, A. Mehta.

RÉSUMÉ: Pour investiguer l'effet de l'AMP cyclique sur la fréquence des battements ciliaires (CBF) dans l'épithélium respiratoire humain, l'on a prélevé des cellules par brossage des cornets nasaux inférieurs chez trois groupes de 10 sujets: des adultes éveillés (âges extrêmes, de 20 à 34 ans), des enfants anesthésiés (âges extrêmes, de 2 à 15 ans), et des adultes anesthésiés (âges extrêmes, de 19 à 61 ans). Les cellules provenant d'adultes éveillés ont été également étudiées après entreposage pendant 24 h dans un milieu de culture tissulaire. CBF a été mesuré *in vitro* par une technique photométrique à la température de la chambre (22.0±1.5°C). Des échantillons ont été montés dans une chambre de perfusion et provoqués, soit par des solutions contrôle, soit par de l'AMP cyclique dibutyryl (10^{-4} ou 10^{-3} M), ou par l'inhibiteur de la protéine kinase dépendant des nucléotides cycliques 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7). L'AMP cyclique dibutyryl (10^{-3} M) a provoqué une augmentation significative de CBF dans tous les groupes étudiés: chez les adultes éveillés (+1.1 Hz; $p<0.01$) chez les enfants anesthésiés (+1.2 Hz; $p<0.01$), chez les adultes anesthésiés (+1.2 Hz; $p<0.01$), et dans les cellules conservées (+1.1 Hz; $p<0.01$). Cette réponse fut inhibée par une pré-incubation au moyen de H-7 (10^{-4} et 10^{-3} M). Nous concluons que l'AMP cyclique est un régulateur de l'activité ciliaire dans l'épithélium respiratoire humain.
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