A REALISTIC APPROACH TO OPTICAL METHODS OF CILIARY BEAT FREQUENCY MEASUREMENT

A.E. Bell, C. Marriott, I.P. Tansey*, Department of Pharmacy, Brighton Polytechnic, Brighton, U.K. *Riker Laboratories, Loughborough, U.K.

The cilia of a transporting epithelium can be studied indirectly by photometrically recording the variation in light intensity reflected from a mucus surface, since this variation mimics the movements of the underlying cilia (Toremalm et al 1974). In order to measure this flickering the cilia must disturb the mucus surface sufficiently to deflect the incident light. Because of the relatively high loss moduli (G") of viscoelastic mucus gels (Bell & Allen 1982), movement of the mucus surface will be inversely proportional to the distance between the cilia and the mucus surface. However differences in thickness from area to area have been reported (Kerrs & Allen 1982). Previously reported methods, using photomultiplier systems, measure all of the available light within a given field of view and make no corrections for localised variations in light intensity.

In this work video recordings of variations in reflected light intensity from different areas of several frog (Rana temporaria) palates have been made using a Vickers photoplan microscope system with incidental illumination (magnification X200). Actual measurements of the ciliary beat frequencies were taken from several areas of the video monitor on playback using a photodiode placed directly over the flickering region (area 100 mm^2, about 5 cilia). In all of the recordings made surface variations in light intensity have only been observed in some areas of the frog palate, even if the angle of the incident light used was varied. However the area of flickering can be considerably increased by gently wiping away some of the surface mucus. In all cases the regions both of flickering and apparent inactivity were not static, interchanging slowly and continuously with time. Optically "inactive" areas were still able to transport marker particles (spheres about 200 µm diameter). Cilia beat frequencies recorded were all between 2 and 11 Hz, the frequency distribution being totally dependent upon the area of frog palate studied. If individual groups of cilia were studied it was common to observe an increase or decrease in beat frequency with time (Table 1). Reduction in amplitude at the lower frequencies effectively prevented measurements below about 2 Hz.

Table 1 Changes in the beat frequency and amplitude of a small group of cilia with time

Time (s)	0	30	60	90	120
Frequency (Hz)	10.1	9.8	8.4	4.7	2.3
Amplitude (mV)	110	90	80	30	10

The fact that the faster cilia have higher signal amplitudes, presumably due to the decreased mucus thickness, tends therefore to skew the observed frequency distribution in any given viewing field toward the higher beat frequencies. Uncoupled cilia e.g. human bronchial beating in cell culture medium with no mucus covering commonly beat at 10-14 Hz (Rutland et al 1982). These observations suggest that when photoelectrical systems are used to assess ciliary beat frequency they may well be selectively measuring values well above the actual operating frequency of cilia covered with mucus, and that the areas of monochronal activity previously observed (Iravani 1969) may be the result of moving areas of reduced mucus thickness. Because of this the method of measurement of cilia beat frequency should be viewed with some caution especially if it is to be used as a criterion for pharmacological activity. Also screening of ciliotoxic drugs using isolated i.e. unloaded cilia, may not be entirely appropriate.

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