Effects of morphine on human nasal cilia beat frequency in vitro†

D. A. SELWYN, J. H. RAPHAEL, D. G. LAMBERT AND J. A. LANGTON

Summary

Using human nasal cytological brushings, we have investigated the effects of morphine on ciliary function by measurement of cilia beat frequency in vitro, and we have also determined opioid receptor binding in these specimens. We obtained ciliated samples from seven volunteers, and measured cilia beat frequency using the transmitted light technique during exposure to morphine 10 μmol litre⁻¹ for 4 h. Mean cilia beat frequency of the samples exposed to morphine was 11.1 (95 % confidence interval 10.9–11.5) Hz and that of the controls 11.3 (11.1–11.7) Hz. There was no significant effect of morphine on human cilia beat frequency in vitro (MANOVA for repeated measures and nested, F = 0.61, P = 0.66). In a separate study, we obtained nasal brushings from 20 patients and measured the binding of the opioid antagonist tritiated diprenorphine[³H] DPN. Mean disintegrations per minute (dpm) for total and non-specific binding were 9036 (8105–9967) dpm and 9130 (8054–9996) dpm, respectively. These values did not differ significantly (paired t test, t = 0.22, P = 0.83). We conclude that morphine had no effect on cilia beat frequency in vitro and we were unable to demonstrate any significant numbers of opioid receptors on nasal ciliated epithelium. (Br. J. Anaesth. 1996; 76: 274–277)

Key words


Opioids are potent analgesics that form the mainstay of perioperative pain relief and are widely used as sedatives in the intensive care unit. A variety of such agents, both natural and synthetic, are available, but one of the commonest agents used in these circumstances is morphine. Several studies have reported a depressant effect of morphine on respiratory mucus transport which is one of the most important defences against respiratory tract infections.

In a study of anaesthetized cats in which barium sulphate was insufflated into the lungs, Van Dongen and Leusink found that the administration of morphine 0.5 mg kg⁻¹ s.c. delayed the clearance of the radiological marker by a factor of 2–3 times, a distal airways phenomenon [1]. Using a radioactive droplet technique, Forbes and Horrigan measured mucociliary flow in the trachea in dogs. Compared with those respiratory tracts ventilated with 40 % nitrous oxide in air, the addition of morphine 6 mg kg⁻¹ i.v. reduced mucus transport rates to 70 % of controls. This reduction was similar to that found with 1.2 MAC of halothane [2].

Chest infections are one of the commonest forms of serious morbidity after surgery, with an incidence of 6–21 % [3–6]. Chest infection is also common in intensive care patients and may develop in about one-third of patients in the ITU [7]. As mucociliary clearance is an important respiratory defence mechanism, its impairment by morphine may have relevance in the postoperative period and in the ITU.

The rate of mucus transport depends on the volume and physical properties of mucus and on the function of the cilia; however, the mechanism by which morphine reduces mucus transport rate has not been elucidated. There are two studies that have investigated the effects of opioids on cilia and have shown a reduction in beat frequency [8, 9]. These studies have been in vitro investigations complicated by the administration of other drugs. We have investigated the effects of morphine on human respiratory ciliary function by measuring cilia beat frequency and opioid receptor density using nasal epithelial cell brushings in vitro.

Materials and methods

EFFECTS OF MORPHINE ON CILIA BEAT FREQUENCY

After obtaining Ethics Committee approval and informed consent, we obtained samples of ciliated epithelium from seven (six female) non-smoking healthy patients, mean age 40.3 (range 21–56) yr. None of the patients was taking any medication or had an upper respiratory tract infection within the previous 4 weeks. No premedication was given. The samples were acquired by passing a bronchoscopy brush over the inferior nasal turbinates after induction of anaesthesia with propofol 2–3 mg kg⁻¹. The tissue was removed from the brush by agitation in Hanks buffered salt solution (HBSS).

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The ciliated samples from each individual were placed between the coverslips of paired perfusion chambers as described previously [10] (fig. 1). Each chamber was perfused from a separate delivery bottle of HBSS into the entry port of the chamber and out from its exit port into a collecting beaker. HBSS was aerated at 1000 ml min\(^{-1}\) and perfused the chambers under the effect of gravity at a constant rate of 0.5 ml min\(^{-1}\). Morphine was added to one delivery bottle in a concentration of 10 \(\mu\text{mol litre}\)\(^{-1}\). The observer did not know which chamber received morphine as the delivery tubes from the bottles to the chambers were wound around one another within a countercurrent water jacket. The bottles of HBSS were immersed in a water bath at 37\((\pm0.1)\)\(^{\circ}\text{C}\) that also surrounded the countercurrent heat exchanger around the delivery tubing. The perfusion chambers were maintained at 37\(^{\circ}\text{C}\) by means of a thermostatically controlled heating element on the underside of the chamber.

To confirm that morphine was reaching the chamber from the delivery bottle in an adequate concentration, we sampled the perfusate downstream of the perfusion chamber and measured morphine concentrations by high performance liquid chromatography (HPLC). We used an 18C column, 25 cm in length, with phosphate buffer at pH 2 running at 1 ml min\(^{-1}\) and electrochemical detection with a carbon electrode. The lower limit of this assay for morphine was of the order of 1 ng ml\(^{-1}\) [11].

For measurement of cilia beat frequency we used a photometric method as described previously [10]. This involved detection of ciliary movements using a differential interference contrast microscope (Nikon Diaphot 200) and transmission of the image to a monitoring screen. Cilia beat frequency is determined by their interference with the light reaching a pinhead photodetector attached to the monitoring screen. Voltage changes across the photodetector were amplified to provide a signal on an oscilloscope greater than 0.2 V and low-pass filtered with a cut-off above 25 Hz. Ciliary movements were recorded for a period of 15 s and the voltage changes across the photodetector were digitized and mathematically processed to give a power spectrum. The signals produced by the beating cilia over a 15-s period were divided into three sequential 5-s intervals for analysis. We computed the peak frequency of one power spectrum obtained by calculating the mean of the power spectra for each epoch. A mean of the dominant frequency of each of the three 5-s epochs was then computed. Although shortening the capture time reduced the resolution of measurement to 0.2 Hz, this was not a limiting factor within the experimental conditions used and it increased the confidence interval of the calculated mean beat frequency. The microscope was mounted on a concrete block to minimize interference produced by extraneous vibration.

When the chambers had equilibrated to 37\(^{\circ}\text{C}\) as indicated by the temperature plate on the chamber base, recordings of cilia beat frequency were commenced. Acceptable ciliated edges for measurement were deemed to be those free of mucus, at least 60 \(\mu\text{m}\) long and not part of single cells. Readings were taken from both chambers before the addition of morphine and 1, 2, 3 and 4 h after morphine had been added to one chamber by analysing as many acceptable ciliated edges as possible from a minimum of six to a maximum of 12 from each chamber in each defined time band.

At the end of each experiment, it was determined which chamber had received morphine and the samples were discarded. The data approximated to a normal distribution and were analysed by MANOVA for repeated measures and nested using polynomial contrasts, with significance taken at \(P < 0.05\) (SPSS for windows v 5.01).

BINDING OF \([3\text{H}]\)DPN TO HUMAN NASAL CILIATED EPITHELIAL CELL MEMBRANES

To determine if opioid receptors were present on cilia, we measured the binding of tritiated diprenorphine, \([3\text{H}]\)DPN, a non-selective opioid antagonist, to ciliated epithelial cell membranes [12].

After obtaining Ethics Committee approval and informed patient consent, we collected nasal ciliated epithelial cells on two separate occasions by the methods described earlier. This involved passing a bronchoscopy brush over the inferior nasal turbinates of 10 healthy patients on each occasion after they had received an anaesthetic induction dose of propofol 2–3.3 mg kg\(^{-1}\). The samples were removed...
from the brushes by agitation in M199. The tissue was washed in Tris HCl 50 mmol litre\(^{-1}\)-NaCl 100 mmol litre\(^{-1}\), buffered to pH 7.4. The tissue was homogenized using three 15-s bursts with a tissue-tearer and then centrifuged at 13500 rpm for 10 min at 40°C. This process of homogenization and centrifugation was repeated three times and the cell membranes were then resuspended in 2 ml of the above buffer. Binding studies were performed in 1-ml assay volumes of Tris HCl for 60 min at 37°C containing a saturating concentration of \([^{3}H]\)DPN 3.7–4.3 nmol litre\(^{-1}\). Non-specific binding was defined in the presence of excess naloxone 10 μmol-litre\(^{-1}\).

Bound and free radioactivity were separated by rapid vacuum filtration using a Brandel cell harvester onto Whatman GF/B filters and washed with three 4-ml aliquots of ice cold buffer. After overnight extraction, bound radioactivity was measured by scintillation spectroscopy with Optiphase-X (LKB Wallac) as a scintillant.

**Results**

We made a total of 739 measurements of cilia beat frequency: 374 from the cilia exposed to morphine and 365 from the controls. Mean cilia beat frequency of the samples exposed to morphine was 11.1 (95% confidence interval 10.9–11.5) Hz and that of the controls 11.3 (11.1–11.7) Hz (MANOVA for repeated measures and nested, \(F = 0.61, P = 0.66\)) (fig. 2).

Mean total and non-specific binding at DPN 3.7–4.3 nmol litre\(^{-1}\) were 9036 (8105–9967) dpm and 9130 (8054–10206) dpm, respectively (table 1). These values were not significantly different, indicating no specific binding (paired \(t\) test, \(t = 0.22, P = 0.83\)).

**Discussion**

We did not find a significant effect of morphine on cilia beat frequency measured in vitro with human nasal cytological samples and were unable to demonstrate the presence of opioid receptors in this tissue.

The variability in the measurements of cilia beat frequency was large. There was greater variability of within-sample measurements than between-sample measurements because of differences in cilia beat frequency between different ciliated edges. Taking \(n\) to be the number of ciliated edges measured at any one time, we have computed a post hoc power of 80% to detect differences greater than 2 Hz at the 5% level of significance. This represents a change in cilia beat frequency of less than 20%; however, because of the non-linear relationship between cilia beat frequency and mucus transport rate [13], it is possible that small changes in cilia beat frequency may be associated with large changes in mucus transport rate. We elected to investigate binding of opioids to ciliated tissue to substantiate our negative finding.

The concentration we investigated, 10 μmol litre\(^{-1}\), is at the upper limit of the range found in clinical practice, that is the plasma concentration found in patients receiving up to 1500 mg of morphine per day for the treatment of cancer pain [14].

We found an initial small increase in cilia beat frequency in both the control and treatment groups. This may have resulted from the effects of sampling or a delay in the chamber medium equilibrating with temperature.

Studies comparing cilia from the nose with those further down the respiratory tract have found a similar beat frequency [15] and the effects of other drugs on nasal and tracheal tissue are similar [16, 17], suggesting that nasal samples are representative of cilia further down the bronchial tree; however, it is possible that they may not have a similar response to morphine.

There are few comparable studies on the effects of morphine on cilia beat frequency. Rutland, Griffen and Cole measured cilia beat frequency of human nasal brushings with a photometric technique after premedication with the opioid papaveretum and atropine [8]. They found that mean cilia beat frequency in premedicated patients was 11.4 (SD

![Figure 2](image-url) Effects of morphine (○) and control (□) on cilia beat frequency (CBF) (mean (95% confidence interval)).

<p>| Table 1 | Binding of ([^{3}H])DPN to human nasal ciliated cell membranes. No specific binding was detected. Data in each experiment are mean (95% confidence interval) for six determination of total and NSB in each experiment. Mean values are 12 determinations |</p>
<table>
<thead>
<tr>
<th>No. of patients</th>
<th>DPN binding (dpm)</th>
<th>Total</th>
<th>NSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>10</td>
<td>8235 (7465–9004)</td>
<td>8106 (6124–10090)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>10</td>
<td>9877 (8129–11544)</td>
<td>10153 (9658–10649)</td>
</tr>
<tr>
<td>Mean</td>
<td>20</td>
<td>9036 (8105–9967)</td>
<td>9130 (8054–10206)</td>
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</tbody>
</table>
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1.4) Hz compared with 13.1 (1.9) Hz in a group of awake and unmedicated controls; this difference was statistically significant ($P < 0.001$). Roth and co-workers used a similar method to directly measure cilia beat frequency in vivo from nasal brushings and compared results from the same patients before and after administration of an opioid-containing premedication [9]. The patients received a mixture of morphine 5–10 mg i.v., atropine 1 mg i.m. and 2% lignocaine 2–3 ml applied topically to the nose. The authors found a small but significant reduction in ciliary beat frequency after medication from a mean of 12.2–11.0 Hz. In both of these studies, the addition of other drugs limits interpretation. Although low doses of lignocaine do not affect ciliary beat frequency in vitro [18], 2% lignocaine has been shown to cause ciliostasis in human nasal biopsy specimens [19]. Atropine is known to inhibit mucus transport rates in humans [20]; however, this appears to be caused by inhibition of mucus secretion and there is little direct evidence for a depressant effect on the cilia [21].

Another explanation for the conflict in our results with those described above may relate to differences in methods. We elected to measure cilia beat frequency from all visible edges whereas other workers appear to have measured the fastest beating ciliated edges. Consequently, the variation in our measurements was large; however, selection of certain ciliated edges, as used by other workers, introduces a bias that we avoided.

Using [3H]DPN at a concentration that would occupy all opioid receptors, we failed to demonstrate any specific binding (receptors) on nasal ciliated epithelial cell membranes. This concurs with our in vitro findings of the absence of effects of morphine on cilia beat frequency.

The mechanisms by which morphine reduces mucociliary transport in vivo remain to be elucidated. They may be related to effects on mucus or to in vivo effects on the cilia. Rogers and Barnes showed that morphine reduced mucus secretion stimulated by capsaicin in bronchitic patients [22]. One could speculate that during anaesthesia which involves procedures and agents that are irritant to the airways, morphine might similarly inhibit mucus secretion. Reduction in the secretion of mucus and its subsequent movement might alter its physical properties which would affect the function of the cilia, an effect that would not be observed with mucus-free in vitro preparations.

Although there is no evidence for neural control of ciliary function in humans, there are free nerve endings adjacent to the basement membrane [23]. An alternative hypothesis is that morphine may have an effect on cilia in vivo because of effects on neural connections which are absent from in vitro preparations.

References


