Influence of perfusate temperature on nasal potential difference

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On behalf of the European Cystic Fibrosis Society – Diagnostic Network Working Group

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Abstract

Nasal potential difference (NPD) quantifies the abnormal ion transport in cystic fibrosis (CF). It has gained acceptance as an outcome measure for investigation of new therapies.

Aim

To quantify the effect of solution temperature on the NPD, we first examined the effect of switching from room temperature (RT: 20-25°C) to warmed solutions (W:32-37°C) and vice versa during each perfusion step. Secondly, standard protocols were repeated at both temperatures in the same subjects.

Results

Changing solution temperature did not alter NPD during perfusion with Ringers solution (<1mV, p>0.1). During perfusion with zero-chloride solution, changing from RT to W tended to decrease absolute NPD (ie became less negative) by 0.9 mV(p>0.1); changing from W to RT increased NPD by 2.1 mV(p<0.05). During isoprenaline perfusion, changing from RT to W increased NPD by 1.5 mV(p<0.01), and from W to RT decreased PD by 1.4 mV(p<0.05).

For full protocols at RT or W in the same subjects, mean values were similar (n=24). During W perfusion, group results for total chloride response had a larger standard deviation. As this increased variability will likely decrease the power of trials, this study suggests that room temperature solutions should be recommended for the measurement of nasal PD.
Keywords

Cystic Fibrosis
Ion transport
**Introduction**

Measurement of nasal potential difference (NPD) evaluates the ion transport across human airway epithelium *in vivo*. NPD measurement has gained acceptance as a diagnostic tool for CF [1, 2] and has been used to measure the effects of disease modifying treatments [3, 4] on transepithelial ion transport.

Over the last 20 years, many groups have measured NPD using the technique initially described by Knowles *et al.* [5] with modifications to the site of measurement and the exact details of the exploring electrode, perfusate temperature and perfusate composition. Over the last 10 years standard operating procedures (SOPs) were developed in the USA [6, 7].

Warming of the perfusates, as originally described by Knowles *et al* [5] but not by others [8] is part of the CFF-SOP. A previous study compared the NPD protocol performed in different subject groups studied with warmed (W) and room temperature (RT) perfusates. This study showed a slightly smaller response to zero-chloride solution and a larger response to isoprenaline when the solutions were warmed, but there was large inter-subject variability [9].

During the development of the SOP for NPD measurements by the European Cystic Fibrosis Society Diagnostic Network Working Group, the large variability in this study was noted, so a second study was undertaken to examine the effect of perfusate temperature on the NPD. Six centres of the European Cystic Fibrosis Society Diagnostic Network Working Group participated. The perfusate temperature was changed *during* each step of the NPD protocol, using a similar design to a recent study comparing
different chloride concentrations in the perfusate [10]. In a separate series of studies, a standard protocol was performed with either W or RT solutions, in the same subjects on different days. The overall aim of this study was to determine the influence of using W compared with RT solutions on NPD.
Methods

Subjects

Six ECFS-DN sites recruited a total of 69 healthy subjects, mean age 32 years (range 16-60). All were non-smokers and were studied at least 4 weeks after any upper respiratory tract infection.

Nasal PD was measured according to the methods of Knowles et al [11] (n=57) using an exploring electrode below the inferior turbinate with a subcutaneous agar filled reference electrode or the London method[8] (n=12) measuring along the floor of the nose compared with a surface reference electrode. The standardised protocol for NPD comprises sequential perfusion of (i) physiological salt solution to measure baseline NPD, followed by the responses to (ii) 100µM amiloride (iii) zero chloride solution with 100 µM amiloride and (iv) zero chloride solution with 100 µM amiloride and 10 µM isoprenaline. The total chloride response is the cumulative change in NPD during (iii) and (iv). To warm the solutions, the perfusion line was placed in a thermostatically controlled water bath or in a warmed water sleeve using counter-current flow to achieve the designated perfusate temperature. Initial validation studies showed that the temperature of the solutions at the tip of the catheter during perfusion was 20 - 25°C for RT and 35 - 38°C for W solutions. Following temperature change between RT and W the temperature at the tip changed by 12-14 °C within 30-40 seconds in both directions.

Protocols
To directly compare the response to temperature changes during a particular step of the perfusion protocol, solutions were perfused at one of the two temperatures for at least 4 minutes, changed to the other temperature for 4 minutes, and then back to the initial temperature for another 4 minutes. The opposite temperature sequence was applied in the other nostril, in random order.

In the second series of experiments, the full perfusion protocol was measured in the same subjects, with either RT or W solutions, on different days, in random order.

Statistical analysis

To simplify discussion, the NPD is reported as absolute values of mean ± standard deviation, with the lumen always negative. Comparison between the different temperatures was made with paired t-tests. In parallel protocols, the results for each individual were averaged to give a single change for temperature increase or decrease for that individual subject.

All studies were conducted with approval from the local Hospitals Ethics Committees, and informed consent was obtained from each participant.
Results

1. Temperature changes during perfusion

The effect of temperature changes during the perfusion are given in Table 1. No significant effect of temperature changes was seen during perfusion with baseline Ringers solution for either the RT – W - RT sequence: RT 12.6 (6.0)mV – W 11.9 (5.8)mV RT 11.8 (5.8)mV or the W – RT – W sequence : W 13.1 (7.5)mV – RT 12.7 (7.0)mV – W 13.1 (8.1)mV.

However, during perfusion with zero chloride solution, warming the perfusate (change from RT to W) decreased the absolute NPD with an average decrease in NPD of 0.9mV. Cooling the perfusate (change from W to RT) increased the NPD by 2.1 mV. For the individual sequences, the absolute responses were: RT – W – RT sequence: RT 24.3 (14.3)mV – W 24.5 (14.3)mV – RT 26.0 (15.4)mV or the W – RT – W sequence : W 22.2 (12.3)mV – RT 25.0(11.8)mV – W 23.0 (11.7)mV.

Conversely, warming the isoprenaline perfusate increased NPD and cooling the perfusate decreased absolute NPD : RT – W – RT sequence: RT 36.4 (13.5)mV – W 38.3 (14.8)mV – RT 37.7(12.6)mV or the W – RT – W sequence W: 35.7(16.0)mV – RT 33.6(14.5) mV – W 34.7(14.7)mV.

The mean (standard deviation) change in NPD during temperature change are reported in Table 1. Time courses of the PD after change between RT and W solutions and back to RT during isoprenaline perfusion are shown in Figure 1.
2. Complete perfusion protocol with room temperature and warmed solutions in same subjects

In 24 subjects, NPD measurement was obtained with RT and W solutions on different days in random order, as shown in Table 2. Individual differences between NPD values are plotted in Figure 2, showing the large intra-subject differences between measurements.
Discussion

This study has confirmed the small but measurable difference in chloride responsiveness when the NPD is measured using warmed solutions. Similar to the previous report by Boyle et al., [9] NPD during perfusion with low chloride solution was smaller (less negative) when using 37°C solutions, while NPD during perfusion with warmed isoprenaline solution was higher. Finally, testing the same subjects on different days with W or RT solutions showed differences of (-15 to +20 mV) in total chloride response, likely reflecting day-to-day variability rather than the different temperatures tested. The differences observed are in line with previous studies of day-to-day variability [2][12].

The important new finding in the current study is that changing perfusate temperature during the measurement allowed delineation of a small response without the difficulty of the day-to-day and subject-to-subject variability. This parallels with the recent demonstration that low chloride responses with 0 mM versus 6 mM Cl⁻ solutions gave an extra 2 mV response [10].

This multicentre study has shown that baseline NPD is not altered by the temperature of the perfusate. Similar to the changes shown earlier,[9] the PD during perfusion of warmed solutions was slightly smaller (less negative) during perfusion with zero-chloride, and slightly larger (more negative) during perfusion with isoprenaline. There was a 1.1 mV difference in the total chloride response, which results in a ~ 3% larger response at 37 °C. Yet this increased response was at the expense of increased between-subject variability, with the group standard deviation increasing by 4 mV.
As the TCS at 37°C were larger than those at 20°C, it could be argued that NPD perfusates should be warmed, as W solutions provide the largest difference between CF and non-CF. However this needs to be balanced against the increased between-subject variability seen during the 37°C perfusion. This increased the variability of the responses may thus affect the statistical power of studies of new treatments for CF. As the power of a study to determine a change is dependent on the ratio between the size of a response and the variability of that response, higher variability may well outweigh any advantage of a larger response.

The use of 37 °C solutions for the nasal PD had its origins in measurement from Ussing chambers and other in vitro testing equipment, where changing apical and basolateral temperatures changes ion transport. However the situation is different for the NPD where the basolateral surface is always perfused at 37°C in vivo. Given that one of the main functions of the nose is to warm the inspired air, intranasal surface temperatures are kept around 30-32°C when breathing air at room temperature [13]. In two of our subjects, intranasal temperature was measured at 31-32°C during perfusion with solutions at room temperature, showing that the effect of solution temperature on the intranasal temperature is small.

The main aim for an SOP is to harmonise a technique to simplify the steps involved, to reduce the risk of error in the procedure, and thus ensure that all investigators are performing the same testing across different sites in different countries. This allows replication during multicentre studies, with maximal accuracy and minimal variability. In turn this gives the smallest number of subjects to be recruited to conclusively demonstrate a response, with least chance of Type I or Type II errors. Thus, the aim of
an SOP is to have the simplest, easiest system which generates the largest response with least variability.

In conclusion, this study has demonstrated that, in volunteer subjects expressing functional CFTR, warmed solutions result in slightly larger total chloride responses, but at the expense of a larger variability. We conclude that the use of warmed solutions has little net overall advantage and thus room temperature solutions should be recommended for simplicity, stability of recording, and reproducibility across centres in different parts of Europe.
Table 1: Mean (SD) change in NPD values after temperature changes from room temperature (RT) to warmed (W) solutions and opposite during perfusion with Ringers, zero chloride and isoprenaline solutions. P-values for paired t-test.

<table>
<thead>
<tr>
<th></th>
<th>RT to W</th>
<th>W to RT</th>
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<tbody>
<tr>
<td>Ringers (n=37)</td>
<td>-0.1 (1.5) mV</td>
<td>-0.2 (1.6) mV</td>
</tr>
<tr>
<td>Zero chloride (n=25)</td>
<td>-0.9 (2.9) mV</td>
<td>+2.1 (4.4) mV*</td>
</tr>
<tr>
<td>Isoprenaline (n=44)</td>
<td>+1.5 (3.2) mV*</td>
<td>-1.4 (3.7) mV</td>
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*p<0.05  *p<0.01
Table 2: Group means +/- SD of NPD parameters and mean differences measured during perfusion with room temperature (RT) and warmed (W) solutions. All p-values for paired t-test are not significant

<table>
<thead>
<tr>
<th></th>
<th>mean (mV) ± SD</th>
<th>Difference (mV)</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>RT</td>
</tr>
<tr>
<td>Baseline potential</td>
<td>24</td>
<td>12.8 ± 7.5</td>
</tr>
<tr>
<td>Amiloride response</td>
<td>24</td>
<td>-7.2 ± 4.4</td>
</tr>
<tr>
<td>Zero chloride response</td>
<td>21</td>
<td>25.0 ± 13.6</td>
</tr>
<tr>
<td>Isoprenaline response</td>
<td>21</td>
<td>9.8 ± 8.4</td>
</tr>
<tr>
<td>Total chloride response</td>
<td>24</td>
<td>32.7 ± 14.0</td>
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Figure 1: Time course of the mean relative change (+/- SEM) in potential after changing the temperature of the solutions during perfusion with isoprenaline (n=16 patients from one site): from room temperature to warm (A) and from warm to room temperature (B).
Figure 2: Individual differences between NPD parameters measured with RT and W solutions in the same subjects. Line depicts mean difference.
References


