EFFECT OF STORAGE ON CHOLINERGIC CONTRACTIONS OF HUMAN BRONCHI IN VITRO

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Cholinergic control is a predominant regulator of human airway tone (Norel et al., 1993). Since human tissue is difficult to obtain for experimental use, when available, it is essential to take advantage of as much tissue as possible. The aim of this study was to examine the effect of cold storage on cholinergic contractions of human bronchi.

Human lung tissue was obtained from patients undergoing surgery for lung carcinoma. Bronchial tissues were used either on the day of surgery (D0) or after storage at 4°C in Krebs-Henseleit solution for one (D1) and two (D2) days, respectively. Bronchial preparations were cut into rings (diameter: 3-6 mm) and set up at a resting tension of 30 mN in 10 ml organ baths containing Krebs-Henseleit solution gassed with 5% CO₂ in O₂ at 37°C. Electrical Field Stimulation (EFS) was performed using platinum electrodes positioned on both sides of the preparations. Biphasic square-wave impulses with a 2 ms duration were delivered at 60 V for 10 s, followed by bath fluid change and a 4 min equilibration period before the next stimulation. For each stimulation a different frequency (4, 10 or 60 Hz) was used. The initial contraction induced by EFS depends on endogenous acetylcholine (ACh) release (De Jongste et al., 1987). At the end of the protocol a single concentration (100 µM) of ACh was added to the baths. Changes in isometric force were recorded using Narco F-60 transducers and Linseis 2016 polygraphs.

Contractions (mean±s.e.m.) are expressed in mN. Statistical evaluation was performed using a one- or two-way analysis of variances (ANOVA), followed by Dunnett’s test. * indicates data significantly different (P<0.05) versus D0 and D1.

The contractions induced by exogenous ACh (100 µM) were 13.7±3.4 mN (D0; n=9). Following storage these contractions were 15.7±1.4 mN (n=13) and 5.9±1.0* mN (n=8) for D1 and D2, respectively. The EFS initial contractions are shown in Table 1. There was a significant decrease in reactivity on D2 compared with either D0 or D1, whereas no difference was observed between D0 and D1. In presence of atropine (1 µM; 30 min; n=3) the contractions were 0±0 mN, 0±0 mN and 2.6±1.5 mN on D0 and 0±0 mN, 0±0 mN and 2.4±0.3 mN on D1, at 4, 10 and 60 Hz, respectively. This significant inhibition of the EFS initial contractions confirms the release of ACh.

In conclusion, cholinergic contractions of human bronchi can be evaluated on D0 or D1 following surgery without major changes in reactivity.

Table 1 Contractions (mN) induced by EFS.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>n</th>
<th>D0</th>
<th>D1</th>
<th>D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Hz</td>
<td>4</td>
<td>10.8±7.0</td>
<td>7.5±4.4</td>
<td>0.8±0.5</td>
</tr>
<tr>
<td>10 Hz</td>
<td>4</td>
<td>18.6±7.0</td>
<td>11.8±5.0</td>
<td>2.0±0.8*</td>
</tr>
<tr>
<td>60 Hz</td>
<td>4</td>
<td>26.5±5.6</td>
<td>26.5±5.6</td>
<td>8.4±1.2*</td>
</tr>
</tbody>
</table>

n indicates the number of different lung samples used.