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Effect of histamine on the electrophysiology of the human parietal pleura

V.K. Kouritas[a,*], A. Tsantsaridou[b], K. Tepetes[c], N. Tsilimingas[b], K.I. Gourgoulianis[d], P.A. Molyvdas[a], C. Hatzoglou[a]

[a] Deparment of Physiology, Larissa Medical School, University of Thessaly, Greece
[b] Department of Cardiothoracic Surgery, Larissa University Hospital, Greece
[c] Department of Surgery, Larissa University Hospital, Greece
[d] Department of Thoracic Diseases, Larissa University Hospital, Greece

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ABSTRACT

Introduction: Histamine is involved in the pathogenesis of numerous diseases and regulates the permeability of different tissues. The aim of this study is to investigate the effects of histamine on the electrophysiology of human parietal pleura and the underlying mechanisms involved.
Materials and methods: Pleural specimens were obtained from patients subjected to thoracic surgery and were mounted in Ussing chambers. Histamine solutions (1 μM to 1 mM) were applied in native and pretreated specimens with dimetindene maleate, cetirizine, ranitidine, amiloride and ouabain. Trans-mesothelial resistance was determined (R_{TM}).
Results: Histamine induced a rapid R_{TM} increase on the mesothelial ($p = 0.008$) and a decrease on the interstitial surface ($p = 0.029$). This effect was dose-dependent and was totally abolished by dimetindene maleate, cetirizine and amiloride and partially by ranitidine and ouabain.
Conclusions: Histamine induces acute electrochemical changes in human pleura mainly via interaction with the H_1 and partially with the H_2 histamine receptors. It also interferes with trans-cellular permeability and therefore may participate in pleural fluid recycling.

1. Introduction

Histamine, following its release by the mast cells, regulates numerous functions in epithelial cells and is involved in the pathogenesis of many diseases (Flynn et al., 2009). These effects are considered to be induced by histamines' interaction with its receptors, namely the H_1 and/or H_2 histamine receptors (Flynn et al., 2009; Downing et al., 1999).

Furthermore, it is now commonly demonstrated that histamine can acutely regulate trans-cellular transportation either via cellular transporters or via the paracellular pathway, influencing the tight junctions of adjacent cells in various epithelial tissues (Flynn et al., 2009; Zabner et al., 2003; Devalia et al., 1994). The aforementioned effect of histamine is considered to be one of the underlining pathophysiologic mechanisms by which clinical manifestations appear following its release (Flynn et al., 2009; Goldie and Pedersen, 1995).

Despite the fact that histamine is believed to interfere with the formation of pleural exudates (Lo et al., 1985; Kikuchi et al., 1996) an explanation for this effect has not yet been provided.

The aim of the present study is to investigate the effects of histamine on the electrophysiological profile of human parietal pleura and to identify the receptors involved. In addition, we examine the role of cellular ion transporters in this effect.

2. Materials and methods

2.1. Materials

Intact sheets of human parietal pleura were obtained from forty (40) patients, who underwent thoracic surgery for lung cancer (via thoracotomy or thoracoscopy). The pleural specimen was obtained from an area distant to the lung tumor. A piece of each dissected specimen was sent for histopathological examination. All specimens included in the study were proven to be free of any disease as per histopathological report. Patients with pleural effusion preoperatively were excluded from the study. After its dissection, the remaining specimen was placed in Krebs (KRB) solution, pre-oxygenated at 95% O_2–5% CO_2 and cooled to 4 °C, and was transferred to the laboratory within 30 min.

The study was approved by the Local Ethics Committee and informed signed consent was obtained from all the patients participating in the study.

2.2. Methods

The KRB solution used throughout the whole study was balanced at pH 7.45 and contained 117.5 mM NaCl, 1.15 mM NaH_2PO_4, 24.99 mM $NaHCO_3$, 5.65 mM KCl, 1.18 mM $MgSO_4$, 2.52 mM $CaCl_2$ and 5.55 mM glucose.

The pleural surface facing the pleural cavity in vivo will be referred to as the mesothelial surface, whereas the surface that faces the chest wall will be referred to as the interstitial surface.

* Corresponding author at: Department of Physiology, Medical School of Thessaly, New Buildings, Mezourlo 41100, PO Box 1400, Greece.
Tel.: +30 2410 685655/697 440 5281; fax: +30 2410 670 240.
E-mail address: vasileios@doctors.org.uk (V.K. Kouritas).

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The pleural specimens were mounted as planar sheets of tissue in Ussing–type chambers (model DVC-1000, World Precision Instruments, Sarasota, Florida, USA), were bathed in Krebs solution on both sides and perfused continuously with 95% O_2–5% CO_2 gas mixture, heated to 37 °C, in order to ensure tissue viability (Hatzoglou et al., 2001; Kouritas et al., 2007, 2008, 2009, 2010a).

Following mounting, the pleural specimens were equilibrated for 30 min. Trans-mesothelial Potential Difference (PD) was then measured for 30 min, with or without application of current of variable intensity (0 ± 400 μA) (Hatzoglou et al., 2001; Kouritas et al., 2007, 2008, 2009, 2010a), constituting the control potential difference. Fifty control experiments were performed.

Following the equilibration period and control measurements, histamine solutions (1 μM to 1 mM, Sigma Chemicals Co., USA) were added sequentially on both surfaces in order to investigate their effects on pleural tissue ($n = 7$ experiments for each concentration, $n = 7$ experiments for each side). The non-specific histamine receptor antagonist dimetindene maleate (Fenistil®, Novartis), the specific and long acting H_1 histamine receptor antagonist cetirizine hydrochloride (Zirtek®, U.C.B.) and the specific H_2 histamine receptor antagonist ranitidine hydrochloride (Zantac®, GlaxoSmithKline) were used at concentrations ranging from 1 μM to 1 mM in order to elucidate the role of histamine receptors in the effect induced by histamine on pleura. The specimens were pretreated with the aforementioned anti-histaminic agents for at least 30 min prior to the addition of 10 μM histamine ($n = 7$ experiments for each drug and $n = 7$ experiments for each side). In another group of experiments, specimens were pretreated with the Na^+ channel blocker amiloride (Sigma Chemical Co., USA, 10 μM, Kouritas et al., 2008, 2009) and the Na^+/K^+ pump inhibitor ouabain (Sigma Chemical Co., USA, 10 μM, Kouritas et al., 2008, 2009) for at least 30 min before the addition of 10 μM histamine, in order to clarify whether ion cellular transporters take part in the histamine effect on human pleura ($n = 7$ experiments for each drug and $n = 7$ for each side). In total, 126 experiments were conducted.

In order to ensure that the recorded results were due to drug action and not due to mechanical perturbation while emptying and re-filling the chambers, the experiments were conducted using solely Krebs solution (data not shown as no electrical changes in PD were observed). The PD following electrical stimulation with application of current (0 ± 400 μA) was measured 1, 5, 10, 30 and 60 min following the addition of each solution. All solutions were freshly prepared before every experiment, heated to 37 °C and continuously perfused with 95% O_2–5% CO_2 gas mixture.

Trans-mesothelial Resistance (R_{TM}) was calculated from the PD (Hatzoglou et al., 2001; Kouritas et al., 2007, 2009).

2.3. Analysis

Statistical analysis was performed using the statistical package SPSS ver. 10.00 for Windows (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). Data are expressed as Mean R_{TM} ($\Omega\,cm^2$) ± standard error of mean (S.E.) or as net R_{TM} change (dR_{TM}) within 1st minute (calculated by subtracting the mean R_{TM} from the mean control values for each pair of measurements). Statistical significance was determined by paired t-test. Comparison among groups was performed by one-way ANOVA (Bonferoni's *post hoc*). p values less than 0.05 were considered significant.

3. Results

3.1. Effects of histamine

The addition of histamine on the mesothelial surface increased the R_{TM} from 19.23 ± 0.7 Ω cm² to 21.58 ± 0.7 Ω cm² ($p = 0.008$ vs. control, Fig. 1a) within 5 min and remained above the control level thereafter. When added on the interstitial surface, histamine induced a decrease in R_{TM} from 19.23 ± 0.7 Ω cm² to 17.4 ± 0.8 Ω cm² ($p = 0.029$ vs. control, Fig. 1b) within 5 min and remained at these levels until the end of the experiments. The R_{TM} change on the mesothelial surface was 2.35 ± 0.6 Ω cm² compared to 1.83 ± 0.7 Ω cm² on the interstitial surface ($p = 0.048$ between the two surfaces). This increase in R_{TM} was dose-dependent and was greater when higher concentrations of histamine were used. The least effective concentration was 10 μM (Fig. 2a and b).

3.2. Effect of histamine receptor antagonists

The addition of dimetindene maleate, cetirizine or ranitidine at concentrations ranging between 1 μM and 1 mM had no effect on the R_{TM} when added on the mesothelial or the interstitial surface of the specimens (Fig. 3a and b).

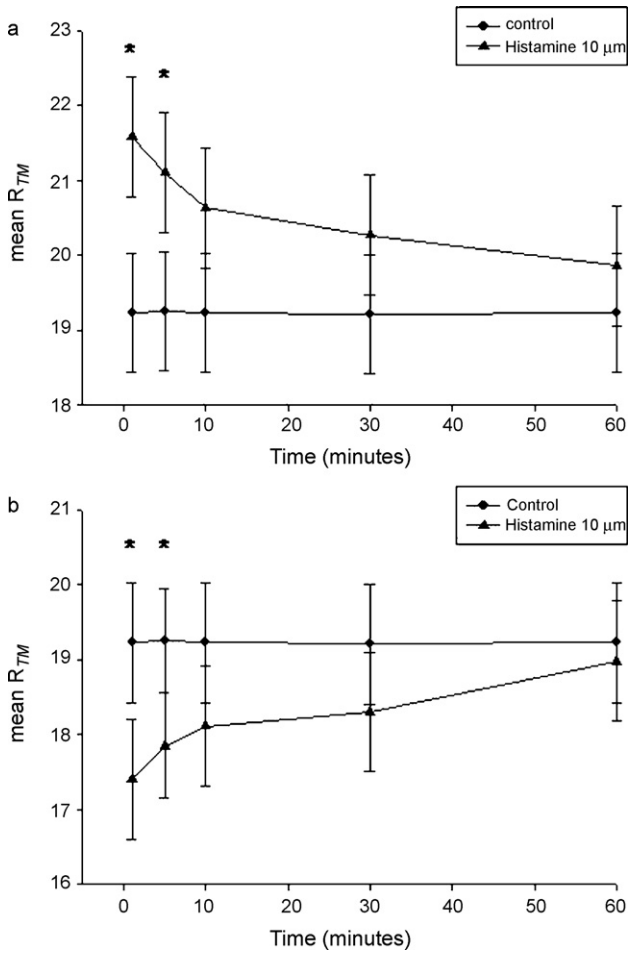

Fig. 1. Effect of HISTAMINE 10 μM on the trans-mesothelial resistance (R_{TM}) when added on the mesothelial (a) and interstitial (b) surface of human parietal pleura, by time. Values are expressed as mean trans-mesothelial resistance R_{TM} ($\Omega\,cm^2$) ± standard error of mean; $n = 7$ experiments. *$p < 0.05$ vs. control.

3.3. Effect of anti-histamine agents on the histamine-induced electrochemical changes

In specimens pretreated with dimetindene maleate the histamine-induced effect was totally inhibited at a lowest concentration of 10 μM, with the addition of histamine on the mesothelial (from 19.23 ± 0.7 Ω cm² to 19.28 ± 0.7 Ω cm², $p > 0.05$ vs. control and $p = 0.018$ vs. histamine, Fig. 4a) or on the interstitial side (from 19.23 ± 0.7 Ω cm² to 19.09 ± 0.8 Ω cm², $p > 0.05$ vs. control and $p = 0.038$ vs. histamine, Fig. 4b) of the specimens.

In tissues pretreated with cetirizine, again, the histamine-induced effect was inhibited at a lowest concentration of 10 μM, with the addition of histamine on the mesothelial (from 19.23 ± 0.7 Ω cm² to 19.14 ± 0.7 Ω cm², $p > 0.05$ vs. control and $p = 0.01$ vs. histamine, Fig. 4a) or on the interstitial surface (from 19.23 ± 0.7 Ω cm² to 19.20 ± 0.8 Ω cm², $p > 0.05$ vs. control and $p = 0.01$ vs. histamine, Fig. 4b) of the specimens.

In specimens pretreated with ranitidine at a lowest concentration of 10 μM, histamine induced a weaker effect when compared to the effect observed when added on non-pretreated tissues; ranging from 19.23 ± 0.7 Ω cm² to 20.84 ± 0.6 Ω cm² ($p = 0.001$ vs. control, Fig. 4a) with the addition of histamine on the mesothelial side and from 19.23 ± 0.7 Ω cm² to 18.22 ± 0.8 Ω cm² ($p = 0.003$ vs. control, Fig. 4b) when added on the interstitial surface. This effect had the tendency to reach statistical significance when compared