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Perspective

# Histamine type 1-receptor activation by low dose of histamine undermines human glomerular slit diaphragm integrity

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ABSTRACT

Histamine has been reported to decrease the ultrafiltration coefficient, which inversely correlates with glomerular permselectivity, however the mechanism(s) underling this effect have never been investigated. This study aimed to assess whether histamine could exert a direct detrimental effect on podocyte permeability and the possible involvement of two key proteins for the glomerular slit diaphragm (SD) integrity, zonula occludens-1 (ZO-1) and P-cadherin.

The effect of histamine (100 pM–1000 nM) on coloured podocytes junctional integrity was evaluated functionally by a transwell assay of monolayer permeability and morphologically by electron microscopy. Histamine receptor ($H_{1\text{-}4}R$) presence was evaluated at both mRNA (RT-PCR) and protein (immunofluorescence) levels. The $K_d$ and $B_{max}$ values for [$^3$H]mepyramine were determined by saturation binding analysis; $IP_1$ and cAMP production evoked by histamine were measured by TR-FRET. ZO-1, P-cadherin and vimentin expression was assessed by qRT-PCR and quantitative immunoblotting.

Histamine elicited a time- and sigmoidal dose-dependent (maximum effect at 8 h, 10 nM) increase in podocyte paracellular permeability widening the paracellular spaces. Only $H_1R$ was predominantly localised to the podocyte membrane. Consistently, histamine elicited a sigmoidal dose-dependent increase in $IP_1$, but not in cAMP. Histamine exposure evoked a concentration-dependent reduction in both ZO-1 and P-cadherin and a parallel induction of vimentin mRNA expression with a maximum effect after 6 h, and protein expression with a maximum effect after 8 h. These effects were prevented by the selective $H_1R$ antagonist chlorpheniramine.

In conclusion, our data demonstrate that histamine, via the $H_1R$, modifies SD morphological and functional integrity, in part, by decreasing the expression of ZO-1 and P-cadherin.



*Abbreviations:* cAMP, cyclic adenosine monophosphate; CDH3, cadherin 3 type 1 P-cadherin gene; ER, endoplasmic reticulum; FITC, fluorescein; GADPH, glyceraldehyde 3-phosphate dehydrogenase gene; GBM, glomerular basement membrane; $H_{1\text{-}4}R$, histamine receptor $_{1\text{-}4}$ subtypes; $K_f$, ultrafiltration coefficient; $IP_1$, inositol monophosphate; $IP_3$, inositol 1,4,5-trisphosphate; PAN, puromycin aminonucleoside; qRT-PCR, quantitative real-time PCR; SD, slit diaphragm; TBP, TATA-binding protein; TJP1, tight junction protein 1 gene; TR-FRET, Time-Resolved Fluorescence Resonance Energy Transfer; VIM, vimentin gene; ZO, Zonula Occludens.

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## 1. Introduction

Histamine is a pleiotropic vasoactive amine, whose pathogenic role in microvascular endothelial paracellular permeability has been extensively studied [1–7]. Most of these studies describe acute events (within seconds to minutes), resulting in a rapid transient increase in permeability, due to a rapid formation of endothelial gaps [2–7]. Moreover, histamine has been suggested to be involved in prolonged vascular leakage by reducing Zonula Occludens (ZO)-1 protein expression in cultured retinal microvascular endothelial cells within hours [8]. The work by Takeuchi et al. suggested that histamine-induced paracellular permeability might be also extended to other epithelial cells. In particular, histamine was shown to significantly downregulate ZO-1 mRNA expression in cultured human nasal epithelial cells [9]. ZO-1 contributes to the functional integrity of different permeability barriers among which the glomerular filter is one [10]. Interestingly, histamine has been previously reported to decrease the ultrafiltration coefficient ($K_f$) [11]. Thus, our hypothesis was that histamine could modify $K_f$ by regulating ZO-1 expression in the podocyte.

Podocytes are parenchymal cells known to be highly dynamic and terminally differentiated. They interact with the glomerular basement membrane (GBM) and communicate through various signalling pathways at the slit diaphragm (SD). The glomerular SD represents the junction structure that links the interdigitating foot processes from neighbouring podocytes and consists of transmembrane-bridging proteins networking with a juxtaposed cytoplasmic platform of protein complexes, which in turn is linked to the actin cytoskeleton [12]. Within this cytoarchitecture, ZO-1 protein is located at the cytoplasmic face of the SD [13] and has been accepted to be one of its functional molecules; a disrupted interaction and distribution of ZO-1 in podocytes results in loss of SD structure and function [14–16]. Besides the transmembrane protein, P-cadherin, a podocyte specific adhesion protein [17] localised on adherens-type junctions, mediates calcium-dependent cell–cell bonds and its loss are recognised as a cause of barrier filtration integrity impairment [18]. Therefore, it is likely that glomerular injury affecting ZO-1 and/or P-cadherin results in loss of SD structure, podocyte detachment, $K_f$ reduction and in a subsequent impairment of the filtration barrier integrity with proteinuria, progressive renal damage and eventual loss of renal function [12,19,20].

Among the histamine receptor subtypes, $H_{1\text{-}4}R$, $H_1R$ and $H_2R$ were first described in mammalian glomeruli [21–23], but these studies were focused on the entire glomerulus or only on stromal cells such as mesangial cells; little is known about parenchymal cells. Indeed, the data on histamine receptors expression on renal parenchymal cells arise only from our recent observations of $H_1R$, $H_2R$, $H_3R$ and $H_4R$ on tubular epithelial cells [24–26]. However, no such studies have to date focused on podocytes.

Thus, the present study was designed to investigate whether histamine could exert a direct detrimental effect on podocyte permeability compromising SD functional integrity, the underlying histamine receptor pharmacology, and the possible involvement of two key SD-associated proteins ZO-1 and P-cadherin.

## 2. Materials and methods

### 2.1. Materials

All reagents and chemicals used were from Sigma–Aldrich (St. Louis, MO) unless otherwise noted. Cell media and reagents were from Lonza group Ltd. (Allendale, NJ, USA). Hans Balanced Salt Solution was from GIBCO (Grand Island, NY). HTS Transwell inserts were from Corning Life Sciences (Lowell, MA). RevertAid™ First Strand cDNA Synthesis Kit, GeneRuler™ 50 bp DNA Ladder, DNA Gel Loading Dye (6×), CellMask™ Orange plasma membrane stain, MagicMark™ XP Western Protein Standard and Alexa-Conjugated secondary antibodies donkey anti-Mouse IgG (A-31570), chicken anti-Goat IgG (A-21469) and goat anti-Rabbit IgG (A-11034) were from Thermo Fisher Scientific Inc. (Rockford, IL, USA). EuroTaq DNA polymerase as well as EuroGOLD Trifast™ were from Euro-clone (Milan, Italy). High Capacity cDNA Reverse Transcription Kit and Power SYBR Green PCR Master Mix were from Applied Biosystems (Foster City, CA). Sequence-specific oligonucleotide primers were purchase from Sigma-Genosys (Milan Italy). The antibodies for histamine $H_1R$ (H300, sc-20633), $H_2R$ (A20, sc-33973), calnexin (AF18, sc-23954), ZO-1 (C-19, sc-8146), P-cadherin (H-105, sc-7893), vimentin (C20, sc-7557) and UltraCruz™ Autoradiography Film were purchased from Santa Cruz Biotechnology Inc. (Dallas, TX, USA), while anti-rabbit and anti-mouse IgG HRP-linked antibodies were from Cell Signaling Technology, Inc. (Danvers, MA, USA) and the swine anti-Goat IgG antibody from Cedarlane Labs (Ontario, Canada). The LANCE® Ultra cAMP Detection Kit, the IP-One HTRF® assay kit, the [$^3$H]mepyramine [PubChemCID 656400; kindly provided by Prof. Rob Leurs (20 Ci/mmol) VU University Amsterdam, Amsterdam] and the Whatman™ GF/C Glass Fiber Filter Paper were from PerkinElmer Inc. (Waltham, MA, USA) and Cisbio Bioassays (France), respectively. Precision Plus Protein™ Dual Color Standards and BCA protein assay were from Pierce Bio-technology Inc. (Rockford, IL, USA) and PVDF membrane from Millipore (Bradford, MA, USA). Visiglo™ HRP chemiluminescent substrate kit was purchased from Amresco llc. (Solon, OH, USA).

Histamine dihydrochloride (PubChem CID 5818), (±) chlorpheniramine maleate (PubChem CID 5281068), [$^3$H]mepyramine and diphenhydramine (Pubmed CID 3100) were dissolved in dimethyl sulfoxide, and the final drug concentrations were obtained by dilution of stock solutions in the experimental buffers. The final concentration of the organic solvent was less than 0.1%, which had no effect on cell viability.

### 2.2. Cell cultures

Immortalised human podocytes were obtained from the respective primary cells, derived from the normal portion of cortex surgically removed kidneys (n = 5) for as described previously [27], by infection with a hybrid Adeno5/SV40 virus as previously described [27–29]. The line was generated in 1997, after the authorization of the local Ethical Committee (Hospital San Giovanni Battista "Molinette", Turin, Italy). Podocytes were isolated from the healthy tissue derived from kidney samples of patients who underwent unilateral nephrectomy due to local renal carcinomas as first-line treatment. To our knowledge, no other relevant pathology was diagnosed in the medical history of each patient enrolled and the derived podocytes can be reasonable assumed as healthy podocytes. Podocytes were characterized for the positive expression of nephrin, podocin, and synaptopodin and for negative expression of von Willebrand factor, CD31, and smooth muscle cell actin. Cells were cultured in DMEM containing 4.5 mg/l glucose supplemented with 10% Fetal Calf Serum, penicillin/streptomycin (100 IU/ml), and l-glutamine and the cultures were maintained at 37 °C in a 95% air/5% $CO_2$ humidified incubator.

### 2.3. Permeability assay

Podocyte monolayer permeability was determined as previously described [30,31]. Human immortalized podocytes (40,000 cells well, 500 μl) were seeded on the top of HTS Transwell inserts (3 μm pore, 24-well plate) and cultured till confluence was achieved. Cells were washed twice with PBS supplemented with