H1 Histamine Receptor Affects the Integrity of Glomerular Slit Diaphragm

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Podocytes are highly dynamic and terminally differentiated cells that interact with the glomerular basement membrane (GBM) and communicate through signalling at the slit diaphragm (SD), playing an essential role for maintenance of an intact glomerular filtration barrier (Pavenstadt et al., 2003). The glomerular SD represents the junction structure linking the interdigitating podocytes foot processes (Lee et al., 2006). Its function is guaranteed by both tight (mostly zonula occludens-1, ZO-1) and P-cadherin-based adherens junctions. Previously, histamine has been demonstrated to affect the ultrafiltration coefficient (kf) (Ichikawa & Brenner, 1979), suggesting a direct effect of the amine on the integrity of the SD.
However this hypothersis has never been fully investigated.
This study was aimed to evaluate whether histamine affects ZO-1 and P-cadherin expression in human immortalized podocytes.
The gene and protein expression of the four histamine receptors was evaluated by RT-PCR and immunofluorescence; co-localization experiments were performed by confocal analysis using calnexin and lamin A/C as marker of endoplasmic reticulum and of nuclear envelope, respectively, while cell mask stain as marker of the plasma membrane. Pharmacological identification of histamine receptors was performed measuring, by TR-FRET, second messenger, both IP3 and cAMP, production evoked by histamine alone (3 pM-10 nM) or in presence of selective antagonists: chlorpheniramine (H_1R), ranitidine (H_2R), GSK189254 (H_3R) and JNJ7777120 (H_4R). The effect of histamine on ZO-1, P-cadherin and vimentin expression was assessed through Real Time RT-PCR and immunoblotting. Finally, the integrity of the SD was evaluated by electron microscopy.
Among the four histamine receptors, only H_1R and H_4R were detected, although only H_1R was predominantly localized on the membrane. Consistently with this observation, histamine did not affect cAMP production, while elicited a sigmoid dose-response curve for IP3 in the range 3 pM-10 nM; the pre-treatment for 10 min with the selective H_1R antagonist chlorpheniramine 10 μM shifted the curve evoked by histamine to the right handside.
Histamine exposure evoked a concentration-dependent reduction of both ZO-1 and P-cadherin and a parallel induction of vimentin gene expression with a maximum after 6h (-50±2.5%, -54±3.1%, +40±5.6%) and protein expression with a maximum after 8h (-60±3.0%, -80±1.96%, +100±10.06%).
All these effects were prevented by the selective H_1R antagonist chlorpheniramine, but not by the H_2R antagonist ranitidine or the H_4R antagonist JNJ7777120 (10 μM).
These observations were strongly validated when the junction integrity was observed by electron microscopy: histamine exerts a detrimental effect on SD prevented by H_1R selective antagonist chlorpheniramine maleate.
In conclusion, our data demonstrate that histamine exerts through H_1R a detrimental direct effect on the GSD integrity by decreasing both ZO-1 and P-cadherin expression, thus probably promoting an epithelial-mesenchimal transition, as suggested by the increase in vimentin expression.

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