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### **H<sub>3</sub> receptor renal expression in normal and diabetic rats.**

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## **Abstract**

### *Introduction*

In order to extend our previous observation of H<sub>4</sub>R upregulation in the kidney of diabetic rats, we evaluated in the same specimens the presence of the H<sub>3</sub>R.

### *Materials and methods*

Kidney specimens from 24 8-week-old male Wistar rats (12 non-diabetic and 12 diabetic animals) were processed for both immunohistochemical and immunofluorescence analyses.

### *Results and conclusion*

H<sub>3</sub>R is expressed in the apical membrane by collecting duct cells in the kidney of rats and it is significantly increased in diabetic animals. These data support the hypothesis that H<sub>3</sub>R could also mediate non-neuronal histamine effects, suggesting its involvement in fluid homeostasis.

**Key Words** histamine H<sub>3</sub> receptor, histamine, kidney, diabetes, collecting ducts

## **Introduction**

Recently, our group demonstrated the presence of the histamine H<sub>4</sub> receptor (H<sub>4</sub>R) in resident renal cells of the loop of Henlé and its profound upregulation in the kidney of diabetic rats[1]. This observation adds to independent evidence of a role for histamine in renal (patho)physiology. In healthy subjects the administration of loading doses of L-histidine led to an increase of histamine concentration in urine. In renal transplant patients the urinary and blood levels of histamine are elevated. In the kidney of diabetic rats, histamine was increased compared with controls[2]. Basically, histamine has been reported to regulate the renal microcirculation, to increase salt and water excretion[3-5], decrease the ultrafiltration coefficient by reducing the total filtration surface area[4], and increase renin release[6].

The aim of this study was to extend our previous observation on H<sub>4</sub>R in healthy and diabetic rats evaluating in the same specimens the renal expression of H<sub>3</sub>R.

## **Materials and Methods**

Animals, protocols, diabetes induction, biochemical and histological parameters have been previously reported[1]. Conventional immunohistochemical procedures were employed as described. Immunoperoxidase staining for H<sub>3</sub>R was performed on 5 µm kidney sections for formalin-fixed tissue from 24 male 8-week-old Wistar rats (12 non-diabetic and 12 diabetic animals). Renal sections were incubated overnight with anti-H<sub>3</sub>R (349–358) (2 µg/ml)[7], followed by a three-layer streptavidin–biotin–peroxidase complex staining method. Photomicrographs were acquired randomly with a digital camera connected to a light microscope equipped with a x40 objective (Leica DM750). Images were processed by ImageJ 1.41 (NIH, USA) software and quantified using the Color Deconvolution image analysis tool. The percentage area was calculated for H<sub>3</sub>R-positive tissue. Values are mean ± SEM of the optical density (in arbitrary units) measurements of individual rats (ten images/zone each) from the different experimental groups. For immunofluorescence and confocal analysis, after antigen retrieval and blocking, kidney sections were incubated with primary anti-H<sub>3</sub>R and anti-AQP2, antibody, followed by incubation with corresponding Alexa Fluor secondary antibodies. After counterstaining with DAPI, photomicrographs were obtained by Apotome systems (Zeiss) at x40 objective. The percentage of positive cells for H<sub>3</sub>R and AQP2 was determined by ImageJ 1.41 software. Values are expressed as mean ± SEM positive cell/collecting duct percentage of individual rats (ten images/zone each) from the different experimental groups.

To confirm the absence of false staining, tissue was also screened in the absence of primary antibodies and following pre-incubation with (349-358) peptide (data not shown). All sections were immunostained in a single session to minimize artifactual differences.

## Results

The immunohistochemical analysis (Fig. 1A) revealed a clear H<sub>3</sub>R-like immunoreactivity in control rats, predominantly in the renal medulla and the papilla. In contrast, in the cortex the immunoreactivity was generally very low, and no signal was detected in the glomeruli. Notably, when diabetic and non-diabetic animals were compared, a significant increase in immunoreactivity, suggestive of H<sub>3</sub>R receptor upregulation, was observed (Fig 1B).

The above data, indicating a higher H<sub>3</sub>R-like immunoreactivity in the apical membrane of epithelial tubular cells mostly located in medulla and papilla, suggested a collecting duct expression profile for the receptor. To confirm this result, immunofluorescence co-staining was performed with AQP2, expressed in the apical membrane by collecting duct cells in the kidney. As shown in Fig. 1C and Fig. 1D, most of the H<sub>3</sub>R positive cells were also positive with AQP2, consistent with a predominant H<sub>3</sub>R expression at the apical membrane of the epithelial cells of the collecting ducts.

## Discussion

We demonstrate for the first time that the H<sub>3</sub>R is mainly expressed in the apical membrane by collecting duct cells of the rat, and that this protein is significantly upregulated in the kidneys of diabetic animals. Our histochemical data add the collecting duct cells to the growing list of non-neuronal H<sub>3</sub>R-expressing cells already reported[7-9], thus providing further evidence for a role of H<sub>3</sub>R in mediating non-neuronal histamine effects. The collecting duct plays a pivotal role in kidney function and homeostasis by regulating ions and water transport. AQP2 abundance in the apical membrane is strictly involved in the mechanisms for regulation of water reabsorption and urine concentration[10]. The ability to concentrate urine is impaired in conditions such as diabetes insipidus, Histamine, whose levels have been reported to be increased in the kidney of diabetic animals[2], has been shown to increase salt and water excretion[8-10]. Consistent with the observation that histamine induces AQP4 internalization in human AQP4-expressing gastric cells[11], we could speculate that H<sub>3</sub>R may subserve an AQP2 trafficking role in renal duct cells. Indeed, It is known that Gi-coupled GPCRs, of which H<sub>3</sub>R is a member, are required for cAMP-triggered trafficking of AQP2[12]. Herein, we observed that all the diabetic animals, which have shown renal damage[1], expressed profoundly higher levels of H<sub>3</sub>R, thus suggesting a strong association. Notably, data obtained on human tubular cells suggested a similar H<sub>3</sub>R intranephron expression. Therefore, we could speculate that our data on diabetic rats could have a positive translation to diabetic patients. However, whether this receptor has a compensatory or pathological implication and its role as a pharmacological target in diabetic nephropathy remains to be established.

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**Fig.1 H<sub>3</sub>R renal expression.** Representative micrographs of transverse kidney sections, immunolabelled with specific anti-H<sub>3</sub>R antibody (A). Quantitative assessment of H<sub>3</sub>R expression in the kidney; results are the mean  $\pm$  SEM of the optical density (arbitrary units) of individual rats (ten images/ zone) performed in duplicate. Data were analyzed by one-way analysis of variance (ANOVA) and the Student–Newman–Keuls test. \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs. control (B). Immunofluorescence double-staining showing both H<sub>3</sub>R (green) and AQP2 (red)-positive cells (C). Quantitative H<sub>3</sub>R- and AQP2-positive cells; results are the mean  $\pm$  SEM of positive cell/collecting ducts percentage of individual rats (ten images/zone) performed in duplicate (D).

