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Original article

Histamine transport and metabolism are deranged in salivary glands in Sjögren's syndrome

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Abstract

Objective. To study histamine transport and metabolism of salivary gland (SG) epithelial cells in healthy controls and SS patients.

Methods. Enzymes and transporters involved in histamine metabolism were analysed in cultured human submandibular salivary gland (HSG) epithelial cells and tissue sections using quantitative real-time PCR and immunostaining. HSG cells were used to study [^{3}H]histamine uptake [(±1-methyl-4-phenylpyridinium (MPP)] and efflux by liquid scintillation counting.

Results. mRNA levels of L-histidine decarboxylase (HDC) and histamine-*N*-methyltransferase (HNMT) were similar in the control and SS glands, but diamine oxidase was not expressed at all. Organic cation transporter 3 (OCT3) in healthy SG was localized in the acinar and ductal cells, whereas OCT2 was restricted to the myoepithelial cells. Both transporters were significantly decreased in SS at mRNA and protein levels. OCT3-mRNA levels in HSG cells were significantly higher than those of the other studied transporters. Uptake of [^{3}H]histamine was inhibited by MPP in a time-dependent manner, whereas [^{3}H]histamine-preloaded HSG cells released it.

Conclusion. Ductal epithelial cells are non-professional histamine-producing cells able to release histamine via OCTs at the resting state up to ~100 nM, enough to excite H_3R/H_4R^+ epithelial cells, but not H_1R, which requires burst release from mast cells. At the stimulated phase, 50–60 μM histamine passes from the interstitial fluid through the acinar cells to saliva, whereas uptake by ductal cells leads to intracellular degradation by HNMT. OCT3/histamine/H_4R-mediated cell maintenance and down-regulation of high histamine levels fail in SS SGs.

Key words: Sjögren's syndrome, salivary glands, histamine receptor, histamine, organic cation transporters.

BASIC SCIENCE

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Introduction

Histamine is a widely distributed biogenic amine that is predominantly synthesized by mast cells, basophils and enterochromaffin-like cells, which produce histamine at a high rate, store it in granules and release it in a regulated manner in bursts so that very high local histamine concentrations are momentarily reached [1]. The novel presynaptic H3 receptor (H_3R) is best known for its ability to regulate neurotransmitter release, whereas H4 receptor (H_4R) linked to immune responses and autoimmune diseases is predominantly expressed in antigen-presenting dendritic cells [2–4] and T and B lymphocytes [5, 6].

The novel histamine receptors have almost 10 000-fold higher affinity for histamine than conventional histamine

receptors (e.g. pK_i is 8.3 for H_4R and 4.2 for H_1R) [7, 8]. Resting plasma histamine 3–8 nM and salivary histamine 3–112 nM can stimulate the novel histamine receptors, almost half maximally. Burst release is required to reach, for example, concentrations of 50–60 μM to stimulate conventional low-affinity histamine receptors. Therefore, during the resting or maintenance phase, low histamine concentrations act at high-affinity receptors, whereas regulated burst release is required to engage the conventional histamine receptors.

H_4R is found in healthy human salivary gland (SG) epithelial cells but is highly decreased in SS [9]. It was therefore analysed if SG epithelial cells contain any non-professional histamine-producing cells, with a potential to produce histamine and via some basal or constitutive release/transport mechanism maintain low histamine concentrations, which are only able to regulate target cells equipped with the novel, high-affinity H_3R or H_4R in an autocrine and paracrine mode, but too low to activate the classical low-affinity receptors. After local mast cell-mediated burst release, the high histamine concentration acts on H_1R/H_2R, which needs to be down-regulated to return to the resting phase. Therefore the histamine degrading potential of SGs was also analysed. Due to the involvement of H_4R in immune responses and in SS [9], the histamine synthesizing, transporting and metabolizing capacity was also analysed in SS, an autoimmune epitheliitis [10], because this has potential relevance for the resting phase, when the non-professional cells and novel high-affinity histamine receptors play a major role, and for the activation phase, when the professional histamine-releasing cells and the conventional low-affinity histamine receptors play a major role.

Earlier only mast cell-derived high micromolar histamine levels and the conventional low-affinity H_1R and H_2R were considered to play in histamine-mediated physiology in healthy individuals and in pathophysiology in patients with SS. However, more recently a novel high-affinity histamine receptor, H_4R, responsive to nanomolar concentrations of histamine was described [11]. Furthermore, non-professional histamine-producing cells containing low-activity histamine-synthesizing 74 kDa HDC enzyme, able to produce and modulate such low nanomolar concentrations, were also described. These non-professional histamine-producing cells include antigen-presenting dendritic cells and lymphocytes, which may play a role in focal sialadenitis in SS [12, 13]. This inspired us to study whether such non-professional histamine-producing cells, able to stimulate their H_4R-provided acinar target cells at nanomolar concentrations, are also present in human SGs.

Non-professional histamine-producing cells and H_4R are such new discoveries that their contribution to the SG physiology and pathology is still unclear. It is very interesting that the former finding of a deficiency of H_4R in SS [9] is now found to be coupled with a deficiency of the equilibrative uniporter-type histamine transporters, which subjects the SGs in SS to a severe combined lack of the ligand and its receptor.

Materials and methods

Labial salivary glands

The ethics committee of the Hospital District of Helsinki and Uusimaa approved the study (19/E5/03) and all subjects gave their informed consent. Five to 10 labial salivary glands (LSGs) were collected from each subject for diagnostic purposes [9]. For real-time quantitative PCR and immunofluorescence, LSGs were snap-frozen in liquid nitrogen and stored at −80°C. For immunohistochemical analysis, LSGs were fixed in formalin and embedded in paraffin. The diagnosis of primary SS was based on the inclusion and exclusion criteria as defined in the American-European Consensus Group criteria [14]. The SS patient group consisted of one man and seven women, with a mean ± S.D. age of 53.9 ± 6.2 years, resting salivary flow of 0.6 ± 0.4 ml/15 min and focus score of 1.6 ± 0.3. SS patients were positive for at least four of the six classification criteria, one of which was focal sialadenitis in all patients. In addition, two of eight patients were SS-A and/or SS-B positive. All exclusions were performed according to the American-European Consensus Group criteria. Healthy controls included one man and five women, with a mean ± S.D. age of 47.1 ± 5.8 years and a resting salivary flow of 0.9 ± 0.3 ml/15 min. All controls had a focus score <1, and none of them fulfilled the SS classification criteria.

HSG cell culture

HSG cells were cultured as described [9] in DMEM/F-12 containing 2.5 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum.

Real-time quantitative reverse-transcription PCR

Total tissue RNA was isolated using the High Pure RNA Tissue Kit (Roche, Basel, Switzerland). Complementary DNA was synthesized using the SuperScript First Strand cDNA Synthesis System (Invitrogen, Carlsbad, CA, USA) [9].

Total HSG cell RNA was extracted using the MirVana miRNA Isolation Kit (Ambion/Life Technologies, Grand Island, NY, USA) and reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems/Life Technologies).

For human L-histidine decarboxylase (HDC), histamine-*N*-methyl transferase (HNMT) and diamine oxidase (DAO) transcripts, quantitative RT-PCR was performed using the iCycler iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories Inc., Hercules, CA, USA) and the iQ SYBR Green Supermix Kit (Bio-Rad). Sequences of the primers were as follows: HDC: forward primer 5′-TTG ATT GCC CTG CTG GCA GC-3′, reverse primer 5′-TGC ACA GAC AAA GAC GGG CAC C-3′; HNMT: forward primer 5′-TGG CAT CTT CCA TGA GGA GCT T-3′, reverse primer 5′-AAA ATC CCA AAG CAG GTC TCC AT-3′; DAO: forward primer 5′-GCT ACG TCC ACG CCA CCT TCT A-3′, reverse primer 5′-CCC AGG CCA CCA GGT CCT CA-3′; and β-actin housekeeping gene: forward primer 5′-TCA CCC ACA CTG TGC CCA TCT ACG A-3′, reverse primer

5′-CAG CGG AAC CGC TCA TTG CCA ATG G-3′. Sequences were searched using the NCBI Entrez Search system and sequence similarities using the NCBI Blastn program. Expression levels of OCT1/SLC22A1, OCT2/SLC22A2 and OCT3/SLC22A3 were quantified as described [15, 16]. PMAT/SLC29A4 expression was quantified using a commercial kit. Messenger RNA expression was measured using TaqMan technology on a 7900HT Real-time PCR system (Life Technologies).

Transport studies

HSG cells (5×10^5/well) were seeded into 24-well cell culture plates and grown for 24 h. All uptake studies were carried out at 37°C as described [17]. Cells were washed with uptake buffer (130 mM NaCl, 25 mM hydroxyethylpiperazine ethanesulphonic acid, 1.2 mM KH_2PO_4, 4.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5.6 mM glucose, pH 7.4) [18] at 37°C. Uptake was initiated by replacing this with uptake buffer supplemented with 100 nM [^{3}H]histamine (392 GBq/mM; PerkinElmer, Waltham, MA, USA), without and with 2 mM 1-methyl-4-phenylpyridinium (MPP, inhibits organic cation transporters). Uptake was stopped by three washes in ice-cold uptake buffer. Cells were lysed in 0.2% SDS and radioactivity was measured by liquid scintillation counting (Hidex 300SL TDCR, Turku, Finland).

For efflux studies, cells were pre-incubated for 60 min at 37°C in uptake buffer containing 100 nM [^{3}H]histamine and washed three times with ice-cold uptake buffer. Efflux was initiated by addition of uptake buffer (without [^{3}H]histamine) at 37°C. At indicated time points, the radioactivity ([^{3}H]histamine) in the cell culture medium and cells was measured.

Immunohistochemical staining

For 4-μm tissue sections, antigen retrieval was performed using a microwave oven. Slides were rinsed in tap water and incubated in (i) 1% H_2O_2 for 10 min and washed 3×5 min in 10 mM phosphate buffered 140 mM saline (PBS, pH 7.4); (ii) 10% normal goat or horse serum for 1 h at 22°C; (iii) monoclonal mouse anti-human HDC IgG produced against the 492–506 fragment of the enzyme located in the carboxyterminal domain of the most active 53 kDa enzyme isoform (1:350; provided by F.S.-J. and J.L.U.), 1:500 diluted monoclonal affinity purified mouse anti-human DAO IgG (HYB313-03) [19], 2.5 μg/ml polyclonal affinity purified rabbit anti-human HNMT IgG (Abcam, Cambridge, UK), overnight at 4°C. Slides were washed 3×5 min in PBS; (iv) appropriate biotin-conjugated secondary antibodies against IgG (1:200 in 1.25% BSA-PBS) for 1 h; (v) avidin–biotin–peroxidase complex (ABC complex, 1:200 in H_2O) for 1 h; (vi) 0.006% H_2O_2 and 0.023% 3,3′-diaminobenzidine tetrahydrochloride (DAB) for 5–7 min, followed by 3×5 min in dH_2O. Slides were counterstained using haematoxylin or fast-red, dehydrated, mounted and covered. Slides were photographed using a Leitz Diaplan microscope and a 5 MP Leica DFC420 digital camera (Leica Microsystems, Wetzlar, Germany). For negative-staining controls, 2.5 μg/ml non-immune rabbit or mouse IgG was used, and human kidney tissue sections were used for positive sample controls.

Immunofluorescence staining

Cryostat LSG sections 4 μm thick were incubated in (i) 10% normal donkey serum for 1 h at 22°C; (ii) polyclonal rabbit anti-human OCT2 and OCT3 antisera [17] combined with 1 μg/ml polyclonal affinity purified goat anti-human smooth muscle α-actin IgG (Everest Biotech Ltd, Upper Heyford, UK) overnight at 4°C, followed by 3×5 min in PBS; (iii) donkey anti-rabbit AlexaFluor 488 and donkey anti-goat AlexaFluor 568 conjugated secondary IgG antibodies for 1 h at 22°C, followed by 3×5 min in PBS; (iv) 4′,6-diamidino-2-phenylindole (DAPI) for nuclear counterstaining for 5 min at 22°C, followed by 3×5 min in dH_2O. The slides were mounted and photographed using a Leica DM6000 microscope and DFC365FX camera (Leica Microsystems, Wetzlar, Germany). Specificities of the OCT2 and OCT3 antisera were confirmed by using antigen absorption controls.

Results

Histamine-metabolizing enzymes in LSG

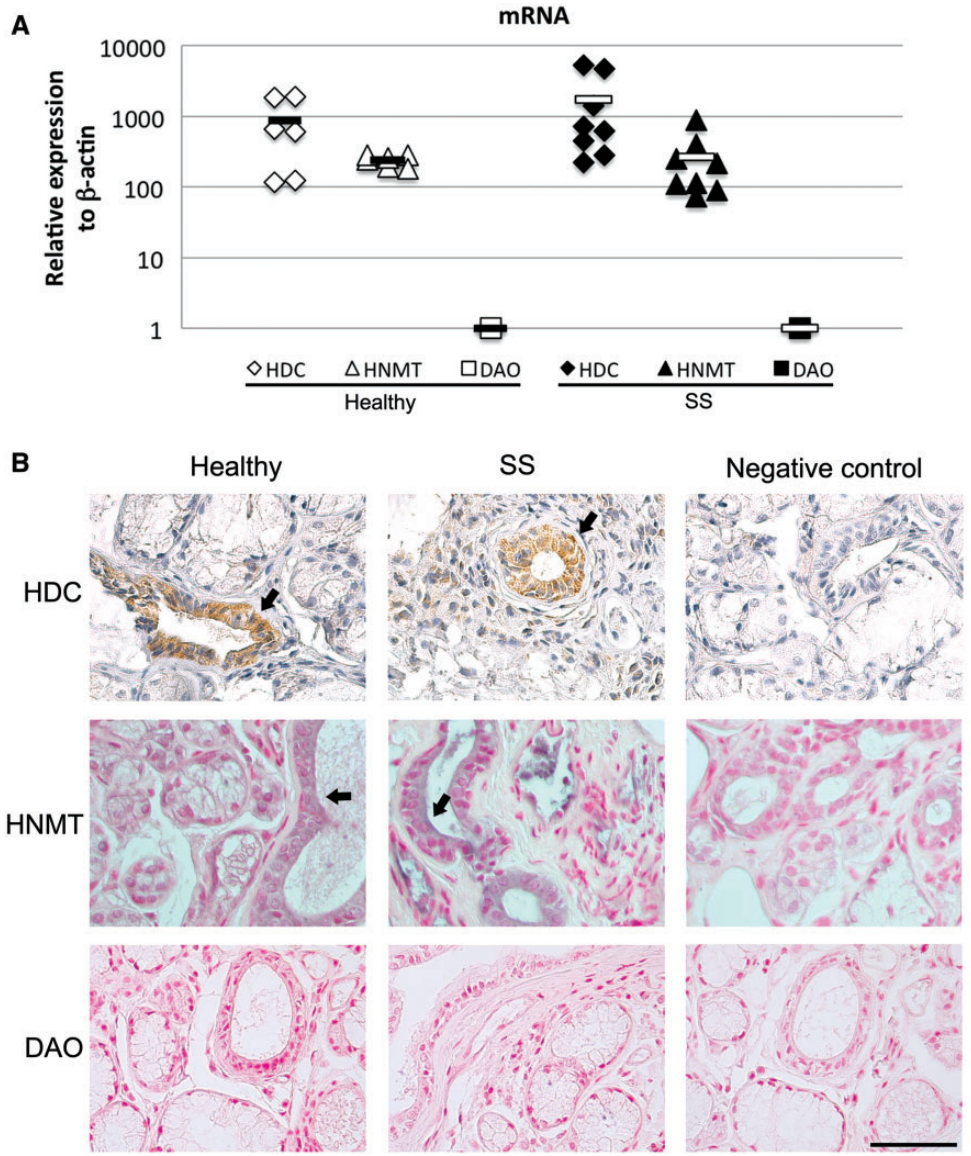
HDC and HNMT mRNA were present in healthy human LSGs, but DAO mRNA was absent. These mRNA values were not different from those measured in LSGs in SS (Fig. 1A). Immunostaining localized HDC and HNMT immunoreactivity in LSGs to epithelial cells in the intralobular excretory salivary ducts in a similar pattern in both healthy individuals and SS patients. In addition, mast cells were strongly HDC immunoreactive (data not shown). DAO immunostaining was negative, although the positive sample controls, tissue sections of human kidney, were strongly positive (data not shown). Negative-staining controls excluded false-positive staining (Fig. 1B).

Histamine transporters in LSG

Expression levels of OCT transporters and ENT4/PMAT were measured because they have been implicated in histamine transport [20, 21]. OCT3 mRNA was present at levels >100-fold higher than OCT1, OCT2 and ENT4/PMAT mRNA, which were barely detectable in healthy and SS SGs. Healthy SGs showed higher expression of OCT3 than SS SGs ($P = 0.0286$, Fig. 2A).

Immunostaining disclosed OCT2 at the protein level in myoepithelial cells, which partly surround the acini and the intercalated and striated ducts. OCT2 staining was much stronger in the myoepithelial cells in LSGs in healthy controls than in patients with SS. OCT3 was immunolocalized to the cell membranes of the acinar and ductal epithelial cells in healthy LSGs, being more strongly expressed at the basolateral than at the apical domain. In contrast, OCT3 immunostaining was very weak or even totally absent in the acinar and ductal epithelial cells in LSGs in SS, although the DAPI-stained blue nuclei disclosed that these cells were organized to tubuloacinar structures (Fig. 2B).

FIG. 1 Expression of major enzymes related to histamine metabolism in the LSGs.



(**A**) mRNA levels of HDC, HNMT and DAO in the salivary glands from healthy individuals did not differ from those of SS patients. (**B**) Immunohistochemical staining revealed HDC and HNMT in the salivary ductal epithelium in both healthy and SS biopsy specimens, whereas DAO expression was not found. Negative control confirmed specificity of the antibodies. Scale bar: 50 μm.

In vitro [^{3}H]histamine transport

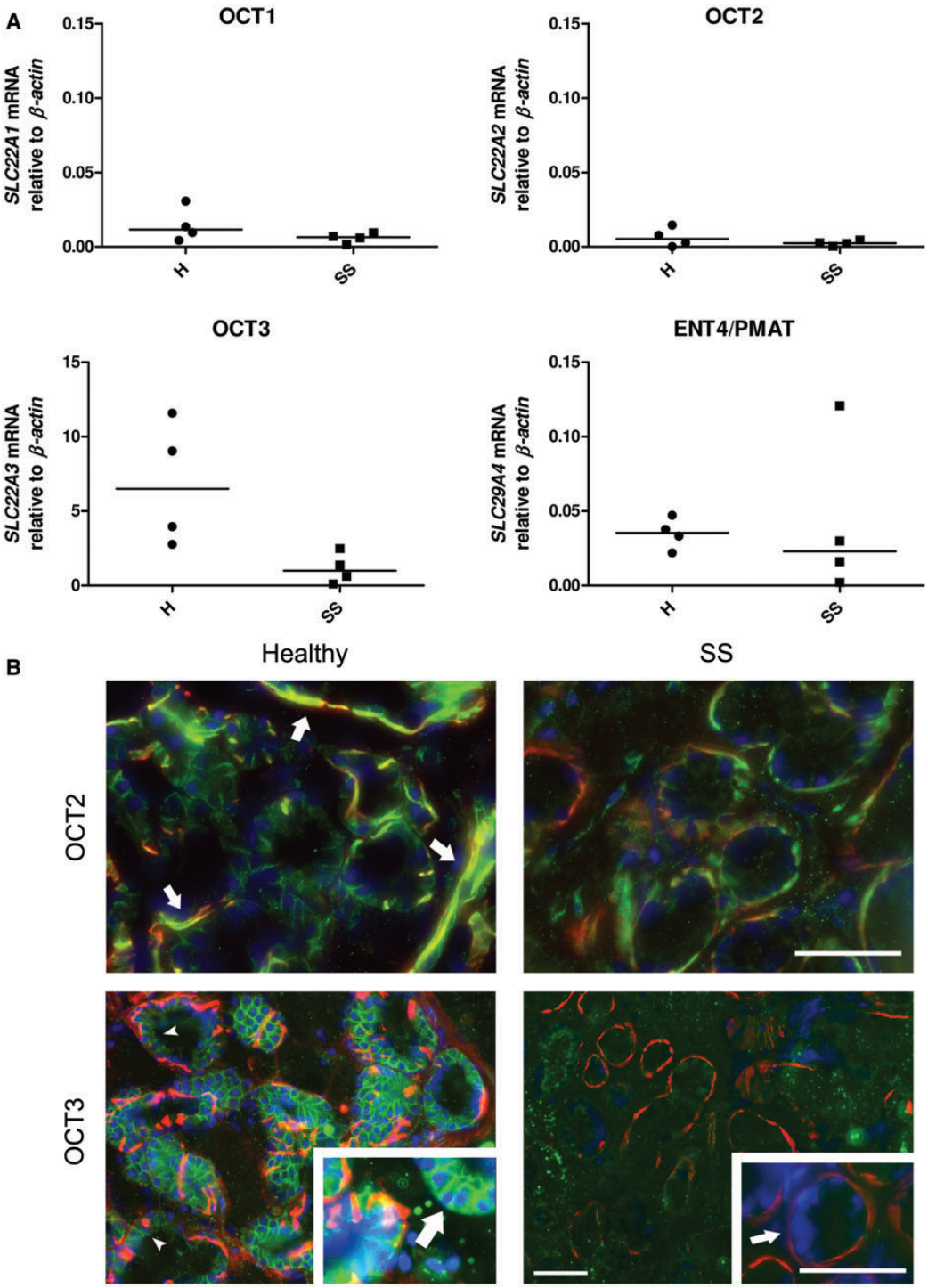
In quantitative RT-PCR experiments, HSG cells expressed OCT3 mRNA at a level >10-fold higher than OCT1 and OCT2, and >2-fold higher than ENT4/PMAT, suggesting a leading role of OCT3 in histamine transport in HSG cells (Fig. 3A).

Cultured HSG cells showed time-dependent [^{3}H]histamine uptake from the loading buffer containing 100 nM radiolabelled histamine and this uptake was inhibited by MPP (Fig. 3B). Conversely, HSG cells preloaded with 100 nM [^{3}H]histamine released it to a histamine-free medium in a time-dependent manner (Fig. 3C and D). Experiments performed in 260 mM glucose showed that [^{3}H]histamine uptake and efflux were sodium independent.

Discussion

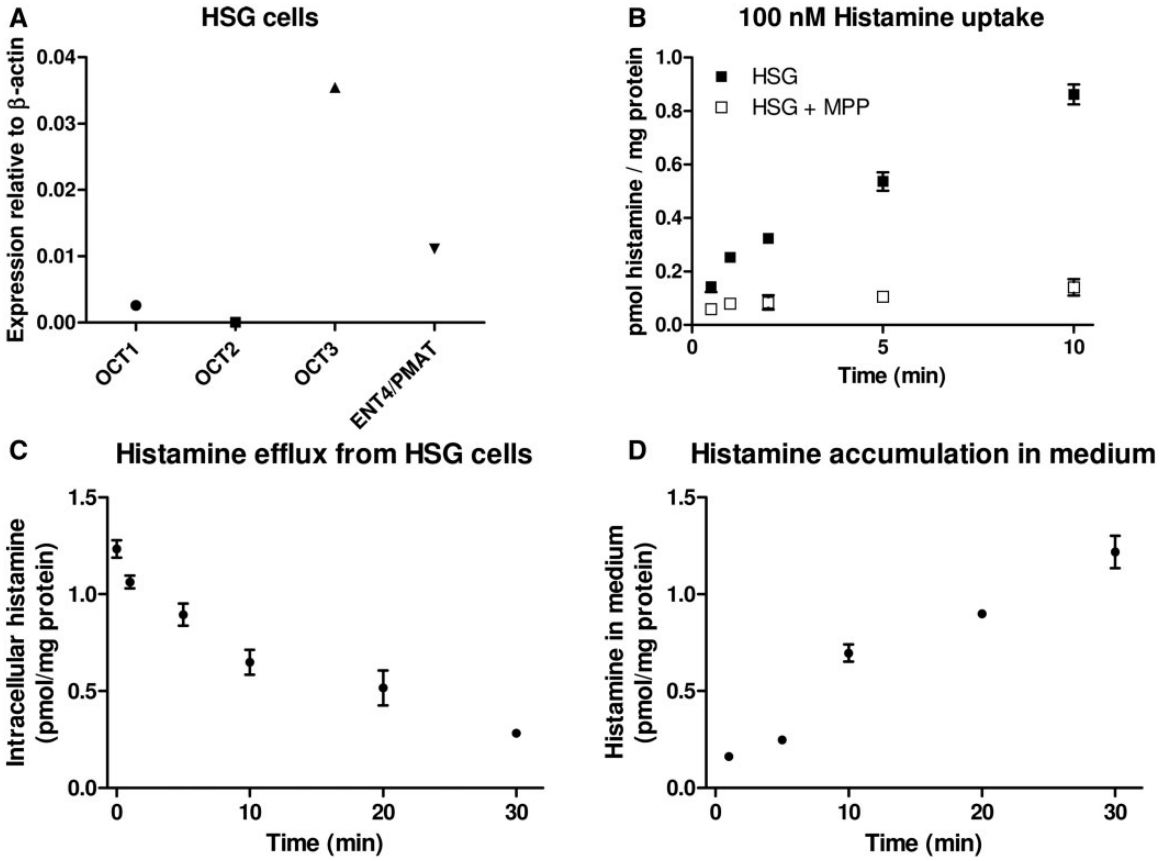
The positively charged monoamine histamine is synthesized from L-histidine by the endoplasmic 53 kDa HDC in

FIG. 2 Expression of major histamine transporters in the LSGs.



(**A**) mRNA levels of organic cation transporters (OCT1, OCT2, OCT3) and plasma membrane monoamine transporter (ENT4/PMAT) in salivary glands from healthy individuals (H) and SS patients. OCT3 showed the highest expression among the transporters in the salivary glands. SS samples expressed OCT3 at lower levels compared with healthy controls. (**B**) Double immunofluorescence staining revealed co-localization of OCT2 and smooth muscle α-actin in the myoepithelial cells (arrows in the OCT2 healthy panel). Double immunofluorescence staining showed strong basolateral (arrows in inserts) and weak apical plasma membrane localization (arrowheads) of OCT3, counterstained with human smooth muscle α-actin. SS samples showed much weaker expression of OCT2 and an almost total lack of OCT3 (arrows in the inserts). Scale bars: 50 μm.

FIG. 3 Expression and function of the transporters *in vitro*.



(**A**) mRNA expression in the human submandibular gland (HSG) cells showed levels of OCT3 >10-fold higher in comparison with OCT1, OCT2 and >2-fold higher in comparison with plasma membrane monoamine transporter (ENT4/PMAT). (**B**) Uptake of 100 nM [^{3}H]histamine by HSG cells in a time-dependent manner (black square) was successfully inhibited (white square) by MPP. (**C**) [^{3}H]histamine efflux from 100 nM [^{3}H]histamine preloaded HSG cells in a time-dependent manner was paralleled by (**D**) an accumulation of [^{3}H]histamine in the cell culture medium during efflux in a time-dependent manner.

professional histamine-synthesizing cells into storage granules, from which it can be rapidly released to reach momentarily high local concentrations as a result of a burst release [1]. These concentrations are high enough to stimulate the conventional low-affinity H_1R and H_2R and apparently reach 50–60 μM [7]. Such professional cells include mast cells, basophils and enterochromaffin-like cells.

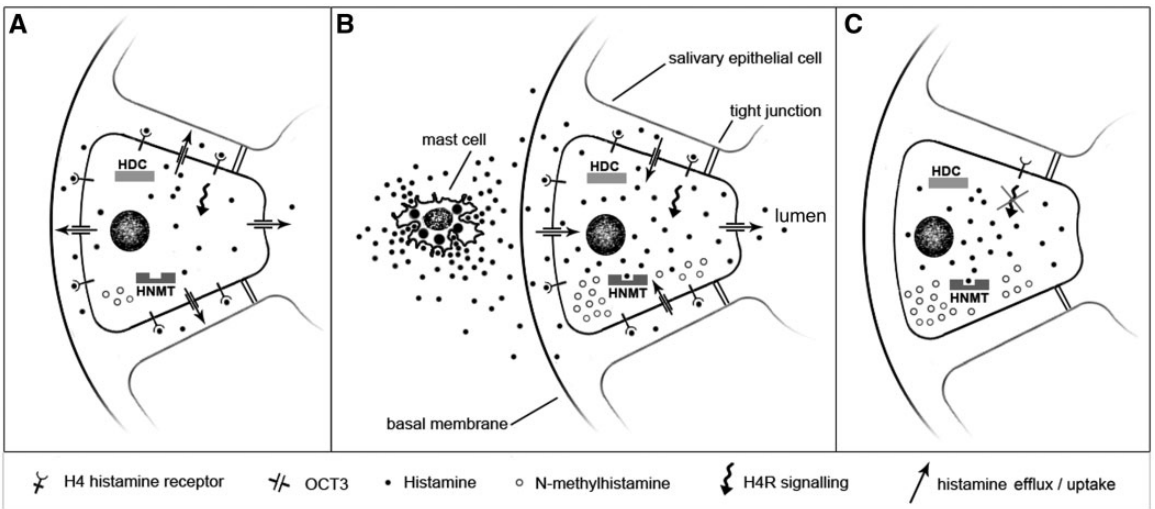
Recently attention has been paid to non-professional histamine-producing cells. They utilize the cytoplasmic 74 kDa HDC isoform to synthesize histamine, but at a ~100–1000-fold lower rate than the professional cells [1]. This histamine is not stored after synthesis but is released into the cytoplasm of the cell, from where it seems to pass into the extracellular space. Because histamine is a monovalent cation at the physiological pH, it needs transporters to cross the plasma membrane. OCT3 is the prime histamine transporter [22] and was highly expressed at the mRNA level in SGs [23] and seems to be responsible for histamine transport across the membrane of the non-professional histamine-producing cells. Our mRNA analyses confirm that OCT3 is the major OCT transporter expressed in human SGs, while ENT4/PMAT [21] was virtually not expressed. Immunolocalization showed a predominantly basolateral localization of OCT3 in the salivary epithelial cells, supporting our hypothesis that histamine produced within the cells can be released via OCT3. In common with other OCTs, OCT3 forms an electrogenic facilitative diffusion system that translocates organic cations in both directions across the plasma membrane [20]. Once outside the cell, the histamine generated by the non-professional histamine-producing cells binds effectively to its high-affinity receptors. It is concluded that the non-professional histamine-producing cells maintain the basal level histamine in tissues and body fluids. Indeed, the resting plasma concentration of histamine is 0.3–1.0 ng/ml (2.7–9 nM) [24] and in saliva is 0.31–12.4 ng/ml (2.8–112.7 nM;

1 nM = 0.11 ng/ml) [25]. Amounts in saliva are enough to stimulate the high-affinity H_3R (pKi 7.8) and H_4R (pKi 8.3) half-maximally, but are too low to affect the conventional low-affinity H_1R and H_2R, which require ~10 000-fold higher concentrations for activation [7]. This is the first study to show, using monoclonal anti-human HDC antibodies, that normal human LSG excretory duct cells contain HDC in excretory ductal cells and have histamine-synthesizing potential. This extends observations on the presence of HDC in the excretory ductal cells in rat submandibular glands [26], but contradicts the universal and indiscriminate presence of HDC in all submandibular gland epithelial cells as was seen in staining with polyclonal anti-rat HDC [27], which might result from cross-reactivity or false-positive staining.

Regarding OCTs, a normalized human RNA master plot disclosed high levels of OCT3 (SLC22A3) mRNA in non-defined human SG extracts [23] and now in the present work OCT3 and OCT2 have been located to acinar and myoepithelial cells, respectively.

Mast cells are increased in SS [28]. Professional histamine-producing cells can momentarily release enough histamine to activate the conventional low-affinity H_1Rs and H_2Rs. This is compatible with our finding of H_1R on human vascular endothelial cells [29]. Stimulation of these cells via H_1R increases cytosolic calcium activation of endothelial nitric oxide synthase (eNOS) [30] and production of NO. This leads to vasodilation and increased vasopermeability, seen in skin as the wheal and flare response (urticaria) upon mast cell activation. SGs did not contain extracellular histamine degrading DAO. It was therefore of interest that 100 nM radiolabelled histamine was effectively taken up by HSG cells from the extracellular medium. A 10 000-fold higher histamine concentration after burst release will probably also lead to rapid uptake of histamine by the OCT2/OCT3-equipped cells [31]. Down-regulation of the activated, high-histamine state is then mediated by the intracellular HNMT-mediated degradation [32, 33], which works optimally first at relatively high histamine concentrations (K_m = 6–13 μM) [24]. Thus a new role is suggested for the ductal $OCT3^+$ cells, a role in the intracellular degradation of histamine released from mast cells in human SGs. Histamine is also taken up by mast cells via OCT3, but there the cytoplasmic histamine is taken up by the recovering storage granules via active VMAT2-mediated transport [1]. This conclusion is also supported by *in vitro* uptake studies, which show effective uptake of 100 nM histamine by cultured HSG cells. Together with immunostaining results, inhibition of this uptake in functional studies by MPP, a monovalent model cation and histamine analogue with a higher affinity for OCT3 than histamine (K_m = 47 μM *vs* 220 μM) [34] suggests a leading role for OCT3 in histamine uptake in human SGs, although these cells also express lower levels of ENT4/PMAT mRNA.

Fig. 4 Different conditions of the salivary gland epithelial cells.



(**A**) A healthy salivary gland at a resting state equipped with intracellular histamine synthesizing HDC. Newly synthesized histamine is transported into the extracellular space via OCT3, where its concentration is high enough to activate histamine receptor 4 (H_4R). (**B**) A healthy salivary gland after mast cell granulation in a stimulated state. Excess extracellular histamine is taken up by the salivary duct epithelial cells via OCT3 and degraded intracellularly by HNMT or passes via the basolateral and apical equilibrative uniporters (OCT3) to saliva. (**C**) In SS at the resting state, histamine is normally synthesized, but due to the lack of OCT3, it cannot pass outside the extracellular space, and even if it could, the deficiency of the high-affinity H_4R restrains stimulation. In SS, at the stimulated high-histamine state after mast cell degranulation histamine cannot be effectively taken up by the cells for subsequent intracellular degradation or transcellular excretion (not shown).

In healthy SGs, OCT3 was localized in the basolateral and more weakly in the apical plasma membrane of the acinar and ductal epithelial cells, compatible with its polarization [15]. OCT2 was restricted to the smooth muscle α-actin containing myoepithelial cells. At the basal resting state, the strong basolateral OCT3 localization would allow histamine efflux from ductal epithelial cells and subsequent binding to acinar and ductal cell H_4R [9] (Fig. 4A).

The predominantly basolateral organization of OCT3 allows the handling of burst-released high histamine concentrations by acinar and ductal epithelial cells by two different mechanisms: intracellular, HNMT-mediated degradation in ductal cells (Fig. 4B) and salivary excretion via the apical OCT3, to which also an eventually apically located ENT4/PMAT could contribute. Even without transport to saliva, OCT3-mediated epithelial cell uptake could down-regulate extracellular histamine by sequestration independent of enzymatic degradation. The present findings suggest that histamine functions at the basal (constitutive) low-level histamine/high-affinity receptor interaction state [9] and at the stimulated high-level histamine/low-affinity receptor interaction state after regulated burst release. At their interface, down-regulation of the stimulated high-level histamine state via ductal cell-mediated, HNMT-dependent intracellular degradation and via acinar and ductal cell-mediated OCT3 (or ENT4/PMAT)-dependent excretion to saliva. Both the constitutive low and stimulated high histamine interactions seem to be severely disturbed in SS, due to the almost total lack of H_4R and OCT3, respectively.

Bone marrow-derived leukocytes, such as antigen-presenting dendritic cells [12] and T and B lymphocytes [13, 35], belong to non-professional histamine-producing cells. This finding was first considered insignificant. This changed upon the detection of the high-affinity histamine receptors, found in both dendritic cells and lymphocytes [6, 36]. Therefore histamine may also have effects on local immune reactions and lymphocyte infiltrates in SS.

In conclusion, intralobular salivary excretory duct epithelial cells are non-professional histamine-producing cells. At the basal constitutive state, they seem able to release enough histamine via OCT3 to the extracellular fluid to stimulate H_4R. If professional histamine-producing mast cells are locally activated, the conventional low affinity H_1R is also activated. At the same time, ductal, acinar and myoepithelial cells take up histamine via OCT2/3 for intracellular degradation, salivary excretion and/or intracellular sequestration. In SS, the basal maintenance phase seems to be disturbed due to the almost total lack of H_4R on the acinar and ductal epithelial cells [9]. At the basal maintenance phase in SS, histamine produced by the non-professional ductal epithelial cells cannot be effectively released to stimulate salivary epithelial cells via H_4Rs. In other words, at the resting phase impaired histamine release and lack of H_4Rs both contribute to the same acinar/ductal cell maintenance and functional defect, therefore potentially contributing to SS development, maintenance and progression. In SS, the down-regulation of histamine after burst release seems to be disturbed due to a deficiency of OCT3 in acinar and ductal cells and of OCT2 in the myoepithelial cells. The monoamine transport system in SGs from SS patients is dramatically altered, identifying new drug targets in this autoimmune disease. Although the treatment of many other rheumatic diseases has greatly improved as a result of the introduction of biologics, there has been little progress in the treatment of SS except for some promise with rituximab treatment. Because low-level histamine/high-affinity H_4R seems to be so important for the maintenance of the tubuloacinar glands and for the dendritic cell and lymphocyte-mediated immune responses against them, there is hope that some new synthetic small molecular weight H_4R-modulating chemical drugs can in the near future provide a new paradigm for the treatment of SS targeting both resident structural and immigrant inflammatory cells.

Rheumatology key messages

- Non-professional histamine-producing cells were found in salivary glands in SS.
- Non-professional salivary epithelial cell histamine-*N*-methyl transferase also metabolizes histamine burst-released by mast cells.
- Impaired histamine transport in the salivary glands in SS may contribute to glandular pathology.

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References

1 Ichikawa A, Sugimoto Y, Tanaka S. Molecular biology of histidine decarboxylase and prostaglandin receptors. Proc Jpn Acad Ser B Phys Biol Sci 2010;86: 848–66.

2 Gschwandtner M, Mommert S, Kother B *et al*. The histamine H4 receptor is highly expressed on plasmacytoid dendritic cells in psoriasis and histamine regulates

their cytokine production and migration. J Invest Dermatol 2011;131:1668–76.

3 Gschwandtner M, Rossbach K, Dijkstra D *et al*. Murine and human Langerhans cells express a functional histamine H4 receptor: modulation of cell migration and function. Allergy 2010;65:840–9.

4 Dijkstra D, Stark H, Chazot PL *et al*. Human inflammatory dendritic epidermal cells express a functional histamine H4 receptor. J Invest Dermatol 2008;128: 1696–703.

5 Gutzmer R, Mommert S, Gschwandtner M *et al*. The histamine H4 receptor is functionally expressed on T(H)2 cells. J Allergy Clin Immunol 2009;123:619–25.

6 Mommert S, Gschwandtner M, Koether B *et al*. Human memory Th17 cells express a functional histamine H4 receptor. Am J Pathol 2012;180:177–85.

7 Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov 2008;7:41–53.

8 Tiligada E, Zampeli E, Sander K *et al*. Histamine H3 and H4 receptors as novel drug targets. Expert Opin Investig Drugs 2009;18:1519–31.

9 Stegaev V, Sillat T, Porola P *et al*. Brief report: first identification of H(4) histamine receptor in healthy salivary glands and in focal sialadenitis in Sjogren's syndrome. Arthritis Rheum 2012;64:2663–8.

10 Skopouli FN, Moutsopoulos HM. Autoimmune epitheliitis: Sjogren's syndrome. Clin Exp Rheumatol 1994;12(Suppl 11):S9–11.

11 Oda T, Morikawa N, Saito Y *et al*. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. J Biol Chem 2000; 275:36781–6.

12 Szeberenyi JB, Pallinger E, Zsinko M *et al*. Inhibition of effects of endogenously synthesized histamine disturbs in vitro human dendritic cell differentiation. Immunol Lett 2001;76:175–82.

13 Kubo Y, Nakano K. Regulation of histamine synthesis in mouse CD4+ and CD8+ T lymphocytes. Inflamm Res 1999;48:149–53.

14 Vitali C, Bombardieri S, Jonsson R *et al*. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61: 554–8.

15 Nies AT, Koepsell H, Winter S *et al*. Expression of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) is affected by genetic factors and cholestasis in human liver. Hepatology 2009;50:1227–40.

16 Schaeffeler E, Hellerbrand C, Nies AT *et al*. DNA methylation is associated with downregulation of the organic cation transporter OCT1 (SLC22A1) in human hepatocellular carcinoma. Genome Med 2011; 3:82.

17 Nies AT, Hofmann U, Resch C *et al*. Proton pump inhibitors inhibit metformin uptake by organic cation transporters (OCTs). PLoS One 2011;6:e22163.

18 Amphoux A, Vialou V, Drescher E *et al*. Differential pharmacological in vitro properties of organic cation transporters and regional distribution in rat brain. Neuropharmacology 2006;50:941–52.

19 Schwelberger HG, Feurle J, Houen G. New tools for studying old questions: antibodies for human diamine oxidase. J Neural Transm 2012 (in press).

20 Nies AT, Koepsell H, Damme K *et al*. Organic cation transporters (OCTs, MATEs), in vitro and in vivo evidence for the importance in drug therapy. Handb Exp Pharmacol 2011;105–67.

21 Itagaki S, Ganapathy V, Ho HT *et al*. Electrophysiological characterization of the polyspecific organic cation transporter plasma membrane monoamine transporter. Drug Metab Dispos 2012;40:1138–43.

22 Grundemann D, Liebich G, Kiefer N *et al*. Selective substrates for non-neuronal monoamine transporters. Mol Pharmacol 1999;56:1–10.

23 Verhaagh S, Schweifer N, Barlow DP *et al*. Cloning of the mouse and human solute carrier 22a3 (Slc22a3/SLC22A3) identifies a conserved cluster of three organic cation transporters on mouse chromosome 17 and human 6q26–q27. Genomics 1998;55:209–18.

24 Maintz L, Novak N. Histamine and histamine intolerance. Am J Clin Nutr 2007;85:1185–96.

25 Kejr A, Gigante C, Hames V *et al*. Receptive music therapy and salivary histamine secretion. Inflamm Res 2010; 59(Suppl 2):S217–8.

26 Medina VA, Prestifilippo JP, Croci M *et al*. Histamine prevents functional and morphological alterations of submandibular glands induced by ionising radiation. Int J Radiat Biol 2011;87:284–92.

27 Kim JH, Park SH, Moon YW *et al*. Histamine H1 receptor induces cytosolic calcium increase and aquaporin translocation in human salivary gland cells. J Pharmacol Exp Ther 2009;330:403–12.

28 Konttinen YT, Tuominen S, Segerberg-Konttinen M *et al*. Mast cells in the labial salivary glands of patients with Sjogren's syndrome: a histochemical, immunohistochemical, and electron microscopical study. Ann Rheum Dis 1990;49:685–9.

29 Okayama M, Baraniuk JN, Hausfeld JN *et al*. Characterization and autoradiographic localization of histamine H1 receptors in human nasal turbinates. J Allergy Clin Immunol 1992;89:1144–50.

30 Kida M, Sugiyama T, Yoshimoto T *et al*. Hydrogen sulfide increases nitric oxide production with calcium-dependent activation of endothelial nitric oxide synthase in endothelial cells. Eur J Pharm Sci 2012;48:211–5.

31 Koepsell H, Schmitt BM, Gorboulev V. Organic cation transporters. Rev Physiol Biochem Pharmacol 2003;150: 36–90.

32 Beaven MA.. Factors regulating availability of histamine at tissue receptors. In: Ganellin CR, Parson ME, eds. Pharmacology of histamine receptors. Bristol, UK: Wright, 1982:103–45.

33 Tahara A, Nishibori M, Ohtsuka A *et al*. Immunohistochemical localization of histamine N-methyltransferase in guinea pig tissues. J Histochem Cytochem 2000;48:943–54.

34 Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and

biopharmaceutical implications. Pharm Res 2007;24: 1227–51.

35 Radvany Z, Darvas Z, Kerekes K *et al*. H1 histamine receptor antagonist inhibits constitutive growth of Jurkat T cells and antigen-specific proliferation of ovalbumin-specific murine T cells. Semin Cancer Biol 2000;10:41–5.

36 Simon T, Laszlo V, Falus A. Impact of histamine on dendritic cell functions. Cell Biol Int 2011;35: 997–1000.