Histamine deficiency induces tissue-specific down-regulation of histamine H2 receptor expression in histidine decarboxylase knockout mice

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Abstract Histidine decarboxylase (HDC) is the single enzyme responsible for histamine synthesis. HDC-deficient mice ($HDC^{-/-}$) have no histamine in their tissues when kept on a histamine-free diet. Therefore, the $HDC^{-/-}$ mice provide a suitable model to investigate the involvement of histamine in the regulation of histamine receptor expression. Gene expression of H1 and H2 histamine receptors was studied in several organs of $HDC^{-/-}$ mice and compared to standard ($HDC^{+/+}$) mice. In many tissues, prolonged absence of histamine induced down-regulation of the H2 receptor subtype. The expression of the H1 receptor was less sensitive to histamine deficiency. Exogenous histamine present in the diet abolished the differences observed in H2 receptor expression. These results suggest that the expression of mouse H2 receptor is under the control of histamine in a tissue-specific manner.

Key words: Histamine receptor; L-Histidine decarboxylase; Knockout mouse; G protein-coupled receptor; Tissue-specific expression; Histamine

1. Introduction

Histamine is a biogenic amine, synthesized exclusively by L-histidine decarboxylase (HDC) in most mammalian tissues. It has multiple biological effects including stimulation of acid gastric secretion, contraction of smooth muscle, vasodilation, regulation of inflammatory reactions and neurotransmission in the central nervous system. These actions of histamine are mediated in humans by four pharmacologically and molecularly defined receptor subtypes [1–3]. In mice, H1, H2 and H3 receptors have been pharmacologically characterized [1], but only the H1 and H2 subtypes have so far been cloned [4,5]. Several studies have demonstrated the participation of the H2 receptor in regulating gastric acid secretion, gastrointestinal motility, heart contractility and cell growth [6].

Recently, the HDC gene has been targeted by homologous recombination in mouse embryonic stem cells, using a construction with deleted exons corresponding to the pyridoxal 5′-phosphate binding site [7]. Homozygous HDC-deficient mice ($HDC^{-/-}$) lack histamine synthesizing activity from histidine and have remarkably low histamine levels in their tissues when they are kept on a histamine-free diet [7]. Since it has been suggested that the function of the H2 receptor could be regulated by histamine in an autocrine or paracrine loop [8], we decided to investigate histamine H1 and H2 receptor expression using histamine-deficient ($HDC^{-/-}$) and normal ($HDC^{+/+}$, wild type) mice using RT-PCR and radioligand binding assays. Our results suggest that in histamine-deficient mice kept on a histamine-free diet, severe histamine deficiency induces down-regulation of the H2 receptor subtype in a tissue-specific manner, while the expression of the H1 subtype is less affected by low histamine levels. Interestingly, the decrease in H2 receptor expression was observed in HDC gene-targeted animals on a histamine-free diet but it was abolished by the presence of histamine in the food.

2. Materials and methods

2.1. Animals

HDC gene-targeted mice were generated as previously described in detail. HDC-deficient ($HDC^{-/-}$) animals were confirmed to be homozygous recombinants by Southern blot [7].

Both $HDC^{-/-}$ and wild type ($HDC^{+/+}$) animals were littermates in a segregating F2 population. About 4-month-old male wild type and $HDC^{-/-}$ mice (CD1 background) were used in all experiments. After killing animals by cervical dislocation the various tissues were removed.

2.2. In vivo treatments

Both $HDC^{+/+}$ and $HDC^{-/-}$ mice were kept on either normal (histamine-rich, i.e. >50 μmol histamine/g food) or histamine-free diet (<0.6 nmol histamine/g food) (Charles River, Hungary) for 15 days before organs were removed, immediately frozen on liquid nitrogen and stored at −70°C until use.

2.3. Membrane preparation

Tissue samples were frozen at −70°C, homogenized in 50 mM $Na_2HPO_4/KHPO_4$ (pH 7.4) buffer and centrifuged at $50\,000\times g$ for 10 min at 4°C. Supernatants were discarded and pellets were washed four times in fresh buffer. Total protein concentration was determined by Bio-Rad dye reagent and compared to bovine serum albumin standards.

2.4. Binding experiments

Equal amounts of membrane preparations obtained from tissues of wild type and $HDC^{-/-}$ mice on either a histamine-free or a histamine-containing diet were incubated for 60 min at 25°C with increasing concentrations of [^{3}H]tiotidine, ranging from 1 to 70 nM. Nonspecific binding was determined in the presence of 5 mM histamine.

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The incubation was stopped by rapid filtration under vacuum using GF/B filters previously humidified in polyethyleneamine. Radioactivity retained in the filters was measured in a liquid scintillation counter. Specific binding was calculated by non-linear regression and expressed as fmol/mg protein.

2.5. Total RNA extraction

Selected tissues were frozen on liquid nitrogen and immediately homogenized at 0°C. The homogenate was centrifuged at 1500×*g* for 3 min at 4°C. The resulting supernatant was subjected to standard phenol/chloroform/guanidinium isothiocyanate RNA extraction. The purity of total RNA was determined by measurement of optical density at 260 nm and 280 nm. The integrity of the 18S and 28S ribosomal RNA was tested by gel electrophoresis in 1% agarose/formaldehyde gels and ethidium bromide staining.

2.6. RT-PCR

RNA samples treated with 1 ng DNase were reverse transcribed to cDNA in a final volume of 20 μl in the presence of 1×PCR buffer, 5 mM $MgCl_2$, 1 mM deoxyribonucleoside triphosphate (dNTP), 50 pmol of random hexamer primers and 50 U of MMLV reverse transcriptase (Promega, Madison, WI, USA). The samples were subsequently incubated at room temperature for 10 min, at 42°C for 45 min, at 99°C for 5 min and cooled on ice immediately afterwards.

The PCR following cDNA synthesis was performed from 10 μl of cDNA mixture using 22 bp oligonucleotides spanning a 517 bp fragment of the coding region of the mouse H1 receptor (sense primer 5′-CTGGTGGTGGTTCTTAGTAGTATC-3′ and antisense primer 5′-CAGCATCAGCAAAGTGGGGAGGTA-3′) and a 468 bp region of the mouse H2 receptor (sense primer 5′-CGTCTGCCTGGCTGTCAGCTTG-3′ and antisense primer 5′-AGAGGCAGGTAGAAGGTGACCA-3′) (Fitzsimons et al., submitted for publication) in a final volume of 50 μl. After an initial denaturation step at 94°C for 2 min, a 30 cycle profile of 30 s at 94°C, 30 s at 55°C and 2 min at 72°C was performed. After the final cycle the temperature was maintained at 72°C for 10 min to allow complete synthesis of the amplified products. In parallel with each sample, a reverse transcriptase-negative reaction was performed where the enzyme was replaced by RNase-free water, to check the amplification products were exclusively derived from RNA. As internal control, mouse glycerol-3-phosphate dehydrogenase (G3PDH) mRNA was amplified from each sample and used to semi-quantify the expression of histamine receptors [9]. 0.5 ng of mouse genomic DNA was used as positive control for the reaction. PCR products were run in 2% agarose gels and the intensity obtained from the ethidium bromide luminescence was digitized and analyzed using Scion Image software (Scion, San Diego, CA, USA). PCR products were sequenced and showed 98% homology with the reported nucleotide sequences [4,5].

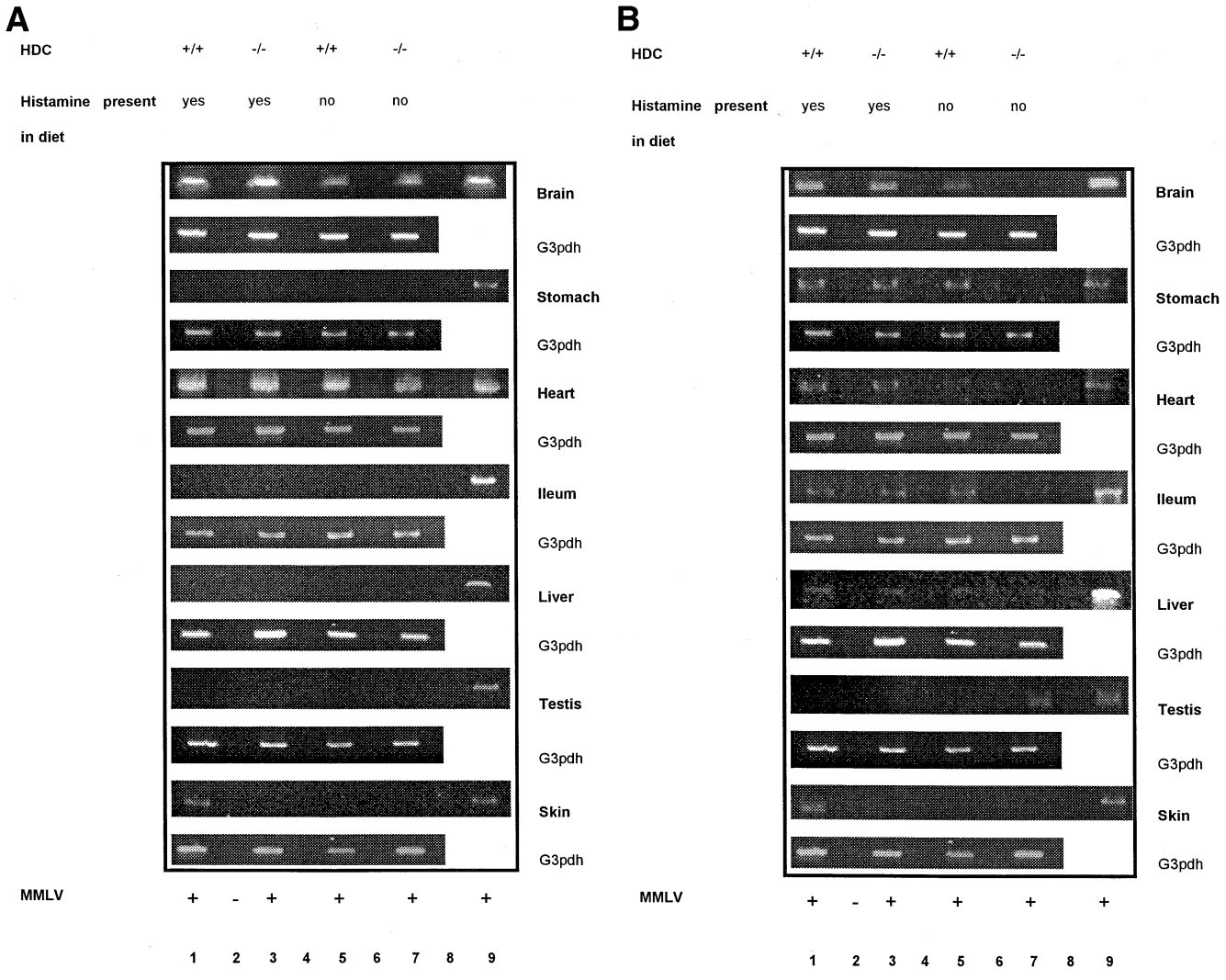

Fig. 1. H1 (A) or H2 (B) receptor mRNA in different mouse tissues by RT-PCR. Lane 1: control wild type mice on histamine-rich diet, lane 3: $HDC^{-/-}$ mice on histamine-rich diet, lane 5: control wild type mice on histamine-free diet, lane 7: $HDC^{-/-}$ mice on histamine-rich diet, lane 9: positive PCR control (mouse genomic DNA). Lanes 2, 4, 6, 8: corresponding samples without MMLV. G3pdh: RT-PCR products from glycerol-3-phosphate dehydrogenase mRNA.

3. Results

When the expression of histamine H1 and H2 receptors was studied by RT-PCR, mRNA of the H1 and H2 receptors was found in several tissues (Fig. 1A,B), in accordance with previous reports [1,4,5]. Prolonged histamine deficiency in $HDC^{-/-}$ mouse tissues induced a marked down-regulation of H2 receptor expression in brain, stomach, heart, ileum, liver and skin. Interestingly, the expression of H2 receptor was detected in testis of $HDC^{-/-}$ mice on a histamine-free diet, while it was undetectable in testis of mice from other groups (Fig. 1B). In most of the tissues analyzed the expression of H1 receptor was undetectable. When found, the expression of H1 receptor was insensitive to histamine levels and no differences were observed between $HDC^{-/-}$ and wild type mice, regardless of the diet they received (Fig. 1A). Particularly, the expression of both subtypes of histamine receptors appeared to be very sensitive to histamine levels in skin of mice, since both H1 and H2 receptors mRNA were detected only in skin of wild type mice kept on histamine-rich diet (Fig. 1A,B).

In order to further characterize the differences observed in the expression of H1 and H2 receptors in several tissues of $HDC^{-/-}$ and wild type ($HDC^{+/+}$) mice, the images obtained from the ethidium bromide staining of the agarose gels were digitized and densitometrically analyzed. The expression of H1 receptor found in brain and heart presented no statistically significant differences among the four groups of mice under study (Fig. 2A). The down-regulation observed in the expression of H2 receptor mRNA was confirmed by this analysis (Fig. 2B). In brain, stomach, heart, ileum, liver and skin of the $HDC^{-/-}$ mice the expression of the H2 receptor mRNA was substantially lower than in the same organs of the control mice. Notably, these differences were abolished by the inclusion of histamine in the diet (Fig. 2B). Even when a down-regulation in the expression of H2 receptor in the control mice kept on a histamine-free diet was apparent in several organs (Fig. 1B), these differences were statistically not significant (Fig. 2B).

In order to correlate differences observed in H2 receptor mRNA levels with the expression of the receptor at the cell membrane, complementary binding experiments were performed in samples from all the organs analyzed, using the H2-specific radioligand [^{3}H]tiotidine. In those tissues where the amounts of histamine receptors were high enough to be detected by binding assays, the results obtained were in accordance with those from mRNA expression studies, except in brain where no differences were detected (Fig. 3).

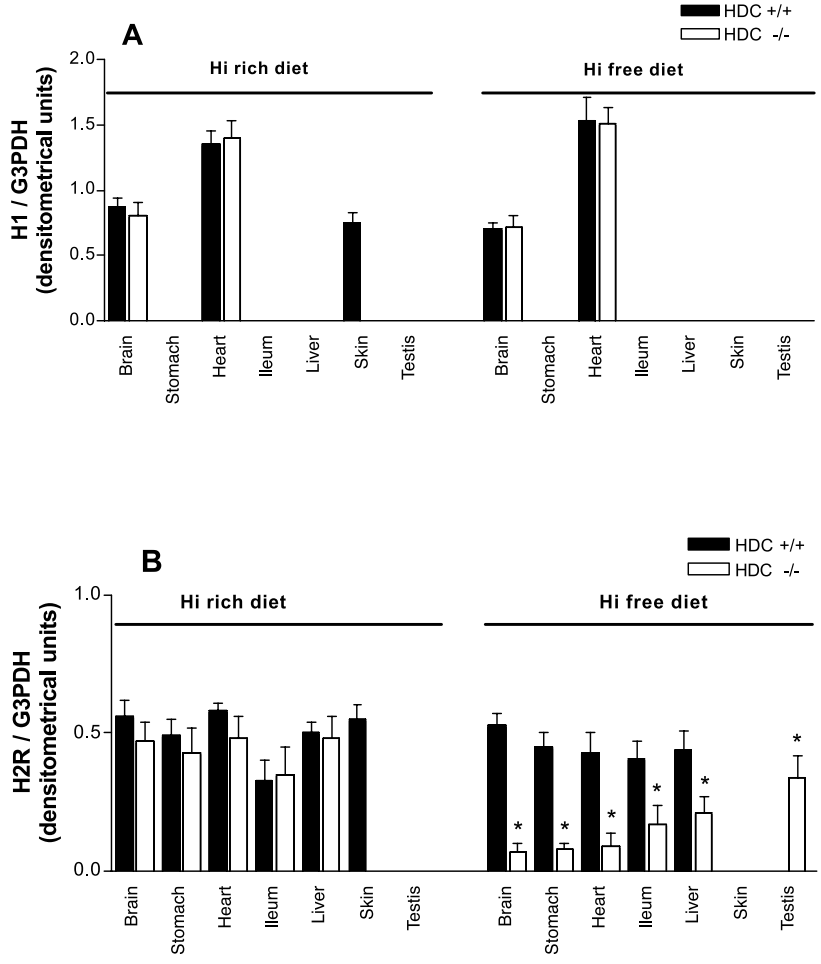

Fig. 2. Densitometric analysis of H1 (A) or H2 (B) receptor mRNA expression in different mouse tissues. The levels of expression of the histamine receptors were semi-quantified using Scion Image software. The values of integrated area units (IAU) obtained from the ethidium bromide luminescent signal corresponding to H1 or H2 receptors were normalized against the values of IAU from the signal corresponding to G3PDH run in the same gel. Results are expressed as mean ± S.E.M. of three independent RT-PCR reactions. *Significantly different as compared with values obtained from control wild type mice ($P < 0.05$, Student's t-test).

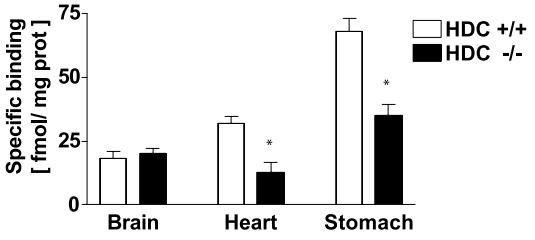

Fig. 3. Binding assays of H2 receptor-specific radioligand [^{3}H]tiotidine to membrane preparations obtained from different mouse tissues. Scatchard analysis of the data revealed single binding sites for [^{3}H]tiotidine. In all cases, the dissociation constant (K_d) values obtained for [^{3}H]tiotidine (20 nM) were similar to those reported in other systems [1]. Each point represents the mean ± S.E.M. of triplicate determinations from three independent experiments. *Significantly different as compared with values obtain from control wild type mice ($P < 0.05$, Student's t-test).

4. Discussion

Histamine, a widespread chemical mediator, exerts its biological actions through the activation of membrane-located G protein-coupled receptors [1]. The expression of histamine H1 and H2 receptor subtypes has been reported in several tissues but how this expression is regulated has still not been completely clarified.

The existence of a tissue-specific expression of human histamine H2 receptor has been reported, but the mechanisms of this specificity are not yet clear [10]. Results obtained from RT-PCR experiments indicate that the regulation of the expression of mouse H2 receptor is also tissue-specific. Severe histamine deficiency in HDC$^{-/-}$ mouse tissues has been previously reported [7]. This prolonged histamine deficiency induced down-regulation of H2 receptor mRNA expression in several organs (i.e. brain, stomach, heart, ileum, liver and skin). Interestingly, in the testis of HDC$^{-/-}$ mice, the expression of H2 receptor mRNA was up-regulated. In the skin, both H1 and H2 receptor mRNAs were detectable only in wild type mice on a histamine-rich diet, indicating that the expression of histamine receptors in this tissue is particularly sensitive to histamine levels. In all tissues in which down-regulation of H2 receptor mRNA was detected, it was reversed by the inclusion of histamine in the food, emphasizing the observation that some phenotypes of the HDC$^{-/-}$ mice are diet-inducible [11]. In general, H1 receptor expression was not affected by histamine deficiency in HDC$^{-/-}$ mouse tissues. This observation suggests that the regulation of the expression of H1 and H2 receptors is regulated by different factors and/or signalling pathways. The existence of distinct control elements in the 5′-untranslated region of both genes strongly supports this conclusion [12,13]. Particularly, the presence of cAMP response elements, GATA motifs and AP2 transcription factor binding sites has been described in the promoter region of the human H_2 receptor gene [13].

Binding experiments showed that changes observed at the mRNA level are correlated with significant changes of the receptor level at the cell membrane from heart and stomach as well. Presently, we have no clear experimental evidence to explain the controversy between the decreased expression of H2 receptor mRNA in brain of HDC$^{-/-}$ mice and the unchanged binding capacity. Interestingly, a significant amount of histamine was detected in brain of HDC$^{-/-}$ mice [7]. Since the occupation of H2 receptor by any ligand, including histamine itself, has been shown to reduce the structural instability of the H2 receptor, resulting in higher expression levels of the receptor protein by binding assays [14], the unexpectedly high amount of histamine in the brain of HDC$^{-/-}$ mice may account for the apparent controversy between the decrease in H2 receptor mRNA and the unchanged number of receptors detected by binding experiments.

In summary, in the present study we demonstrated, using genetically histamine-deficient mice, the involvement of histamine in the regulation of H2 receptor expression. The mechanisms by which histamine exerts this effect are not yet clear, but it is conceivable that histamine could induce the expression of transcription factors that have response elements in the promoter region of H2 receptor gene. Considering these observations we also speculate that some phenotypical alterations observed in HDC$^{-/-}$ mice could be diet-inducible and attributable to changes in histamine H2 receptor expression described herein.

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