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Research Article

Histamine deficiency suppresses murine haptoglobin production and modifies hepatic protein tyrosine phosphorylation

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Abstract. Histidine decarboxylase (HDC) synthesizes endogenous histamine from histidine in mammals. HDC-deficient mice (HDC–/–), if kept on a histamine-free diet, have no histamine in their tissues. HDC–/– mice show multiple phenotypes. In this study we show that both the constitutively expressed and turpentine-induced level of an acute-phase protein, haptoglobin, is significantly lower in the serum of HDC–/– mice compared to that of wild-type animals. This effect was abolished if HDC gene-targeted mice received histamine-rich food. No differences were found when lipopolysaccharide (LPS) was used to induce the acute-phase reaction. Using specific antibodies to phosphorylated tyrosine, we showed that protein tyrosine phosphorylation (Y-P) of ~50- and 26- to 27-kDa liver proteins is significantly decreased in HDC–/– mice, but that the difference was largely diminished if the animals were kept on a histamine-rich diet, suggesting that the phenotype with lower haptoglobin production is diet inducible. Upon in vivo treatment with LPS, Y-P band intensity decreased, regardless of the presence or absence of histamine. Identification of elements of the signalling pathway with decreased phosphorylation may elucidate the molecular background of the effect of endogenous histamine in the hepatic acute-phase reaction.

Key words. Histamine; knockout; acute phase protein; haptoglobin; tyrosine phosphorylation.

Introduction

Histamine plays a basic role in many physiological, developmental, and pathophysiological processes, including gastric acid secretion, allergic skin reactions, neurotransmission, wound repair, embryogenesis, hematopoiesis, and malignant growth, levels of histamine production [1–6] suggesting a role for histamine in regulating cellular growth, differentiation, and proliferation.

The biological role of histamine has been extensively studied with pharmacological approaches using specific receptor agonists and antagonists for the three known histamine receptors (H1, H2, and H3) [7–8] or histamine synthesis inhibitors (e.g., α-fluoro-methyl-histamine) [9]. Histidine decarboxylase (HDC) is the sole enzyme responsible for histamine biosynthesis, by decarboxylating L-histidine [10]. Wide expression of HDC marks the his-

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tamine-producing cell types, such as mast cells, platelets, basophils, enterochromaffin-like cells (ECLs), hypothalamic tuberomammillar neurons, and most tumor cells [11]. High histamine and HDC levels occur in regenerating liver, bone marrow, embryonic tissues and experimental tumors [12]. The activity of HDC depends on the presence of a co-enzyme, pyridoxal 5′-phosphate, which binds to HDC at a putative binding site (TFNPSKW) [13]. Recently, using mouse embryonic stem (ES) cells, the HDC gene has been targeted by homologous recombination using a targeting construct with deleted exons corresponding to the co-enzyme-binding site [Ohtsu et al., unpublished data]. Since histamine plays a multiple modulatory role in the regulation of the acute-phase reaction [14], in this study we tested the inducibility of the acute-phase reaction in histamine-deficient and normal mice by testing the serum level of an acute-phase protein, and by examining changes in the tyrosine phosphorylation pattern in the liver. Our data suggest that in histamine-deficient mice kept on a histamine-free diet, both constitutive and turpentine-provoked haptoglobin synthesis are significantly lower than in wild-type mice. Moreover, the decrease in basic [but not lipopolysaccharide (LPS)-induced] tyrosine phosphorylation of hepatic proteins with molecular masses of ca. 50 and 26–27 kDa was observed in HDC–/– animals, but this difference was abolished if histamine was added to the food.

Materials and methods

Mice

HDC gene-targeted mice were generated as described in detaila elsewhere [Ohtsu et al., unpublished data]. Briefly, using isogenic mouse genomic DNA obtained by amplification of 129Sv-derived E14 ES cell DNA, we designed the HDC-targeting construct to replace an ~2.4-kb fragment extending from the *Spe*I site in intron 5 to the *Pst*I site in exon 9 with a PGK-*neo*^{r} cassette. The cloned fragments [15] were linearized and introduced into the ES cell line R1 [16], and selected by G418 and gancyclovir. Of six LA-PCR-positive clones aggregated with CD1 morulae [17], three of confirmed to be homologous recombinants by Southern blotting.

In vivo treatments

Both wild-type (HDC+/+) and HDC knock out (HDC–/–) mice were kept on either normal (histamine-rich, i.e., >50 µmol/g) or histamine-free (<0.6 nmol/g histamine/g food) diet (Charles River, Hungary) and treated subcutaneously with turpentine (25 µl), or with *Escherichia coli* bacterial LPS (both from Sigma, St. Louis, Mo.) intraperitoneally (10 mg/kg body weight). Control mice received the same volume of saline. After 24 h (selected in previous experiments), blood samples were taken. In another group of mice, livers were removed 1 h after the treatments. Serum samples were kept at –70 °C, until use.

Liver extracts and Western blot analysis

For total lysates, livers were snap frozen in liquid N_2, and then lyses [18] in a lysis buffer (10 mM Tris-HCl pH 8.0, 10 µg/ml leupeptin, 0.5 mM EGTA, 2% NaF, 1% Triton-X 100, 25 mM phenyl-methyl-sulphonyl-fluoride and 2% Na-orthovanadate). Cytoplasmic and nuclear fractions were separated from fresh liver tissue by centrifugation (150 *g*, 15 min, 4 °C), then lysed as described above. Each gel (10% polyacrylamide) lane was loaded with 20 μg of total extracted protein. Western blots were performed on PVDF membrane [19]. The transfers were blocked with PBS containing 0.1% (v/v) Tween-20 and 5% (w/v) low-fat milk, probed with monoclonal anti-phosphorylated-tyrosine mouse IgG1 antibody at 1:333 dilution (Sigma) and then by peroxidase labelled goat anti-mouse antibody (1:20,000) (Sigma). Dilutions had been optimized in earlier experiments and an ECL (Amersham Pharmacia Biotech, UK) detection system was used.

Indirect enzyme immunoassay for measurements of haptoglobin and α_2-macroglobulin

For overnight antigen coating at 4 °C, we used plasma or serum samples diluted 1:8000 in carbonate buffer (pH 9.5). BSA-Tris (1.0%) was applied for blocking at room temperature for 1 h. The primary antibodies, rabbit anti human haptoglobin and anti-human α_2-macroglobulin (both from Sigma) were diluted to 1:500. The dilution of the secondary antibody, monoclonal anti-rabbit immunoglobulin-peroxidase conjugate (Sigma) was 1:1200. The antibody reactions were carried out at 37 °C for 1 h. For development, OPD substrate (Reanal, Budapest) was applied. After stopping the reaction, the plates were read at 492 and 620 nm in a microplate reader. Statistical significance was determined by Student's t-test (two-tailed, heteroscedastic) and ANOVA.

Results

Both constitutive (basic) and turpentine-induced serum haptoglobin levels are significantly decreased in HDC–/– mice; however, upon LPS treatment, the difference between HDC–/– and wild-type mice was not significant (fig. 1A). When the serum level of α_2-macroglobulin, which does not react to the acute-phase stimuli turpentine or LPS, was determined, no difference was measured between wild-type and HDC–/– mice (fig. 1B). The differences in constitutively expressed haptoglobin between HDC-targeted and wild-type mice disappeared if mice were fed histamine-containing food (fig. 2). Using liver tissue extract, Western blots were stained for phosphoryl-

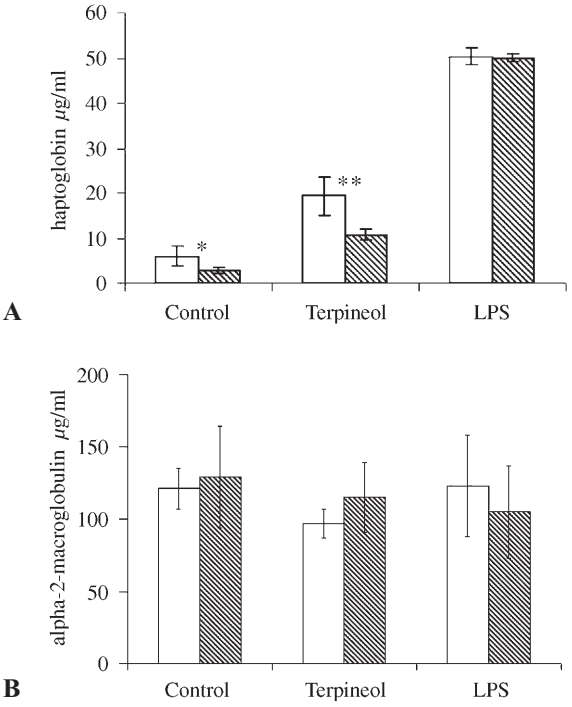

Figure 1. Serum levels of haptoglobin (*A*) and α_2-macroglobulin (*B*) in wild-type (open bars) and histidine decarboxylase knockout (HDC–/–) (shaded bars) mice (n = 4) treated with physiological saline (control), turpentine (terpineol) and LPS for 24 h. *: $p < 0.05$, **: $p < 0.01$.

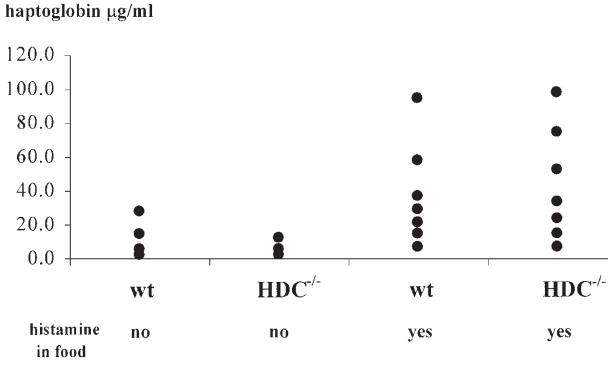

Figure 2. Serum levels of haptoglobin in wild-type (wt) and histidine decarboxylase knockout (HDC–/–) mice kept on food with (n = 7) or without (n = 4) histamine. Values of individual samples are shown.

ated tyrosine (Y-P) (fig. 3). In both cytoplasmic (lanes 1–4) and nuclear (lanes 5–8) lysates from HDC–/– (lanes 1, 3, 5, 7) mice, a major reduction in both the 50-kDa and the 26- to 27-kDa band is seen compared to corresponding lanes (2, 4, 6, 8) for wild-type animals (densitometric analysis suggested about 30- and 5-fold differences, respectively; data not shown). After LPS treatment (lanes 1, 2, 5, 6), the intensity of both proteins was lower, regardless the presence or absence of an intact HDC gene. This difference between the constitutively expressed Y-P protein fractions (fig. 3b) was much higher if the animals were kept on histamine-free (lanes 1, 2) than on normal (lanes 3, 4) food.

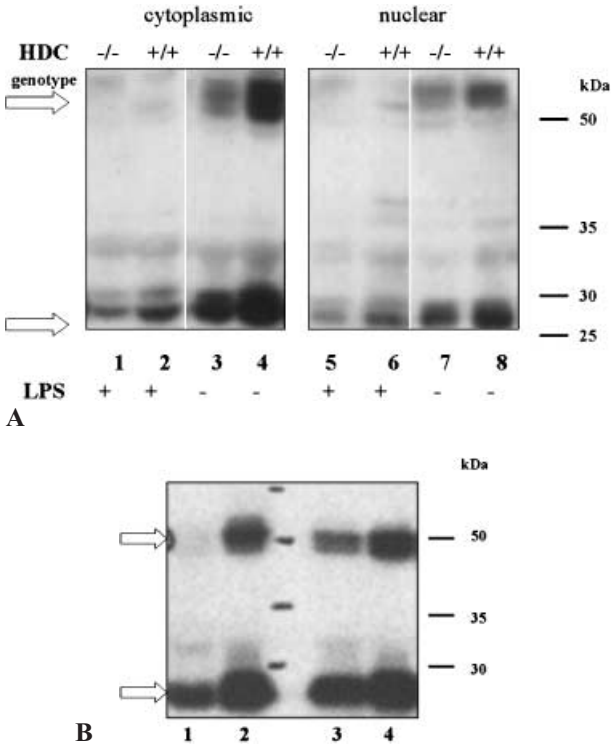

Figure 3. Western blot analysis of phosphorylated-tyrosine (Y-P)-containing proteins from liver lysates of wild-type and HDC–/– mice. (*A*) Cytoplasmic (lanes 1–4) and nuclear (lanes 5–8) lysates from liver of wild-type (lanes 2, 4, 6, 8) and HDC–/– (lanes 1, 3, 5, 7) mice with (lanes 1, 2, 5, 6) or without (3, 4, 7, 8) LPS treatment. (*B*) Total liver lysates from HDC–/– (lanes 1, 3) and wild-type (lanes 2, 4) mice fed histamine-free (lanes 1, 2) or normal (histamine-rich) (lanes 3, 4) food. Arrows show an approximately 50-kDa and a 26- to 27-kDa band; molecular mass markers are indicated.

Discussion

Acute-phase-reactions (APRs) play a fundamental role for avoiding the harmful effects of external stimuli and inner pathogenic changes as well as in re-establishing the homeostatic balance. The hepatic APR is a systemic and well-regulated process, resulting in more or less transient changes in the biosynthetic pattern of the liver. The proteins produced during the acute-phase response can carry out various functions that moderate inflammation [20, 21]. In a few hours or days, the production of acute-phase proteins (APPs) increases of 1.5 to 2-times (e.g., ceruloplasmin, C3, B factor), 2- to 4-fold (e.g. α_1-antitrypsin, haptoglobin, fibrinogen), 6- to 8-fold (C1-inhibitor), or even 100- to 1000-fold (C-reactive protein:, serum amyloid A), or, indeed, decreases about 40–60% (e.g., transferrin, albumin, and fibronectin) [21].The mediators (e.g.,

interleukin (IL)-6, IL-1, tumor necrosis factor-α, arachidonic acid products) that influence the acute phase principally affect the quantitative characteristics of the biosynthesis and glycosylation of certain proteins produced in the liver [22–23]. Histamine influences the acute-phase response, not only by influencing the production of APPs (haptoglobin, complement factors C3, C2, C4, and factor B) [24–26] but also by elevating the gene expression of IL-6 [27] and influencing IL-6 receptor expression [28]. Thus, the decrease in APP (such as haptoglobin) production after turpentine stimulation may be closely related to the absence of endogeneous histamine in HDC–/– mice. We found, however, no difference in up-regulation of APPs after LPS stimulation. This finding could be explained either by the lack of histamine dependence of LPS-induced APP production, or by the fact that the concentration of LPS was probably high enough (or supraoptimal) to provoke maximal stimulation after 24 h. Similarly, in IL-1 type I receptor knockout mice, the effect of only turpentine, but not that of LPS is diminished [29], suggesting different cytokine and/or signalling pathways in genetically deficient mice. To elucidate this question, the protein and mRNA levels of many other APPS in HDC–/– and wild-type mice following various inflammatory signals are being examined.

The considerable and simultaneous differences in constitutive tyrosine phosphorylation of two proteins (~50 and 26–27 kDa) suggest a major interaction of endogenous histamine with hepatic signalling pathway(s). In contrast to the differences without stimulation, their similar disappearance in both HDC–/– and normal (HDC+/+) mice even after just 1 h of LPS treatment emphasizes again that LPS-induced APP production is probably less dependent (or independent) on histamine than that of basic synthesis or turpentine-induced synthesis. The lack of inducibility of α_2-macroglobulin is probably due the considerable heterogeneity in its response behaviour to inflammatory stimuli among mouse strains [30, 31].

Exogenous histamine in the food seems able to replace the endogenous defect in histamine synthesis in HDC–/– mice, since mice on the histamine-rich diet did not show lower serum levels of haptoglobin of in tyrosine phosphorylation of hepatic proteins. These data suggest that at least the phenotype of decreased haptoglobin production and weaker tyrosine phosphorylation is diet inducible. This hepatic change in histamine-deficient mice can be induced at any age and in other experimental conditions (e.g., inflammation) just by withdrawing histamine from the food.

The (in)direct relationship between haptoglobin production and altered tyrosine phosphorylation is not known: identification of these Y-P proteins in further analytical studies will provide some information. Possible candidates may be MAP kinases (jun kinase JNK p46, p40, p42/40), protein tyrosine kinases (p53/56^{lyn}, p58/p64^{hck}, p59^{frg}), and NF-κB kinases (IKKα, IKKβ, CAPK) which are in the same molecular range [32, 33]. JNK p46 has a substrate (p27) with molecular mass (26–27 kDa) similar to that of the smaller band. In one of our preliminary studies (not shown), significant elevations of NF-κB were found, suggesting that the histamine-dependent tyrosine phosphorylation likely affects one or more kinds of transcriptional factor. This study, by using genetically histamine-deficient mice, emphasizes the important but still unclarified role of histamine in the acute-phase reaction.

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