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Inflammation Research

Expression of histamine degrading enzymes in guinea pig tissues

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Introduction

Histamine can be inactivated by diamine oxidase (DAO) catalysed oxidative deamination or by histamine N-methyltransferase (HNMT) catalysed ring methylation [1]. Although the guinea pig has historically been one of the most important models for studying histamine function, relatively little information is available regarding the expression and localisation of the histamine inactivating enzymes in this species. Therefore we used enzymatic activity measurements and reverse transcription-PCR (RT-PCR) to study the distribution of DAO and HNMT in the guinea pig.

Materials and methods

Bioptic samples were obtained from twelve anaesthetized Hartley guinea pigs of both sexes (weight 360–570 g, age 16–24 weeks) and immediately frozen in liquid nitrogen. For enzymatic measurements, tissue samples were mechanically homogenized in 20 mM bis-Tris hydrochloride pH 7.0 containing 5 mM 1,4-dithiothreitol and 1 mM phenylmethane sulfonyl fluoride and homogenates were cleared by centrifugation for 10 min at 20,000 x g. DAO activity was determined by a radiometric assay with [1,4^{-14}C]putrescine as the substrate [2]. HNMT activity was measured by transmethylation of histamine with S-adenosyl-L-[methyl^{-14}C]methionine [3]. Protein concentration was determined according to Bradford [4]. Each sample was measured at least in duplicate and mean values of activity in μU/mg (1 μU = pmol/min) were calculated from parallel samples of 6–12 animals.

Total cellular RNA was prepared from tissue samples using TRI Reagent® (MRC, Cincinnati, OH) according to manufacturer's instructions and reverse transcribed into cDNA for 90min at 42°C using M-MLV Reverse Transcriptase (Promega, Mannheim, Germany) with $(dT)_{18}$ as primer. DAO, HNMT and a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), cDNA fragments of 448 bp, 320 bp, and 576 bp, respectively, were amplified by PCR using 35 cycles of 15 s 94°C/15 s 65°C/30 s 72°C in reactions containing 5 ng cDNA, 200 μM dNTPs, 200 nM primers (DAO: f-ACCCACCTGTTGCACTACCGTGTC, r-TGTCCTCATTGTTGTGCAGAAACTTC, HNMT: f-GAACCAAGTGCTGAACAAATCACCAAG, r-AGGTCATTCTGGGGTAGGCGGGAT, GAPDH: f-CATCACCATCTTCCAGGAGCGA, r-GCCTGCTTCACCACCTTCTTGA), and 0.5 U Taq Polymerase (Eppendorf, Hamburg, Germany). PCR products were analysed by agarose gel electrophoresis. The study was carried out according to Slovenian regulations on animal experimentation.

Results and discussion

As compared with other species DAO activity was found to be quite low in most guinea pig tissues with highest values determined in liver, gastrointestinal tract, and bladder (Fig. 1A). Low DAO activity was consistently present in most other tissues analysed including kidney, spleen, lung, cerebellum, and blood vessels, whereas no activity was detectable in cerebrum and blood cells. The DAO activity data corresponded well with the results of DAO mRNA expression analyses in these tissues determined by RT-PCR (Fig. 2).

As in other mammalian species, HNMT activity was present in all guinea pig tissues analysed except in blood cells (Fig. 1B). The highest HNMT activity was found in spleen, intestine and brain. In accordance with these activity measurements, HNMT mRNA was detected by RT-PCR in all tissues analysed even though expression levels appeared to be low in many cases (Fig. 2). For both DAO and HNMT, tissue activities showed considerable inter-individual variation as has already been noted for other species.

In the guinea pig, DAO is present at low levels in many tissues with a less restricted expression pattern compared to other species [5]. Our data complement previous work showing prominent DAO expression in guinea pig liver and small intestine where DAO appears to be synthesized in hepatocytes and enterocytes, respectively [6]. Despite low overall DAO tissue levels considerable DAO activity can be released into the guinea pig circulation by injection of heparin (data not shown) indicating an adequate extracellular histamine inactivation capacity.

In agreement with earlier work, our results confirm that HNMT is expressed in most tissues and in many cell types [5, 7, 8]. As a cytosolic enzyme HNMT is responsible for the inactivation of intracellular histamine and the presence of a widespread intracellular histamine inactivation capacity implies that many cells might have to deal with histamine. This

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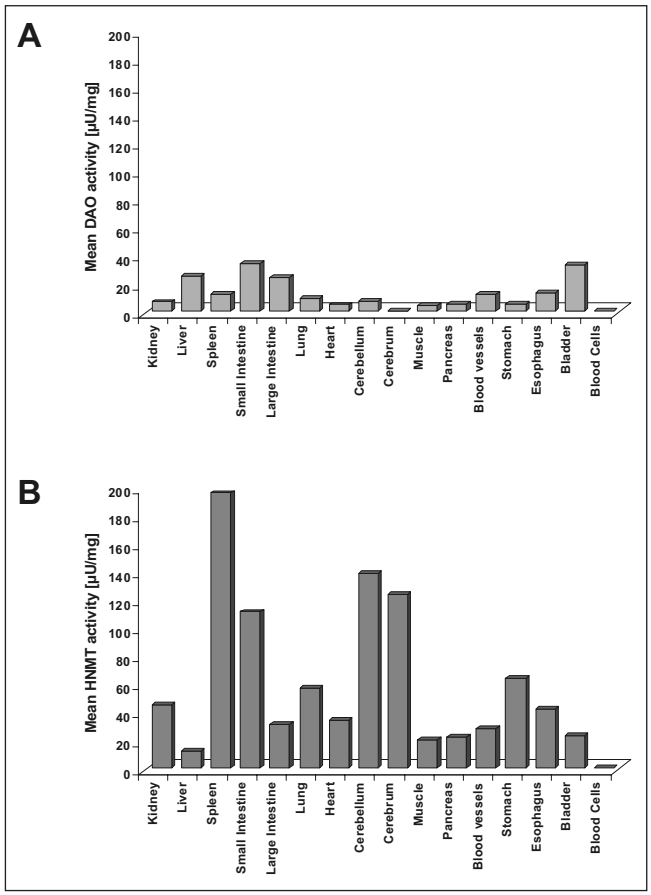

Fig. 1. Mean enzymatic activities of (A) DAO and (B) HNMT determined in cleared homogenates of guinea pig tissues (1 µU = 1 pmol/min).

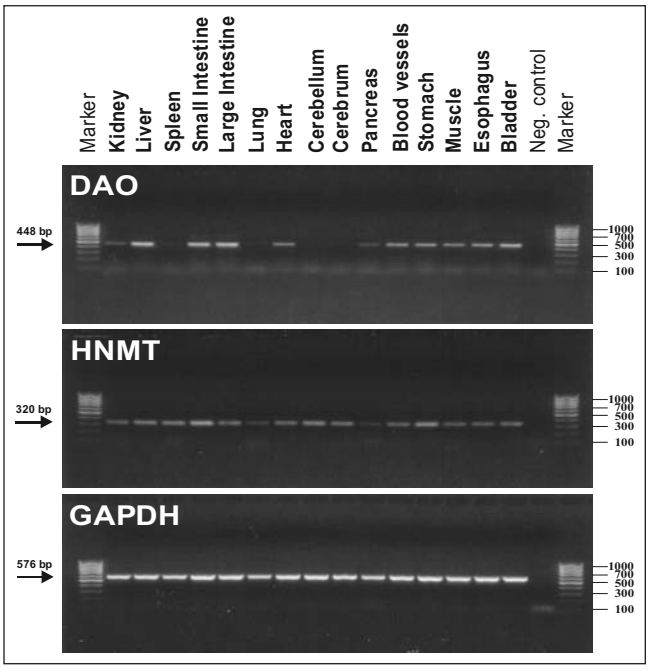

Fig. 2. Expression of DAO, HNMT and GAPDH mRNAs in guinea pig tissues. Representative agarose gels with ethidium bromide stained RT-PCR products are shown.

supports the notion that histamine constitutes an important signalling molecule in most mammalian tissues, which is supported by widespread expression of histamine receptors.

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