Determinations of histamine and some of tis metabolites and their clinical applications

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SUMMARY AND CONCLUSIONS

Histamine is a biogenic amine that is biosynthesized by the decarboxylation of the amino acid histidine. Its synthesis and storage mainly take place in tissue mast cells and in certain white blood cells, the basophilic granulocytes. In response to some immunological and non-immunological stimuli, histamine is "explosively" released from these cells. Histamine release can be either local or general. Localized release leads to itching and oedema in the skin or bronchoconstriction in the lung. A generalized, massive histamine release causes profound changes in the cardiovascular system and can induce a life-threatening anaphylactic shock. After its release histamine is rapidly deactivated by enzymatic transformations. The most important histamine metabolites are N^{τ}-methylhistamine, N^{τ}-methylimidazoleacetic acid and imidazoleacetic acid. As imidazoleacetic acid also originates from the metabolism of histidine this metabolite is less useful in studying the role of histamine. A small part of the released histamine and the vast majority of the histamine metabolites leave the body by urinary excretion.

For the study of the role of histamine in several diseases it is necessary to dispose of routinely feasible, sensitive and specific techniques, that are able to measure the low concentrations of both histamine and its metabolites in body fluids. As these were not available in our laboratory at the start of this study, in this thesis a number of newly developed procedures for the determination of histamine and its metabolites N^{τ}-methylhistamine and N^{τ}-methylimidazoleacetic acid are described.

For the determination of histamine and N^{τ}-methylhistamine in plasma and urine a so-called isotope dilution technique with mass spectrometric detection is used. In this technique a fixed amount of non-radioactively labeled histamine (c.q. N^{τ}-methylhistamine) is added to the samples. These stable isotopically labeled analoga are some mass units higher in mass than the naturally occurring compounds. After some extraction and derivatization procedures a gas chromatographic separation is performed using capillary columns, followed by mass spectrometric detection. Only the detection method discriminates between the naturally occurring compound and its labeled analog, and the ratio between the amount of both compounds accurately reflects the amount of the amine present in the original sample.

N^{τ}-methylhistamine and N^{τ}-methylimidazoleacetic acid in urine can also be determined by means of a gas chromatographic method with nitro-

gen-phosphorus detection. In this technique a fixed amount of synthetic homologues of these histamine metabolites is added to the urine as internal standard. After extraction and derivatization the mixtures are separated on a capillary column and the nitrogen-containing compounds selectively detected. The ratio between the naturally occurring substance and the internal standard is related to the amount of the histamine metabolite in the urine sample.

The normal values of histamine and N^{τ}-methylhistamine in blood plasma, and those of histamine, N^{τ}-methylhistamine and N^{τ}-methylimidazoleacetic acid in urine have been determined for healthy control persons. In contrast to the concentration of histamine and N^{τ}-methylhistmine in urine, the concentration of N^{τ}-methylimidazoleacetic acid appeared to be strongly dependent on the dietary histamine intake. Therefore, in case the urinary excretion of the latter metabolite is used for the determination of the histamine turnover, histamine containing foodstuffs (like sauerkraut and cheese) have to be avoided. The amount of histamine excreted in the urine is, especially for females, sometimes increased, without the occurrence of increased urinary histamine metabolites or symptoms associated with enhanced histamine production and/or liberation. These increases are most probably caused by bacterial histamine production in the vagina and/or the urological tract. For this reason, the amount of histamine in urine is not a reliable index for the plasma level of this amine. Under normal circumstances the histamine production by the bacterial flora in the gastrointestinal tract only contributes to a little extent to the amount of histamine and histamine metabolites excreted in urine.

The role of histamine in bronchial obstructive reactions of patients with bronchial asthma after allergen inhalation was studied by the determination of N^{τ}-methylhistamine in fractionated urine samples. It was demonstrated that the urinary excretion of this histamine metabolite is enhanced after the early bronchial obstructive reaction, whereas no increase was observed after the late bronchial obstructive reaction. It is concluded that during the early reaction mast cell degranulation does take place, but not during the late reaction.

Patients with a pathologically increased number of mast cells or basophilic granulocytes exhibit a strongly enhanced histamine production. In studying the urinary excretion of histamine and its metabolites it became clear that determinations of the metabolites of histamine provide a more reliable parameter for this enhanced production than the determination of histamine

itself. The determinations of N^{τ}-methylhistamine and N^{τ}-methylimidazoleacetic acid in urine appear to be useful parameters for the diagnosis and follow-up of patients with mastocytosis. This is especially important because this disease often shows no characteristic symptoms by which the diagnosis can be stated with certainty.

The release of histamine occurring during hypersensitivity reactions after wasp-stings or after intravenously administrated iodinated contrast media was studied by means of the determination of N^{τ}-methylhistamine in urine. The urinary excretion of this histamine metabolite is clearly enhanced in patients showing the symptoms of a hypersensitivity reaction, and the amount of this amine in urine correlates well with the severity of the reaction.

Finally the plasma histamine levels during surgical hip replacement were determined. It was investigated whether cement used for attaching the prothesis to the bone gives rise to histamine release. It was demonstrated that the plasma histamine levels are about two times enhanced during cement application. However, before it can be stated with certainty that this increase is indeed caused by the cement application it will be necessary to investigate the plasma histamine levels of a control group of patients receiving the same anaesthesia and comparable blood transfusions.

In conclusion it can be stated that the newly developed methods for the determination of histamine and its metabolites N^{τ}-methylhistamine and N^{τ}-methylimidazoleacetic acid are sensitive and specific, and offer the possibility to measure these compounds in urine routinely. The determination of histamine and N^{τ}-methylhistamine in plasma are less easy to perform and require, in addition to sample collection according to a strict protocol, an optimal condition of the measuring-instrument. The determinations of histamine metabolites in urine collected over a certain period of time provide a parameter for the mean histamine production- and/or liberation rate during this time. Because it is possible that bacteria in the urological tract and/or the vagina produce histamine, the determination of histamine in normally collected urines is of limited value. It is possible that urine collection by catheterization can overcome this problem. The level of histamine in plasma is, especially because of the very short half-life time of this amine in plasma, only related to the very moment of collection. Generally this can be considered as a disadvantage. Only when rapid and consecutive events have to be studied, each possibly inducing histamine

release, the determination of histamine in plasma will be preferable to the determination of histamine metabolites in urine.

Thus, surveying the significance of histamine and its metabolites in clinical chemistry, the determination of histamine metabolites in urine seems to acquire an important place in the diagnosis of patients with mastocytosis, and in the study of mechanisms of allergic and other hypersensitivity reactions. The determination of histamine in plasma, however, appears to be suitable only for some specific research questions.