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IN VIVO DEACETYLATION OF *N*-ACETYL AMINO ACIDS BY KIDNEY ACYLASES IN MICE AND RATS

A POSSIBLE ROLE OF ACYLASE SYSTEM IN MAMMALIAN KIDNEYS

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Summary

Deacetylations of *N*-acetylhistidine and *N*-acetyltryptophan were examined in vivo by their administration to mice and rats. *N*-Acetylhistidine accumulated preferentially in the kidney and was converted to histidine effectively by acylase I. Similar deacetylation of *N*-acetyltryptophan by acylase III was also observed. Acylase I and III activities in mouse kidney increased in parallel remarkably at the period of weaning. A hypothesis that the acylase system in mammalian kidneys is a mechanism acquired to utilize amino acids from exogenous and endogenous acyl derivatives including those derived from protein hydrolysis was offered.

Introduction

Mammalian tissues contain three acylases of different substrate specificities towards α-*N*-acyl amino acids. Acylase I (EC 3.5.1.14) acts on *N*-acyl derivatives of many amino acids, but it shows little or no activity towards *N*-acyl derivatives of aspartate and aromatic amino acids except for histidine [1,2]. Acylase II (EC 3.5.1.15) is specific to *N*-acylaspartate [1,2]. Recently, I found an acylase that acts preferentially on *N*-acyl-L-aromatic amino acids and the enzyme designated as acylase III [3]. Therefore, *N*-acyl derivatives of almost all amino acids are covered by these three acylases as substrates. In addition to these acylases, two specific acylases are known to be present in mammalian tissues, i.e., acyl-lysine deacylase (EC 3.5.1.17) [4] and *N*-acetyl-β-alanine deacetylase (EC 3.5.1.21) [5]. Comparing *N*-acetyl amino acids and *N*-formyl

amino acids as substrates toward acylase I, II and III, the former shows higher K_m values and the latter higher V values [6]. No sufficient acetyl or formyl amino acid as the substrate of acylases is known in mammalian tissues except for *N*-acetyl-L-aspartate in the brain [7], and the physiological role of these acylases has not been established. The common property of all these acylases is that their activities are particularly higher in the kidney than any other tissue [1,3—5,7]. In the present study, in vivo deacetylation of *N*-acetyl amino acids administered to mice and rats and the age dependency of acylases were examined and the physiological role of these acylases in the kidney was discussed.

Materials and Methods

Materials. For intraperitoneal injection, *N*-acetyl-L-amino acids and L-histidine HCl (monohydrate) were dissolved in 0.14 M NaCl and water, respectively. The pH of each solution was adjusted to about 7 with HCl or NaOH. The injection volume of the solution was 0.1 ml/10 g body weight. Male mice (ddI, 19—21 g, 4—5 weeks old) and rats (Wistar, 190—220 g) were used unless mentioned.

Enzymic activities in vitro. The reaction mixture contained 0.3 ml 0.2 M borate buffer (pH 8.0), a substrate (4 μmol) and enzyme solution in a final volume 1.0 ml. The reaction was carried out at 37° C for an appropriate period. The amount of free amino acids formed by the reaction was estimated as described previously [3,8].

Preparation of tissue extracts. Animals were decapitated and tissues were removed and stored in a solid CO_2 box (1—3 days). The tissues (0.1—1.5 g) were homogenized in 4—8 ml of ice-cold 0.4 M $HClO_4$. The homogenate was centrifuged (3000 rev./min, 5 min). The supernatant (4.0 ml) was neutralized to pH 5—6 with 2 M KOH in an ice bath and the precipitate was removed by centrifugation (3000 rev./min, 5 min). In the case of the experiments on mice, tissues from three mice were combined. The blood was also collected by the decapitation and treated in the same way as described on tissues. The neutralized supernatant (4—5 ml) was applied to a phosphocellulose column (0.6 × 3 cm) equilibrated with 0.03 M phosphate buffer (pH 6.2), and 2 ml of the buffer was passed through the column. The eluate from the column, of which volume was adjusted to 7.0 ml with water, was used for the determination of histidine, tryptophan and *N*-acetylhistidine. By this treatment, histamine, putrescine, spermidine and spermine that interfere with the determination of histidine were removed completely from the sample. Histidine, tryptophan and *N*-acetylhistidine were recovered completely (more than 90%) in the eluate from the phosphocellulose column.

Determination of histidine and tryptophan. An aliquot (1.0 ml) of the eluate described above was diluted to 3 ml with 0.02 M acetate buffer (pH 4.7) (in the assay of histidine) or 0.02 M acetic acid (in the assay of tryptophan) and applied to a SP-Sephadex column (0.6 × 3 cm). The procedures for the separation of both amino acids and fluorometrical determination were followed as described previously [8].

Determination of N-acetylhistidine. *N*-Acetylhistidine in the sample was con-

verted to free histidine by hydrolysis as follows. To an aliquot (3.0 ml) of the eluate described above, 70% $HClO_4$ was added to a final concentration of 1 M, and the mixture was incubated at 98—100°C for 12 h in the same way as described previously [9]. After the hydrolysis, the mixture was neutralized and histidine was determined as described above. From the value obtained from the hydrolyzed sample, the value obtained from non-hydrolyzed sample was subtracted. Although the values obtained in this way are the sum of histidine derived from *N*-acetylhistidine and other histidine-containing peptides, the values were expressed as those of *N*-acetylhistidine for convenience.

Results

Deacetylase activities in mouse tissues in vitro

In the present study, acylase I and III activities were represented with the activities towards *N*-acetyl-L-methionine and *N*-acetyl-L-tryptophan, respectively. Extracts from mouse tissues were assayed for the activities towards these substrates and *N*-acetyl-L-histidine (Table I). All activities were highest in the kidney and little in the blood. Separation of acylase I and III in mouse kidney by DEAE-cellulose column chromatography [3] showed that most of *N*-acetylhistidine was deacetylated by acylase I fraction. The deacetylation of *N*-acetylhistidine in this fraction was inhibited by *N*-acetylmethionine, i.e., 57% at 1 mM of *N*-acetylmethionine, 82% at 2 mM and 99% at 3 mM. This indicates that *N*-acetylhistidine is deacetylated by acylase I. In the following experiments in vivo, *N*-acetylhistidine and *N*-acetyltryptophan were used as substrates of acylase I and III, respectively, because of their sensitive fluorometrical estimation.

Deacetylation of N-acetylamino acids in mice and rats in vivo

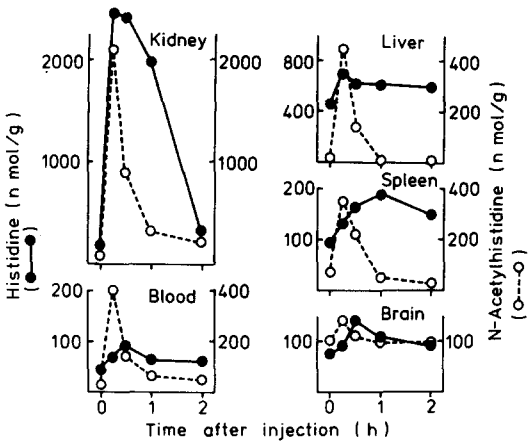
The changes in tissue levels of both histidine and *N*-acetylhistidine were examined on mice as a function of time after the injection of *N*-acetylhistidine (500 mg/kg, i.e., 2.5 μmol/g) (Fig. 1). In the kidney, extreme increase of histidine level was observed and the level was higher than that of *N*-acetylhistidine. The histidine level lagged behind the level of *N*-acetylhistidine. Both levels in the kidney at 15 min were similar to those at 7 min. In other tissues, the ele-

TABLE I

DEACETYLASE ACTIVITY TOWARD *N*-ACETYL AMINO ACIDS IN MOUSE TISSUES

Tissues from three mice were combined and homogenized with 10 vols. of 0.04 M borate buffer (pH 8.0) and the homogenate was centrifuged (20 000 × *g*, 20 min). An appropriate amount of the supernatant was assayed for the activities at the concentration of each substrate (4 mM). The values (mean from two experiments) are expressed as μmol/h per g tissues. Mice: 8 weeks old.

	N-Acetyl-L-methionine	*N*-Acetyl-L-histidine	*N*-Acetyl-L-tryptophan
Kidney	443	33.0	40.5
Liver	75	3.9	1.4
Brain	5	0.3	0.05
Spleen	7	1.1	0.08
Thymus	5	1.0	0.08
Blood	0	0.08	0.00



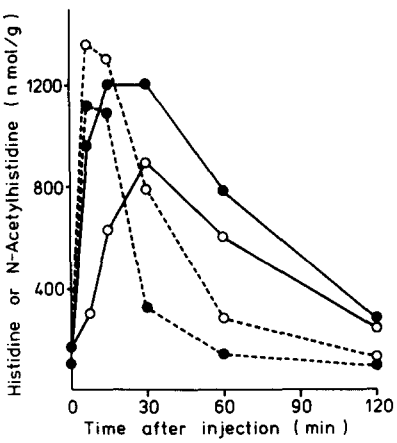


Fig. 1. Deacetylation of *N*-acetylhistidine in mice in vivo.

Fig. 2. Effect of *N*-acetylmethionine on the deacetylation of *N*-acetylhistidine in mouse kidney in vivo. ○———○, ○- - - - -○, the levels of histidine and *N*-acetylhistidine, respectively, after the mixed administration of *N*-acetylhistidine and *N*-acetylmethionine (each 250 mg/kg). ●———●, ●- - - - -●, the levels of histidine and *N*-acetylhistidine, respectively, after the administration of *N*-acetylhistidine only (250 mg/kg).

vation of histidine level was poor compared with that of the kidney and the elevation was retained longer than in the case of the kidney. These patterns except for the kidney are similar to that in the blood where acylase activity was little. In rat kidney, of which acylase I activity is about twice that of mouse kidney [3], the administration of 500 mg/kg *N*-acetylhistidine caused more elevation of histidine than that of mouse. The peak levels of histidine (at 1 h) and *N*-acetylhistidine (at 15 min) were about 3000 nmol/g and 500 nmol/g, respectively. The administration of *N*-acetyltryptophan (150 mg/kg, i.e., 0.6 μmol/g) to mice caused also remarkable elevation of tryptophan in the kidney (about 500 nmol/g at 15 min) and slight elevation in the liver (about 70 nmol/g at 15 min). These elevated tryptophan levels returned to the normal level within 1 h.

Inhibition of the deacetylation of N-acetylhistidine by N-acetylmethionine in mouse kidney in vivo

In order to check whether *N*-acetylhistidine is deacetylated by acylase I also in vivo like in vitro, the inhibition of its deacetylation by *N*-acetylmethionine was examined also in vivo (Fig. 2). As expected from the result in vitro, the deacetylation was inhibited by the administration of *N*-acetylmethionine. The inhibition was strongest at 7 min when the concentration of *N*-acetylmethionine seems to be highest, and the deacetylation appears to be restored with the decrease of the inhibitor. This indicates that *N*-acetylhistidine is deacetylated by acylase I also in vivo. Comparing the results in Fig. 1, the data in Fig. 2 in the absence of *N*-acetylmethionine shows that the increase of histidine in the kidney is dose dependent.

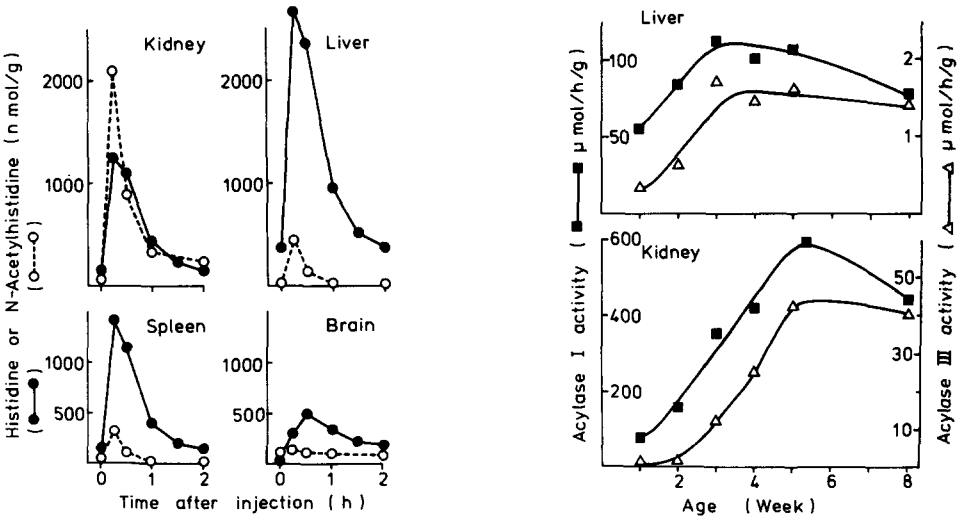


Fig. 3. The levels of *N*-acetylhistidine and histidine in mouse tissues after their respective administrations.

Fig. 4. Age dependency of acylase I and III in mouse kidney and liver. Acylase I and III activities were represented with the activities toward *N*-acetylmethionine and *N*-acetyltryptophan, respectively.

Comparison of the levels of N-acetylhistidine and histidine in mouse tissues after their administrations

In order to examine the uptake of *N*-acetylhistidine and histidine into mouse tissues, both levels were compared after their respective injection (each 2.5 μmol/g) (Fig. 3). Histidine accumulates in tissues more than *N*-acetylhistidine except for the kidney. On the other hand, the accumulation of *N*-acetylhistidine in the kidney is higher than that of histidine and is very low in other tissues with a low acylase I activity, on which the amount of accumulation depends also, i.e., its low activity is expected to increase the accumulation. Considering the loss of *N*-acetylhistidine by the deacetylation in the kidney and that the accumulation of histidine by the administration of histidine itself is lower than that caused by the administration of the same dose of *N*-acetylhistidine (see Fig. 1), *N*-acetylhistidine is more easily taken up in the kidney than histidine. These results indicate that *N*-acetylhistidine is taken up preferentially in the kidney with a high acylase activity.

Age-dependent increase of acylase activities

Fig. 4 shows the age dependency of the activities of acylase I and III in the kidney and liver of mice. Parallel increase of both activities was observed in both tissues. In the kidney, the activities increased remarkably at the period of weaning (3 weeks) and reached their plateau values at 5 weeks.

Discussion

The points clarified on acylases in mammalian kidneys from the present and previous studies are summarized as follows.

(1) The activities of all acylases that act on *N*-acyl amino acids are particularly higher in the kidney than any other tissue [1,3—5,7], especially in the cortex region of the kidney [3]. (2) Acyl derivatives of almost all amino acids are covered by acylase I—III as substrates. (3) *N*-Acetylhistidine administered to mice accumulated preferentially in the kidney and was converted effectively to free hsitidine by acylase I. (4) Acylase I and III activities in mouse kidney were very low for 2 weeks after birth, increased in parallel remarkably at the period of weaning (3 weeks) and reached plateau values at 5 weeks. Similar age dependency was also reported on rat acylase II [7]. (5) Dipeptides with uncharged N-terminal amino acids are also substrates of acylase I [10,11]. (6) No sufficient acetyl or formyl amino acids are known in mammalian tissues as substrates of acylases except for *N*-acetyl-L-aspartate in the brain [7], while many proteins are known to include *N*-acetyl N-terminal amino acid and *ε*-*N*-acetyllysine.

Additionally, marked deacetylation of *N*-acetylhistidine was also observed in rat kidney and *N*-acetyltryptophan that is deacetylated by acylase III was also deacetylated in mouse kidney. A part of hsitidine increase in tissues except for the kidney appears to be derived from the kidney, because acylase activities and the uptake of *N*-acetylhistidine are very low in these tissues, while, histidine uptake is easy, and histidine elevation in these tissues is corresponding with that in the blood with little acylase activity.

Considering these points, I speculate about the physiological role of acylases in the kidney as follows. The acylase system in mammalian kidneys is one of the mechanisms acquired to utilize amino acids from exogenous and endogenous acyl derivatives including dipeptides and *N*-acetyl amino acids derived from protein hydrolysis. The observation by Rose et al. [12] that acetylation of L-tryptophan enhances its usefulness in the maintenance of nitrogen equilibrium in human deprived of tryptophan appears to support this hypothesis.

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