METABOLISM OF N\textsuperscript{\textalpha}-METHYLHISTIDINE IN RATS

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N\textsuperscript{\textalpha}-Methylhistidine exists mostly, if not all, as one constituent of anserine in normal muscle (1). However, the free form of N\textsuperscript{\textalpha}-methylhistidine came to appear in the muscle of rats fed on a methionine-excess diet (2). Urinary excretion of N\textsuperscript{\textalpha}-methylhistidine was increased by a meat diet (3), a vitamin E-deficient (4), a histidine-excess (5) or a methionine-excess diet (2). On the other hand, orally or parenterally administered N\textsuperscript{\textalpha}-methylhistidine was quantitatively recovered in the urine and feces, and the urine contained unchanged N\textsuperscript{\textalpha}-methylhistidine together with a metabolite, N-acetyl-N\textsuperscript{\textalpha}-methylhistidine (6). However, the metabolism of administered N\textsuperscript{\textalpha}-methylhistidine remains obscure.

For the tritium labeling of N\textsuperscript{\textalpha}-methylhistidine, \textsuperscript{3}H\textsubscript{2}O was used. The labeled samples were purified with repeated lyophilization, recrystallization and ion-exchange chromatography of Dowex-50. The radiochemical purity was shown with paper chromatography using three different solvent systems; \textit{n}-butanol: acetic acid: water (4:4:1), phenol: water: NH\textsubscript{4}OH (775:215:10.4) and pyridine:\textit{n}-butanol (2:1).

Male Sprague-Dawley rats, weighing 70 to 80 g, were housed in individual screen bottom cages in a room maintained at 23 ± 1°C with 50% humidity and light regulation (12 hr light a day). Five animals were used in each experimental subgroup. Rats were given 250 \mu Ci of \textsuperscript{3}H-N\textsuperscript{\textalpha}-methylhistidine, having a specific activity of 100 \mu Ci per \mu mole, by subcutaneous injection. After the sacrifice of the rats, the liver, kidney and gastrocnemius muscle were quickly removed and provided for a sample of amino acid and peptide analysis. The maximum radioactivity in the blood was attained at fifteen minutes after the injection of \textsuperscript{3}H-N\textsuperscript{\textalpha}-methylhistidine and then exponentially dropped. The calculated half-life of the radioactivity in blood was 30 min. The incorporation of radioactivity into histidine and histidine derivatives in rat gastrocnemius muscle was analyzed with a preparative amino acid analyzer. No other radioactive peak except N\textsuperscript{\textalpha}-methylhistidine in rat gastrocnemius muscle was shown at 5, 10, 30, 60 or 120 min after \textsuperscript{3}H-N\textsuperscript{\textalpha}-methylhistidine
administration. Figure 1 showed the incorporation of radioactivity into histidine derivatives in muscle after one hour of $^3$H-$N^\alpha$-methylhistidine administration. At 24 hr after injection, no radioactivity of histidine derivatives, including $N^\alpha$-methylhistidine, was observed in gastrocnemius muscle.

The recoveries of radioactivity in 24-hr and 48-hr urines were 93.9 ± 4.5% and 98.4 ± 4.6%, respectively. $^3$H-$N^\alpha$-Acetyl-$N^\alpha$-methylhistidine was synthesized from $^3$H-$N^\alpha$-methylhistidine and acetic anhydride according to the method by Young et al. (6). The radiochemical purity was shown with paper and thin layer chromatographies and the ninhydrin reaction was negative for the authentic compound. The spectral data of $N^\alpha$-acetyl-$N^\alpha$-methylhistidine were confirmed with an infrared spectrometer. $N^\alpha$-Methylhistidine and $N$-acetyl-$N^\alpha$-methylhistidine were able to separate with thin layer chromatography in three different solvent systems. Figure 2 shows a thin layer chromatogram of the methanol-water solvent system. The urine contained unchanged $N^\alpha$-methylhistidine together with a metabolite identified by thin layer chromatography as $N$-acetyl-$N^\alpha$-methylhistidine (Fig. 2). In addition, $N^\alpha$-methylhistidine fractions revealed higher radioactivity in hydrolyzed urine than that in untreated urine with column chromatography indicated in Fig. 1. When the doses of administered $N^\alpha$-methylhistidine were changed to 0.02, 0.5, 1, 2 or 5 mg per rat, urinary radioactivities in 24-hr urines were over 90% at all dose levels and the radioactivities originating in $N$-acetyl-$N^\alpha$-methylhistidine were 64.5 ± 3.4, 64.8 ± 4.5, 73.0 ± 8.4, 66.6 ± 5.8 and 66.8 ± 5.6% of total radioactivity, respectively. From the analyses of amino acid and peptide fractions of the serum, liver and kidney at one hour after the injection of $^3$H-$N^\alpha$-methylhistidine, it was suggested that the greatest portion of administered $N^\alpha$-methylhistidine was
acetylated in the liver and kidney, then excreted in urine (Fig. 2). The acylation enzyme of N\textsuperscript{\alpha}-methylhistidine is under investigation. However, the above results revealed that following acetylation N\textsuperscript{\alpha}-methylhistidine was mostly recovered in urine as shown in N\textsuperscript{\alpha}-methylhistidine (6).

REFERENCES


