Effects of L-lysine administration on certain aspects of ascorbic acid metabolism in weanling rats

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Summary. L-lysine administration to male weanling rats at a dose of 110.4 mg (25% LD_{50}) per 100 g body weight per day for 15 days reduced the liver total ascorbic acid level. The biosynthesis as well as the degradation of L-ascorbic acid was diminished under these conditions. The fall in liver total ascorbic acid level after L-lysine administration was ascribed to its reduced synthesis.

Alterations in the growth patterns of animals have been reported by various investigators to result from ingestion of lysine in excess[2-5]. Supplementation of lysine has been found to produce biochemical changes in the animals[6,7] and also to alter the activities of different enzyme-systems[8-10]. High levels of dietary tyrosine have been reported to result in a marked reduction in the biosynthesis of L-ascorbic acid[11]. As weanling rats are found to be more susceptible to disproportionate amounts of amino acid than matured ones[12], the present investigation was carried out on them in order to evaluate the effects of L-lysine administration on ascorbic acid metabolism.

Male weanling albino rats weighing 25–35 g were divided into 2 groups: A and B, of equal average body weight and were maintained on a 18% casein diet. The other ingredients of the diet were the same as reported elsewhere[13,14]. Fat-soluble and water-soluble vitamins were furnished in the diet according to Berg[15].

The animals of the group A were injected intraperitoneally with L-lysine hydrochloride (solution adjusted to pH 7.4 with $NaHCO_3$) at a dose of 110.4 mg per 100 g body weight per day which is equivalent to 25% LD_{50}[16]. The treatment was done for a period of 15 days. The animals of the other group serving as pair-fed controls received injections of only the medium used to dissolve L-lysine.

After the last injection, the animals were fasted overnight and sacrificed under light ether anesthesia. Blood was collected from the hepatic vein by a heparinized syringe and plasma was separated by centrifugation. Their liver and kidney tissues were removed, chilled in ice and weighed. Liver tissue was homogenized in an all glass homogenizer with 4 vol. of cold 0.15 M KCl. The tissue extract was freed from nuclear and mitochondrial fractions by centrifugation at 9605 × g for 15 min. The supernatant was then centrifuged at 86,443 × g for 60 min in a Beckman L3-50 ultracentrifuge. The supernatant fraction was utilized for estimating dehydroascorbatase activity[17]. The sediment was resuspended in 0.15 M KCl (1 ml suspension = 0.5 g fresh liver tissue) and was subsequently utilized as enzyme source for determining L-ascorbic acid synthesizing ability[18]. The protein content of the liver homogenates used in the enzymatic studies were determined by the biuret method[19]. The total ascorbic acid content of the plasma, liver and kidney was estimated according to the method employed by Chatterjee et al.[13]. The results presented in table 1 demonstrated that the kidney and the liver weights remained unaffected following L-lysine administration for a period of 15 days. The total

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Table 1. Effect of L-lysine administration on liver and kidney weights and on the plasma and their total ascorbic acid content (the values are means ± S. E. M.)

Groups of animals	Plasma (mg/100 ml)	Liver Weight (g/100 g body weight)	Total ascorbic acid (μg/100 mg tissue)	Kidney Weight (g/100 g body weight)	Total ascorbic acid (μg/100 mg tissue)
Pair-fed control	1.91 ± 0.09 (5)	4.62 ± 0.10 (6)	25.0 ± 1.3 (5)	0.926 ± 0.40 (6)	20.0 ± 0.8 (6)
Treated	1.94 ± 0.10 (4) $p > 0.05$	4.89 ± 0.27 (6) $p > 0.05$	20.5 ± 1.2 (5) $p < 0.05$	0.924 ± 0.020 (6) $p > 0.05$	21.6 ± 0.9 (6) $p > 0.05$

The figures in the parentheses indicate the number of animals.

Table 2. Effect of L-lysine administration on the biosynthesis and degradation of L-ascorbic acid by the liver (the values are means ± S. E. M.)

Group of animals	L-ascorbic acid synthesized from D-glucuronolactone (μmoles/g microsomal protein)	from L-gulonolactone (μmoles/g microsomal protein)	Dehydroascorbatase activity (μmoles of 2–3 diketogulonic acid formed/g soluble supernatant protein)
Pair-fed control	97.4 ± 9.9 (4)	280 ± 19 (4)	639 ± 27 (5)
Treated	57.6 ± 4.9 (5) $p < 0.01$	188 ± 12 (5) $p < 0.01$	485 ± 29 (5) $p < 0.01$

The figures in the parentheses indicate the number of animals.

ascorbic acid content of the plasma and the kidney remained unaltered, whilst the liver total ascorbic acid level was markedly reduced following lysine treatment. The in vitro biosynthesis of L-ascorbic acid from D-glucuronolactone as well as from L-gulonolactone, as demonstrated in table 2, was found to be significantly reduced after lysine administration. This suggests that the fall in liver total ascorbic acid level after L-lysine administration might arise from the diminished synthesis of L-ascorbic acid. L-tyrosine at 5% level in the diet has also been found to depress the synthesis of L-ascorbic acid from D-glucuronolactone[11]. Administration of phenylalanine was reported to result in an inhibition of protein synthesis[12]. The diminution in the activities of D-glucuronoreductase and L-gulonooxidase after L-lysine administration may likewise be attributed to the reduced synthesis of the enzyme protein resulting from a depression of synthesis of protein as a whole.

Administration of L-lysine also produced a marked depression in the liver dehydroascorbatase activity (table 2). This suggests that the animal tries to compensate the loss in tissue ascorbic acid produced by the reduction in the rate of its biosynthesis. But the fact that the liver total ascorbic acid level still remained diminished signifies that the magnitude of reduction in the rate of ascorbic acid biosynthesis was more than that of the reduced rate of its degradation.

The absence of alteration in the kidney total ascorbic acid level after L-lysine administration may be ascribed to the unchanged plasma total ascorbic acid level under the same condition. Inspite of diminished synthesis of L-ascorbic acid by the liver, the plasma total ascorbic acid level remained unaltered after L-lysine administration. This absence of alteration in the plasma total ascorbic acid level might be due to supposedly diminished excretion of ascorbic acid in the urine. The possibility that the changes in ascorbic acid metabolism following repeated injections of L-lysine might be an artefact may be ruled out by the fact that the pair-fed control rats also received repeated injections of the medium used to dissolve the amino acid.

In the course of this investigation, it was also noted that food intake by the treated animals did not differ significantly from those of the control groups, and their body weight too remained unchanged. It therefore suggests that L-lysine administration at the present dose appears to have no effect on the general growth pattern or on the growth of different organs of the animal.

Hydrolysis of histones by horse urinary kallikreins

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Summary. Horse urinary kallikrein was shown to hydrolyze histones from calf thymus and chicken nuclei erythrocytes. The hydrolysis is inhibited by benzamidine and hyposulfite but not by soy-bean trypsin inhibitor; horse plasma kallikrein also produces this hydrolysis.

Kallikrein (EC 3.4. 21.8) is the general designation for serine proteases which liberate hypotensive peptides (kinins) from a plasma protein, kininogen. These enzymes have been found in several tissues and biological fluids such as plasma, glandular secretions and urine. Although they have some properties similar to those of trypsin, mainly in their ability to hydrolyze N-α-substituted-arginine esters, they show a much higher proteolytic specificity towards its natural substrate, kininogen[3]. Horse urinary kallikrein (HoUK), a glandular kallikrein which liberates lysyl-bradykinin from horse kininogen, does not cleave either casein or hemoglobin[4,5] but it was reported to hydrolyze salmine[5] and polyarginine[6]. These findings raised some doubts whether kallikreins or contaminants were responsible for this hydrolysis[7], but the results on specific inhibition are in favour of hydrolysis by kallikrein itself; hog pancreatic kallikrein was also said to hydrolyze salmine[8].

The studies reported in this communication were undertaken as an attempt to confirm the observation of non-specific proteolysis of salmine and to extend these studies to the action of HoUK on histones, proteins which have also a large basic amino acid content.

Material and methods. Horse urinary kallikrein (HoUK) was prepared by procedures already described[4,9]. The enzyme activity was followed by its esterase activity on N-α-tosyl-L-arginine-methyl ester (TAME), under the conditions described for human plasma kallikrein[10]. An enzyme preparation with specific activity 10.1 units/mg (μmoles TAME hydrolyzed per min) was used throughout