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Studies on urea synthesis: effect of protein intake on deamination of L-amino acids

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Biochimica. — *Studies on urea synthesis: effect of protein intake on deamination of* L*–amino acids.* Nota di FRANCESCO CEDRANGOLO, VINCENZO ZAPPIA, PATRIZIA GALLETTI e ADRIANA OLIVA, presentata (*) dal Corrisp. F. CEDRANGOLO.

RIASSUNTO. — Si studia sperimentalmente la velocità di deaminazione di vari L–aminoacidi nel fegato di ratti a dieta normale (gruppo I) e in paragone in quello di ratti tenuti per un certo tempo a dieta iperproteica (gruppo II). Si trova che la velocità di questa reazione è praticamente la stessa nei due gruppi di animali. Un aumento della deaminazione si riscontra tuttavia nei ratti del gruppo II per l'aminoacido L–istidina e per la L–treonina. I risultati sono discussi anche sulla base dei lavori di Schimke e *coll.*, secondo i quali una dieta iperproteica sarebbe in grado di indurre gli enzimi ureogenetici.

INTRODUCTION

It is generally accepted that " free " ammonia, originated from L-amino acids, is an intermediate in the urea biosynthesis [1, 2].

Several lines of evidences have been collected in this laboratory indicating an alternative urea pathway apart from the Krebs-Henseleit cycle, which does not imply ammonia as precursor of urea nitrogen [3–8].

In recent studies [9, 10] ammonia production *in vitro* from homogenates of tissues has been compared in two groups of rats, fed respectively with normal (group I) or high protein diet (group II). The increase in urea excretion *in vivo*, observed in rats of group II, was not paralleled by an increase in ammonia formation *in vitro* [10].

On the other hand Schimke and coworkers [11–13] have reported that rat liver adapts quite readily to an increase of protein intake by increasing the levels of urea cycle enzymes.

Similar results have been reported from studies performed with primates [14].

In order to evaluate if deamination of L-amino acids is the mechanism responsible for the production of " free " ammonia which, according to the cycle of Krebs, is transformed into urea, the effect of dietary protein intake on L-amino acid deamination has been investigated.

Preliminary results of this work have been reported [15].

MATERIALS AND METHODS

Glycine and L-amino acids were obtained from Sigma Chemical Co. The presence of the only L-isomer was checked with purified D-amino-acid-oxidase (Hog kidney), obtained from Worthington Biochemical Corp. Preparation with D-isomer impurities were discarded.

(*) Nella seduta del 9 marzo 1974.

All other chemicals were analytical-grade preparations obtained from the usual commercial sources. Glass-distilled water was passed through ion-exchange resin as Amberlite IR (H^+ form) in order to avoid the presence of traces of ammonia. Nessler's reagent was prepared following Vanselow's directions [16]. Saturated borate NaOH buffer, for ammonia microdiffusion, was prepared according to Reinhold and Chung [17].

The enzymatic deamination of natural amino acids was measured on the basis of ammonia production. The values were corrected for the ammonia produced in blanks without substrate.

Ammonia was assayed by the Conway-Seligson [18] microdiffusion technique modified by Cedrangolo *et al.* [19]. The pH during microdiffusion was controlled at 11 ± 0.2 to obtain complete recovery of free ammonia present in the tissue and to avoid formation of artifactual ammonia from unstable amides, proteins etc. [19].

TABLE I

Ammonia production from L-amino acids in rat liver

AMINO ACIDS	GROUP I (controls)	GROUP II (high protein intake)
	Ammonia formed (μmoles/sample/hour)	
Glycine	+ 0.43±0.09 (*)	+ 0.53±0.10
L-leucine	+ 0.28±0.08	+ 0.18±0.09
L-arginine	+ 0.25±0.11	— 0.48±0.07
L-citrulline	+ 0.83±0.20	+ 0.37±0.10
L-threonine	+ 0.56±0.08	+ 5.03±0.80
L-cysteine	+ 0.91±0.08	+ 0.63±0.07
L-histidine	+ 1.77±0.09	+13.47±1.40
L-valine	+ 0.14±0.06	+ 0.18±0.15

(*) Standard deviation.

The incubation mixture contained: 1 ml of 30% homogenate, 100 μmoles of L-amino acids as indicated in the table, 10 μmoles of $MgCl_2$, 100 μmoles of phosphate-buffer pH 7.4, in a final volume of 3 ml. The incubation was carried out at 37° C for 1 hour.

Wistar male rats, weighing about 200 g were obtained from a breeding farm (Morini, Reggio Emilia, Italia). They were divided in two groups: the first one fed with normal diet (Group I), the second fed with high protein diet (raw cow meat) (Group II). After four days the animals were sacrificed and the freshly excised livers were rinsed with cold phosphate-buffer (KH_2PO_4—

—K_2HPO_4) 0.1 M pH 7.4. The homogenates (30% *v/v*) were performed in the same buffer in a glass Potter-Elvehjem apparatus with teflon pestle at 1,000 rpm. 1 ml homogenate was transferred in 25 ml flasks and incubated at 37° C in a Dubnoff shaking–bath in presence of the various L-amino acids. The composition of incubation mixture is indicated in the Table I. At the end of the incubation 1 ml samples were mixed directly with 2 ml of borate-buffer in microdiffusion bottles. These were rapidly stoppered with a rubber cap fitted with a glass rod. The bottom end of the rod was wetted by dipping into 5 N H_2SO_4. After microdiffusion the ammonia was measured by rinsing the glass rod end with 10 ml of diluted Nessler's reagent.

RESULTS AND DISCUSSION

Table I shows that deamination of glycine, L-leucine, L-arginine, L-citrulline, L-cysteine and L-valine is not increased in rats of group II.

This results suggest that the " general L-amino-acid-oxidase " (leucine and valine are substrates of this enzyme) is not induced by the increased protein intake, and indirectly confirm that this enzyme is not involved in the pathway of urea biosynthesis. In fact if L-amino acid-oxidase had a physiological role connected with the five enzymes of urea cycle (carbamyl phosphate synthetase, ornithine carbamyl transferase, arginino succinate synthetase, arginino succinate lyase and arginase), the demonstrated induction of the latter enzymes [11-14] should be paralleled by an induction of L-amino acid-oxidase.

In addition a decrease of deamination of L-arginine and L-citrulline is observable in rats of group II. This finding could be explained taking into account that these amino acids are substrates of two enzymes of urea cycle, which are induced by high protein diet [11-14].

The reported results, together with previous data [10] indicating the absence of ammonia increase in liver of rats of group II, suggest that " free " ammonia is not an obligate metabolite in the pathway of urea biosynthesis.

Among the amino acids tested, only L-threonine and L-histidine showed a significant increase of deamination in the group of rats fed with high protein diet (see Table I). Deamination of threonine in liver homogenates could be catalyzed by threonine dehydrase (E.C. 4.2.1.16) present in this tissue and ammonia can be produced from histidine through urocanic acid pathway [20]. The physiological significance of the induction of these latter enzymes is not clear at the moment and deserves further investigation.

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