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INHIBITION OF ARGININE-GLYCINE AMIDINOTRANSFERASE BY ORNITHINE

A POSSIBLE MECHANISM FOR THE MUSCULAR AND CHORIORETINAL ATROPHIES IN GYRATE ATROPHY OF THE CHOROID AND RETINA WITH HYPERORNITHINEMIA

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Summary

The inhibitory effect of ornithine on L-arginine:glycine amidinotransferase (EC 2.1.4.1) was studied in crude rat kidney homogenates. The enzyme activity was linear with time up to 45 min and with protein up to 200 μg. The apparent K_m and V of amidinotransferase were 9.21 mM and 1.53 μmol/g protein per min, respectively. The enzyme was competitively inhibited by ornithine, with a K_i of 0.253 mM. Kidney arginase was inhibited only slightly and non-competitively. The inhibition of amidinotransferase by ornithine may thus be important in creatine biosynthesis. In gyrate atrophy of the choroid and retina with hyperornithinemia, a human autosomal recessive disease caused by decreased ornithine aminotransferase activity, plasma ornithine concentrations are elevated 10—20-fold (0.65—1.35 mM during fasting). In consequence, endogenous creatine production probably is severely decreased because of inhibition of the rate-limiting transamidination step by ornithine. The deficiency of creatine and further of readily available energy in the form of phosphocreatine is suggested to be involved in the pathogenesis of the choroidal, retinal and type II muscle fiver atrophies in gyrate atrophy.

Introduction

The rate-limiting step in the biosynthesis of creatine is L-arginine:glycine amidinotransferase (EC 2.1.4.1) (glycine amidinotransferase). In the ureotelic

rat, the enzyme has its greatest activity in kidney and pancreas [1] in the uricotelic chicken in kidney and liver [2] and in man in kidney and pancreas, although it is present in several other human tissues, including liver, muscle and brain [3]. In rat kidney the enzyme is located in the inner mitochondrial membrane [2] and no coenzymes or activators are needed for the reaction. Endogenous and exogenous creatine regulate amidinotransferase activity through a negative feedback system.

Arginine is broken down in the urea cycle through cleavage by arginase and in creatine biosynthesis by amidinotransferase. The bulk of the arginine is probably used in the first reaction. Both enzymes produce ornithine, which is oxidized further, used for polyamine synthesis, or recycled to arginine in the urea cycle.

The creatine concentrations in serum and urine are low in hyperornithinemia with gyrate atrophy of the choroid and retina [4,5], an autosomal recessive disease. Night blindness and progressive myopia are noticed by the age of 5 to 9 years, and the visual fields gradually become restricted; by age 30 the patients are practically blind. They also have type II muscle fiber atrophy [6]. Plasma and urinary ornithine concentrations are increased 10—20-fold. As inhibition of amidinotransferase by ornithine is a possible mediator in the pathogenesis of the eye and muscle changes in gyrate atrophy, I studied the influence of ornithine on the activity of rat kidney amidinotransferase.

Materials and Methods

Animals and tissue preparation. Three male rats of the Sprague-Dawley strain (weighing 300—400 g) were killed by decapitation. The animals had been allowed to eat and drink ad libitum. The kidneys were removed and decapsulated, and cortexes dissected out, weighed, homogenized in a Potter-Elvehjem homogenizer in 4 vols. (w/v) 0.1 M sodium phosphate buffer (pH 7.5), and freeze-thawed three times. The homogenized tissues were stored at −18°C without any loss of enzyme activity during one month. Kidney homogenates of individual rats were handled separately. The values presented are means of the results for individual homogenates.

Chemicals. L-Arginine-HCl was purchased from Sigma (St. Louis, MO, U.S.A.), glycine from Merck (Darmstadt, F.R.G.), and L-ornithine-HCl from Fluka (Buchs, Switzerland). L-[*guanidino*-^{14}C]Arginine-HCl came from the Radiochemical Centre (Amersham, U.K.).

Analytical methods

Amidinotransferase and arginine assays. The 250 µl incubation volume contained 50 µl homogenate, 1.25—3.75 µmol L-arginine, 2—6 µmol glycine and 1 µCi L-(guanidino-^{14}C)arginine in 0.1 M sodium phosphate buffer (pH 7.5). For blanks, the same mixtures were used without glycine in amidinotransferase assay and water was used instead of enzyme in arginase assay. Closed tubes were incubated in a Dubnoff shaker at 37°C for 20 min and the reaction was stopped by heating at 100°C for 2 min. After cooling and centrifugation, 10 µl of the supernatant was pipetted onto a silica gel thin-layer chromatography plate (Machery and Nagel Co, Düren, F.R.G.) and run 10 cm in one direction in

n-butanol/acetone/glacial acetic acid/water (35 : 35 : 10 : 20, v/v). Guanidinoacetate, urea and arginine were located by cutting the plate into 0.5 cm strips, eluting guanidinoacetate in 0.25 ml 1 N NaOH and developing color with the Voges-Proskauer reaction [7]. The R_F values for arginine, guanidinoacetate and urea were 0.5, 4.5 and 6.5 cm, respectively. In ultraviolet light fluorescent spots were present at the site of guanidinoacetate and urea and in further experiments these were successfully used for location. The bands corresponding to guanidinoacetate and urea were cut out, 200 μl water were added, and the bands counted in Aqualuma (Lumac, Basel, Switzerland) with an Ultrabeta (Wallac, Turku, Finland) liquid scintillation counter. Urea was also determined separately by trapping the CO_2 liberated from the reaction mixture by urease. The mean count was 10% lower, either because some impurities were counted in the first system with urea or because some counts were lost during collection of CO_2.

Results

Autoradiographs of the TLC plates contained by far the highest radioactivity at the site of arginine and urea was more prominent than guanidinoacetate. No other radioactive products were found. Amidinotransferase activity was linear with time for more than 45 min and the homogenate up to 200 μg protein. The Lineweaver-Burk double-reciprocal plot of the amidinotransferase activity in the rat kidney homogenate gave a K_m for arginine of 9.21 mM. The V was 1.53 μmol/g protein per min.

Inhibition of the amidinotransferase by ornithine was clear and linear up to 4.17 mM ornithine. The guanidinoacetate produced at higher ornithine concentration was below the detection limit of the assay. The K_i of ornithine for amidinotransferase was determined at 4.17, 8.33 and 12.5 mM arginine with ornithine concentrations between 1.67 and 3.33 mM. The reciprocals of the

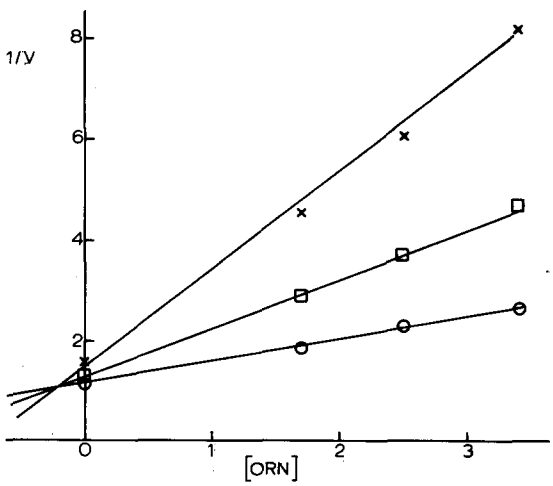

Fig. 1. The inhibition constant of ornithine for the amidinotransferase reaction according to the method of Dixon. The different arginine concentrations were 4.17 (X); 8.33 (□); and 9.21 mM (○).

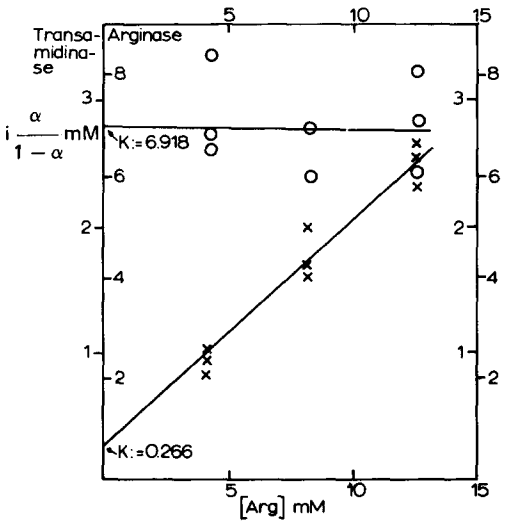

Fig. 2. Ornithine inhibition of amidinotransferase and arginase reactions, according to Hunter and Downs [20]. Arginase (o———o); amidinotransferase (X———X).

reaction velocities plotted against the inhibitor concentration by the method of Dixon [8] gave a common point of intersection (Fig. 1). The inhibition was competitive and the inhibition constant K_i for ornithine was 0.253 ± 0.039 mM. In these assay conditions, which are not ideal for arginase, the molar production of urea by rat kidney homogenate is 4—5 times that of guanidinoacetate. Inhibition of arginase by ornithine is weak and noncompetitive, the K_i for ornithine being 6.9 mM (Fig. 2) far above the physiologic level.

To emphasize the differences between the types of inhibition in the amidinotransferase and arginase reactions, the inhibitor constants were presented according to the method of Hunter and Downs [20]. For each observation a velocity ratio was calculated ($\alpha = v_i/v$). When the substrate concentrations were plotted against the corresponding $i(\alpha/1-\alpha)$, the noncompetitive inhibition in the arginase reaction gave a horizontal straight line, which was independent of substrate concentration and cut the vertical axis at K_i = 6.9 mM. The amidinotransferase reaction was presented by a line with a slope K_i/K_m = 0.180 (Fig. 2).

Discussion

The K_m of amidinotransferase in rat kidney homogenate (9.21 mM) was greater than formerly published for hog kidney amidinotransferase (2.27 mM) [8]. Both assays were done with crude tissue homogenates, but the present measurements were based on a system that is probably more reliable; the differences observed are presumably due to differences in both methodology and species, and in the diets of animals.

The end product negative feedback control of amidinotransferase by creatine concentration in vivo is well documented [1]. This study shows that ornithine concentration is also an important inhibitor of the amidinotransferase reaction in vitro and thus is able to suppress creatine synthesis. As the kidney is the

most important organ for amidinotransferase in the rat and in man [3], these results can probably be applied directly to the whole creatine production. The inhibitor constant for ornithine (253 ± 29 μM) was clearly higher than the plasma ornithine concentration in normal man (58 ± 14 μM). Thus, the inhibition of amidinotransferase by ornithine probably is mild at physiologic ornithine and arginine concentrations.

Another possible mechanism for controlling amidinotransferase activity in vivo could be the subcellular localization of the enzyme together with selective transport of the substrate and inhibitors. In rat kidney, amidinotransferase is located on the outside of the inner mitochondrial membrane [11], but in chicken liver it is present in the mitochondrial matrix [2]. Thus, in rat kidney, amidinotransferase and arginase compete for the same cytoplasmic arginine pool, but in chicken liver creatine synthesis involves the transport of arginine through the mitochondrial membrane.

In man, amidinotransferase activity is diminished in thyrotoxicosis [12] and myotonic muscular dystrophy [12]. In the latter acreatinuria is also present, probably reflecting low creatine production of the body. Neither plasma nor tissue ornithine concentrations have been reported in these diseases but they are presumably normal.

Another disease with a constant hypocreatinemia and hypocreatinuria is gyrate atrophy of the choroid retina, in which the plasma and urinary ornithine concentrations are increased 10—20-fold [6]. The fibroblasts and lymphocytes of the patients show an almost total absence of L-ornithine-2-oxoacid aminotransferase (EC 2.6.1.13) [13—16]. The mechanism of the atrophies in the choroid and retina and in the type II muscle cells has remained obscure. According to the present results, ornithine is a potent inhibitor of amidinotransferase in homogenized rat kidney but has only a small effect on arginase. If these results apply to other tissues as well, a rat would be totally or almost totally dependent on exogenous creatine if it had ornithine concentrations 10—20 times that of arginine. We suggest that this inhibition of transamidination by ornithine could be one of the mechanisms responsible for the degenerative processes in gyrate atrophy. Patients with this disease are deficient in creatine and in phosphocreatine. In some situation this would create a lack of readily available energy, which might then lead to atrophy. The facts in favor of this hypothesis so far are the low plasma and urinary concentrations of creatine in patients with gyrate atrophy [6] and the inhibition of amidinotransferase by ornithine concentrations of the magnitude regularly found in the patients. The activity of amidinotransferase in retina or pigment epithelium has not been reported, but they have a high ATP concentration and ATPase activity [17]. In muscle almost the sole source of energy for ATP formation is phosphocreatine [18]. The brain is also normally capable of producing energy and has amidinotransferase activity [19]. Thus it is possible that in all these organs the atrophies result from inhibition of creatine synthesis by ornithine in the rate-limiting amidinotransferase reaction.

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