In vitro INFLUENCING OF BRAIN γ-GLUTAMYL TRANSPEPTIDASE ACTIVITY BY SOME AMINO ACIDS

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Partly purified mouse brain γ-glutamyl transpeptidase was used as a tool for studying differences in the ability of some amino acids to stimulate the reaction catalyzed by this enzyme. The maximum enhancement of the reaction rate was achieved at pH 6·5—7·5. The values of the kinetic constants, K_m and V_{max} of this reaction were determined for various amino acids as substrates. The possible causes of differences in influencing the γ-glutamyl transpeptidase reaction by the individual amino acids tested are discussed from the viewpoint of relations and differences between these constants.

γ-Glutamyl transpeptidase catalyzes the transfer of the γ-glutamyl residue from glutathione to acceptor amino acids according to the following reaction:

$$\text{glutathione} + \text{amino acid} \rightleftarrows \gamma\text{-glutamyl amino acid} + \text{cysteinyl-glycine}$$

This reaction was reported first by Hanes and coworkers[1,2]. It is assumed that the interaction of γ-glutamyl transpeptidase with amino acids and the γ-glutamyl residue is one of the main steps in amino acid transport mediated by the γ-glutamyl cycle[3-5]. It has been shown in histochemical studies that γ-glutamyl transpeptidase activity is connected with cell membranes especially where a high rate of amino acid transport is assumed. The enzyme is heavily concentrated in the brush border of proximal convoluted tubulus of the kidney, in intestital epithelium and also in *plexus chorioideus* and brain capillaries[6-10]. The activity of γ-glutamyl transpeptidase in brain homogenate and isolated brain capillaries and its changes during the postnatal development were determined also biochemically[11-13]. Since *plexus chorioideus* and brain capillaries are considered a functional part of the blood-brain barrier, γ-glutamyl transpeptidase, which is significantly located in these parts, is regarded as the enzyme connected with the transport of certain amino acids between blood and brain[11,13].

The model substrate most commonly used for the determination of the activity of γ-glutamyl transpeptidase preparations is γ-glutamyl-*p*-nitroanilide[14] which simultaneously serves as donor and acceptor of the γ-glutamyl residue:

$$2(\gamma\text{-glutamyl-}p\text{-nitroanilide}) \rightarrow \gamma\text{-glutamyl-}\gamma\text{-glutamyl-}p\text{-nitroanilide} + p\text{-nitroaniline}$$

The rate of this reaction catalyzed by the enzyme from rat kidney can significantly be enhanced

by the addition of acceptors of the γ-glutamyl residue, which are various amino acids and peptides[15]:

$$\gamma\text{-glutamyl-}p\text{-nitroanilide} + \text{acceptor} \rightarrow \gamma\text{-glutamyl acceptor} + p\text{-nitroaniline}$$

In this study the effect is examined of certain amino acids on the activity of a partly purified preparation of mouse brain γ-glutamyl transpeptidase. The problem of differences in pH necessary to attain both the optimal rate of reaction catalyzed by this enzyme and also maximal stimulation by the addition of acceptor amino acids are considered. The ability of individual amino acids to increase the reaction rate is compared from the viewpoint of the corresponding values of Michaelis constants (K_m) and maximal reaction rates (V_{max}).

EXPERIMENTAL

Material: γ-L-Glutamyl-*p*-nitroanilide and L-amino acids were purchased from Sigma Chemical Co. γ-Glutamyl transpeptidase was isolated from cerebral cortex of 6—7 weeks old white male mice (inbred strain A) and partly purified as described elsewhere[15,16].

The activity of γ-glutamyl transpeptidase was determined with γ-glutamyl-*p*-nitroanilide as γ-glutamyl donor[15,17]. The reaction mixture (1 ml) contained 2·5 mM γ-glutamyl-*p*-nitroanilide, 80 mM Tris-HCl at various pH-values, 75 mM-NaCl, the L-amino acid (the concentrations are given in Fig. 2), and the enzyme (about 0·5 mg of protein). Following 60-min incubation at 37°C, the reaction was discontinued by the addition of 2 ml of 1·6M acetic acid. The quantity of *p*-nitro aniline liberated was determined at 410 nm in a Specord-UV-VIS spectrophotometer (Zeiss, Jena). The specific activity of the enzyme was expressed in units per mg of protein. One γ-glutamyl transpeptidase unit liberates one nanomol of *p*-nitroaniline at 37°C in one minute under the conditions described above.

The protein content of the enzyme preparation was determined by the method of Lowry and coworkers[18].

The maximum reaction rates (V_{max}) and Michaelis constants (K_m) were determined from Lineweaver-Burk reciprocal plot of reaction rate values *versus* the corresponding concentrations of acceptor amino acids[19].

The results given here are means of five experiments at least.

RESULTS AND DISCUSSION

The effect of pH on the transpeptidation reaction catalyzed by kidney γ-glutamyl transpeptidase has been investigated earlier with γ-glutamyl-*p*-nitroanilide and γ-glutamyl-α-naphthylamide as donors of the γ-glutamyl residue. It has been shown that the optimum pH-values for this reaction vary between 7·0 and 9·5 (ref.[15,20–22]). In this study, the rate was examined of the reaction catalyzed by partly purified mouse brain γ-glutamyl transpeptidase at various pH-values. It is shown in Fig. 1 that both in the absence and in the presence of acceptor amino acids the maximum reaction rate was achieved at pH-value close to 9·0. This pH value, however, cannot be

regarded as optimal from the viewpoint of relative increase of reaction rate resulting from the addition of acceptor amino acids. Maximum stimulation of the reaction rate after the addition of L-alanine or L-glutamine was observed at pH 6·5, after the addition of L-glutamate or L-methionine at pH 7·0, and after the addition of L-asparagine or L-methionine-D,L-sulfoximine at pH 7·5. The postulated physiological role of γ-glutamyl transpeptidase in the transport of amino acids in the brain[11,13] manifests itself most likely according to the scheme[15] which consists in the reaction of a temporary γ-glutamyl–enzyme complex with the acceptor amino acid giving rise to the γ-glutamyl amino acid. Since with brain γ-glutamyl transpeptidase the difference between the nonstimulated rate of transpeptidation reaction and the enhanced rate after the addition of the acceptor amino acid is the greatest at pH 6·5 to 7·5, it can indirectly be concluded that these pH-values are optimal for the transport function of the enzyme discussed also *in vivo*.

The ability of certain amino acids to stimulate the reaction catalyzed by partly purified brain γ-glutamyl transpeptidase follows from Fig. 2. Each amino acid was tested at pH 9·0 and at the pH-value at which the enhancement of reaction rate is

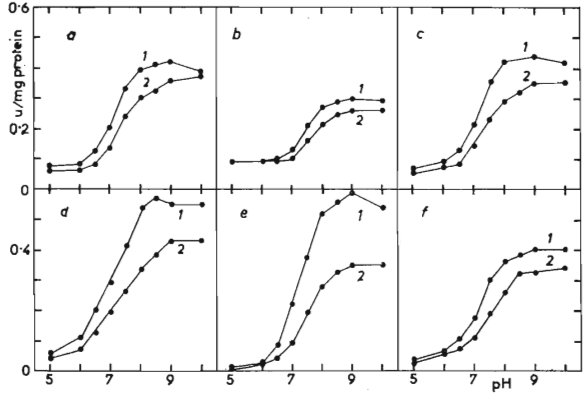

FIG. 1

Effect of pH on Activity of Mouse Brain γ-Glutamyl Transpeptidase

The enzymatic activity was determined with γ-glutamyl-*p*-nitroanilide as substrate as described in the text. As acceptors served 30 mM L-alanine (*a*), 10 mM L-glutamate (*b*), 10 mM L-methionine-D,L-sulfoximine (*c*), 10 mM L-glutamine (*d*), 10 mM L-methionine (*e*), and 30 mM L-asparagine (*f*). *1* activity in presence of acceptor amino acid, *2* activity in absence of acceptor amino acid.

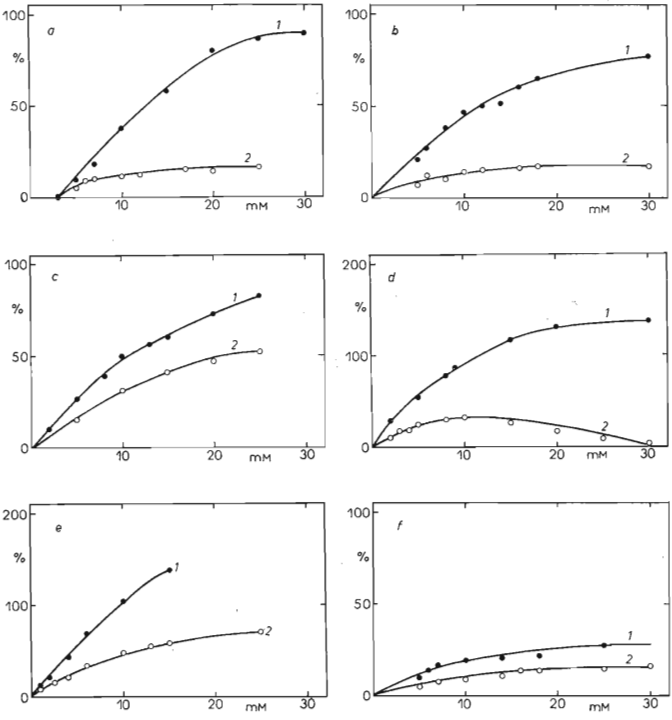

FIG. 2

Dependence of Activity of Mouse Brain γ-Glutamyl Transpeptidase on Increasing Concentration of Acceptor Amino Acid

The activity of the enzyme was measured as described in the text. The effect of various concentrations of acceptor amino acids on the activity of the enzyme was examined at pH 9·0 and at the pH at which maximum enhancement of enzymatic activity was observed. The size of the stimulation of reaction rate by the individual amino acids is expressed as increase of enzymatic activity (in per cent) above the level corresponding to the reaction in the absence of the acceptor. As acceptors served L-alanine (*a*), L-glutamate (*b*), L-methionine-D,L-sulfoximine (*c*), L-glutamine (*d*), L-methionine (*e*), and L-asparagine (*f*); 1 pH 7·5 (L-asparagine and L-methionine-D,L-sulfoximine), pH 7·0 (L-methionine and L-glutamate), pH 6·5 (L-alanine and L-glutamine); 2 pH 9·0.

maximum. The increase of the concentration of individual amino acids resulted (except for L-glutamine at pH 9·0) in an increase of the reaction rate. The decrease of the reaction rate at pH 9·0 as a result of increasing the concentration of L-glutamine above 10 mM has been observed and reported by this Laboratory earlier[16]. An increase of the reaction rate above the level corresponding to the reaction in the absence of the amino acid acceptor was at all concentrations more marked at pH 6·5–7·5 than at pH 9·0. It also follows from Fig. 2 that the ability of the individual amino acids to stimulate the γ-glutamyl transpeptidase activity considerably differs. Of the amino acids examined the stimulation at pH 9·0 is best with methionine, methionine-D,L-sulfoximine, and glutamine (up to 10 mM concentration). Very good stimulators at pH 6·5–7·5 are besides methionine and glutamine also alanine, slightly worse are methionine-D,L-sulfoximinine and glutamate. This finding is in very good agreement with the data on the acceptor activity of various amino acids at pH 8·0 or 8·8 as regards the reaction catalyzed by kidney γ-glutamyl transpeptidase[15,22].

The Lineweaver–Burk plot of reciprocal values of concentrations of amino acids and the corresponding reaction rates was used for the determination of K_m- and V_{max}-values for the individual amino acids and various pH-values (Table I). The K_m-values for the reactions proceeding at pH 9·0 were lower with all amino acids tested than the K_m-values for the reactions proceeding at pH 6·5–7·5. This is in agreement with the finding that pH 9·0 is the optimum value for the reaction catalyzed by γ-glutamyl transpeptidase. The differences between the individual amino acids as regards their ability to stimulate the reaction rate are best illustrated by the V_{max}-values. The differences in K_m-values are inadequate to the differences in the V_{max}-values from this viewpoint and most likely reflect other phenomena than differences in the affinity of substrates (in this case of acceptor amino acids) for the enzyme. Even though e.g. the K_m-value is lower in the presence of asparagine at pH 7·5 than the K_m-value obtained with L-methionine-D,L-sulfoximine, the maximum reaction rate is clearly higher in the presence of L-methionine-D,L-sulfoximine. To explain these apparent discrepancies we must take into account the fact that not only the measure of affinity of the amino acid for the enzyme indicates the size of the rate of increase of the enzymatic reaction but that a role may also play the inhibitory effect (if any) of the reaction product accumulated. The corresponding γ-glutamyl amino acid is formed during the reaction catalyzed by γ-glutamyl transpeptidase in the presence of the acceptor amino acid and the relation between the rate of accumulation of this product and the rate of its subsequent degradation can affect the resulting reaction rate. It is known that γ-glutamyl amino acids can be converted either by the action of γ-glutamyl transpeptidase or of γ-glutamyl cyclotransferase (another enzyme of the γ-glutamyl cycle)[23]. The rates of degradation of various γ-glutamyl amino acids by these enzymes significantly differ in their dependence on the nature of the amino acid residue[12,15]. A higher K_m-value (Table I) for some amino acid may reflect a lower affinity of this substrate for γ-glutamyl transpeptidase yet the rate of the subsequent degradation

of the corresponding γ-glutamyl amino acid by this enzyme can be the factor decisive for the final magnitude of stimulation of the rate of the γ-glutamyl transpeptidase reaction. Similarly, a contamination, if any, of the partly purified preparation of brain γ-glutamyl transpeptidase by γ-glutamyl cyclotransferase may affect the resulting stimulation of the reaction rate by the acceptor amino acid. It should be noted in this respect that of the γ-glutamyl amino acid series the best substrates of γ-glutamyl transpeptidase and γ-glutamyl cyclotransferase are γ-glutamyl-methionine and γ-glutamyl-glutamine[12,15,24,25]. These are γ-glutamyl derivatives of those amino acids whose stimulating activity in the case of the reaction catalyzed by partly purified brain γ-glutamyl transpeptidase is high (see corresponding V_{max}-values in Table I), even though the K_m of one of these amino acids, of methionine, is relatively high at pH 9·0. It follows from Table I that the highest V_{max}-values can be obtained in the case of the reaction catalyzed by brain γ-glutamyl transpeptidase by the addition of L-methionine as acceptor amino acid. This finding is from the viewpoint of the role of γ-glutamyl transpeptidase in amino acid transport in agreement with the

TABLE I

Kinetic Constants of Mouse Brain γ-Glutamyl Transpeptidase with Various Acceptor Amino Acids as Substrates

The K_m- and V_{max}-values were determined from the Lineweaver–Burk plot of the data given in Fig. 2. The K_m-value is given in mM and the V_{max}-value in per cent of nonstimulated activity of the enzyme.

Acceptor amino acid	Apparent K_m apparent V_{max}			
	pH 9·0	7·5	7·0	6·5
L-Alanine	0·56 *117*			5·60 *225*
L-Glutamate	0·60 *120*		3·00 *185*	
L-Methionine-D,L-sulfoximine	1·50 *156*	2·80 *195*		
L-Glutamine	0·70 *138*			3·40 *268*
L-Methionine	1·70 *175*		5·10 *315*	
L-Asparagine	0·55 *117*	0·93 *130*		

observation that L-methionine is well accepted by the brain[26]. A high V_{max}-value also corresponds to the reaction stimulated by L-methionine-D,L-sulfoximine. This compound is an anomalous amino acid whose administration to experimental animals causes convulsion[27]. It is known that L-methionine-D,L-sulfoximine is an inhibitor of one of the enzymes of the γ-glutamyl cycle in brain, of γ-glutamyl-cysteine synthetase[28]. Its rapid transport to the brain after intraperitoneal application has been clearly proved[29]. The high glutamine concentration of brain compared to blood plasma is conditioned, besides others, by the action of transport mechanisms. It is therefore obvious that there is a certain correlation between the acceptor activity of at least some amino acids in the brain γ-glutamyl transpeptidase reaction *in vitro* and their transport in the brain. The amino acid transport in brain follows several pathways: from blood to brain, from brain to blood, and from brain to the cerebrospinal fluid. The activity and certain characteristics of partly purified brain γ-glutamyl transpeptidase support the view that this enzyme plays a role in at least some of these transport pathways. A more detailed elucidation of the physiological role of this enzyme in the brain expecially from the viewpoint of its localization at anatomical sites of the blood-brain barrier awaits future studies.

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