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The effect of ammonia and pH on brain γ-glutamyl transpeptidase in young rats

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Acute hyperammonemia, induced by two consecutive injections of ammonium acetate (550 and 450 mg per kg b.wt.), decreased the activity of γ-glutamyl transpeptidase (GGT) in most brain regions of 18- and 30-day-old rats. This decrease in the brain GGT activity was more pronounced in younger than in older rats. After the addition of NH_4Cl to the incubation medium, the inhibitory action of NH_4^+ on this enzyme activity was also demonstrated in crude synaptosomal membranes at pH 7.4, but in a range of NH_4^+ concentrations many-times higher than those found in the plasma or brains of young hyperammonemic rats. Because similar concentrations of NH_4^+ stimulated the activity of the purified enzyme from rat kidney (mainly at pH 9.0), the inhibition of GGT activity in the young rat brain is probably mediated indirectly and not by a direct interaction of ammonia with the enzyme molecules.

γ-Glutamyl transpeptidase; Ammonia; pH conditions; Kidney; Developing brain; Synaptosome

1. INTRODUCTION

Glutamine (Gln), the regional concentrations of which are significantly elevated in the rat brain following hyperammonemia [1], is generally assumed to be the non-toxic storage form of NH_3 in the central nervous system (CNS). Gln released from astroglia is almost completely taken up by a Na^+-independent mechanism into nerve endings the membrane-bound γ-glutamyl transpeptidase ((5-glutamyl)-peptide:aminoacid 5-glutamyltransferase, GGT, EC 2.3.2.2) of which seems to be involved [2–4] in its transfer. Subsequent conversion of Gln to the excitatory transmitter glutamate (Glu) is preferentially mediated by mitochondrial, phosphate-activated glutaminase (PAG) the synaptosomal activity of which is inhibited by ammonium ions (NH_4^+) [5]. In contrast, NH_4^+ stimulates the activity of synaptosomal GGT [3] which hydrolyzes Gln to produce ammonia and transfers the γ-glutamyl group to an appropriate acceptor. This means that this enzyme catalyzes not only the transpeptidation reaction, involving the group translocation from a donor molecule (glutathione, Gln) to various acceptors (amino acids, short peptides), but also the hydrolysis of the donor compounds, functioning as glutathionase or glutaminase. The transpeptidase and hydrolytic reactions exhibit different pH optima, but at physiological pH the GGT mediated hydrolysis of Gln seems to predominate over transpeptidation [6]. Further characterization of this hydrolytic reaction revealed phosphate independence and maleate activation of this extramitochondrial glutaminase (MAG) [7].

Synaptosomal membranes also contain a relatively high activity of GGT [3,4], which seems to be almost devoid of MAG activity [8]. Cooper and Plum [9] therefore concluded that GGT probably does not play an appreciable role in brain Gln hydrolysis. However, under hyperammonemic conditions, high concentrations of Gln enable this amino acid to compete for binding to the donor site of purified GGT [10]. Moreover, glycylglycine (GlyGly) as a γ-glutamyl acceptor can further stimulate the production of ammonia and, in this way increase the formation of Glu from Gln, without an influencing PAG [11].

The lack of information about the role of GGT under the conditions of brain hyperammonemia led us to study not only the possible regional differences of GGT activity in the brain of young hyperammonemic rats, but also the direct effects of NH_4^+ and GlyGly at pH 7.4 and 9.0 on synaptosomal, membrane-bound GGT isolated from the cerebral cortex of young rats in comparison to the enzyme purified from rat kidneys.

2. MATERIALS AND METHODS

Female Wistar rats (18- and 30-day-old animals) were treated with 2 injections of ammonium acetate in isotonic saline (550 and 450 mg per kg b.w. injected i.p. with a 30 min interval) or 0.9% NaCl alone.

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Hyperammonemic and control animals were killed 10 min after the second injection [12]. Cerebral cortices and cerebella were rapidly dissected together with hippocampal formations which were further subdivided into the area CA1, CA2/Ca3 and dentate gyrus as described elsewhere [12,13]. Pooled samples were homogenized, centrifuged for 20 min at 12.000×g and the obtained sediments were used for GGT assay. The preparation of crude synaptosomes (P_2 fraction) from brain cortices of 30-day-old rats was based on the method of Cotman et al. [14]. The P_2 sediments were subjected to lysis in a hypotonic buffer (5 mM Tris-HCl, pH 7.4) as described by Sanderson and Murphy [15]. Purified GGT from kidneys of adult rats was prepared by a rapid method described by Murphy and co-workers [16].

GGT activity was measured by adding 0.1 ml suspension of crude synaptosomal membranes or the purified enzyme to a reaction mixture (final volume 1.0 ml) containing L-γ-glutamyl-*p*-nitroanilide (2.5 mM) and Tris-HCl buffer (80 mM; pH 7.4 or 9.0) at 37°C. In most cases GlyGly (20 mM) and NaCl (75 mM) were added to stimulate the enzyme reaction [3]. In experiments with ammonium ions NaCl was substituted by various concentrations of NH_4Cl. The release of *p*-nitroaniline and the content of protein were determined as described previously [3,13,16]. Statistical evaluation was carried out by means of Student's *t*-test.

3. RESULTS

Acute hyperammonemia, induced by two consecutive injections of NH_4-acetate, caused an 11-fold increase in blood ammonia and a 5-fold increase of brain ammonia above control levels (here not shown). The hyperammonemic conditions had a diverse effect on GGT activity in several brain regions investigated in 18-day-old and 30-day-old rats. The statistically significant decrease was observed in the cerebral cortex, cerebellum and dentate gyrus regardless of pH values used during the enzyme estimation. However, in most cases the decrease was more pronounced at pH 7.4 than at pH 9.0. GGT activity in the hippocampus proper (CA1 and CA2/3 areas) of 30-day-old rats did not seem to be significantly influenced. However, GGT activity decreases in these brain regions found at pH 7.4 or 9.0 were statistically significant in the hippocampal Ca1 area of 18-day-old rats (Table I).

The in vitro effect of NH_4^+ on the activity of GGT in crude synaptosomes and in the purified kidney enzyme at pH 7.4 and 9.0 pointed to a higher activity in the incubation medium without GlyGly at higher pH (Table II). The addition of GlyGly considerably increased the activity of GGT in synatosomal and kidney preparations (10- to 13-fold at pH 7.4 or 4- to 5-fold at pH 9.0). At pH 7.4, a slight decrease of synaptosomal GGT activity was apparent when NH_4^+ concentrations were higher than 10 mM. Under these conditions, the activity of the purified enzyme seemed to be moderately and transiently stimulated. At pH 9.0, NH_4^+ stimulated GGT activity in both enzyme preparations, but the increase was more evident in the purified enzyme from adult kidneys than in crude synaptosomal membranes prepared from the cerebral cortex of young rats. Moreover, the synaptosomal GGT was gradually stimulated with increasing concentrations of NH_4Cl (up to 100 mM), whereas maximal stimulation of GGT activity was reached at 37.5 mM NH_4Cl with the purified enzyme.

Table I

Effect of acute hyperammonemia on γ-glutamyl transpeptidase activity in homogenates prepared from various brain areas of 18- and 30-day-old rats

The enzyme acitivity was assayed in Tris-HCl buffer (80 mM, pH 7.4 or 9.0) in the presence of GlyGly (20 mM) and NaCl (75 mM). Values are given as the mean of 3–9 measurements (in triplicates) ±S.E.M.

Brain region (area)	Age (days)	γ-Glutamyl transpeptidase activity (nM *p*-nitroaniline·mg^{-1} protein·min^{-1})			
		pH 7.4		pH 9.0	
		Control	Ammonia	Control	Ammonia
Cerebral cortex	18	4.97±0.23	3.98±0.27 (80%)*	4.35±0.15	3.64±0.16 (84%)*
	30	6.14±0.07	5.38±0.09 (88%)**	5.82±0.11	5.28±0.19 (91%)*
Cerebellum	18	2.64±0.09	1.95±0.18 (74%)**	2.38±0.20	1.96±0.15 (82%)*
	30	4.60±0.21	3.43±0.15 (75%)**	3.96±0.16	3.57±0.13 (90%)*
Hippocampus (Area CA3)	18	4.35±0.34	3.98±0.37 (91%)	3.64±0.16	3.26±0.29 (90%)
	30	4.92±0.11	4.83±0.10 (102%)	4.67±0.22	4.67±0.28 (115%)
Hippocampus (area CA1)	18	5.56±0.25	4.10±0.35 (74%)**	4.48±0.24	3.73±0.13 (83%)*
	30	5.69±0.16	5.87±0.14 (103%)	5.14±0.17	5.56±0.13 (107%)
Dentate gyrus	18	4.28±0.26	2.73±0.15 (63%)***	3.77±0.14	2.95±0.17 (78%)**
	30	5.85±0.35	5.41±0.55 (92%)	5.33±0.14	4.84±0.14 (91%)*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.002$. Numbers in brackets denote values ammonia vs. control in percentage.

4. DISCUSSION

In contrast to adult animals [12], acute hyperammonemia decreased GGT activity not only in the dentate gyrus, but also in the frontal cortex and cerebellum of 30-day-old rats. The decrease found in the cerebellum was even in contradiction to the stimulation of GGT activity in this brain region found in adult rats [12]. The fall of this enzyme activity was more evident at physiological pH than at higher pH values, usually used for the assay of GGT activity [3,6,12]. These findings suggest that the brain GGT activity is affected differently by acute hyperammonemia in young and adult rats.

It has been reported that the addition of NH_4^+ [3,12] or some amines [17,17] increased the GGT activity in crude and purified preparations isolated from different organs. Comparable stimulation of this enzyme activity was demonstrated after continuous addition of NH_4Cl to cultured cells [19] or after prolonged hyperammonemia [3,12] suggesting that ammonia may profoundly affect certain cellular functions [20–23].

Besides hyperammonemia, the activity of GGT can be further influenced by changing the pH. The enhanced activity of this enzyme assayed in the crude synaptosomal membranes or as the purified kidney enzyme at physiological pH (with GlyGly in the medium) could be explained by previous findings [6] demonstrating that most enzyme molecules were in the active form for transpeptidation. The fact that the enzyme activity was conveniently measured at pH > 8.0 reflected higher actual concentrations of deprotonated acceptors at these pH values. In the absence of an acceptor, however, the hydrolytic activity was higher at pH 9.0 in both preparations which could be due to additional autotranspeptidation is more likely to occur at physiological pH than at higher pH values, in spite of the fact that actual concentrations of GlyGly in the deprotonated form were lower.

Comparable specific activity increases of GGT in the crude synaptosomal membranes and in the purified kidney preparation (expressed in percentages) were reached at pH 9.0 when the concentration of NH_4Cl in the medium with the purified enzyme was 25 mM. However, the action of ammonia was weaker in the synaptosomal membranes isolated from corticles in 30-day-old rats than from adults whereas the stimulation of purified enzyme was comparable to the percentually expressed stimulation observed in synaptosomes isolated from the cerebral cortex of adult rats. [3]. As molecular characteristics of GGT were comparable in the brains of young (7-day-old) and young adult rats (L. Dvořáková, personal communication), the age-dependent changes in the lipid composition of cell membranes in various regions of the developing rat [23] or differences in phosphorylation of membrane proteins, including GGT [24], could play a role in the observed changes induced by elevated concentrations of ammonia.

The results indicate that acute hyperammonemia decreases the GGT activity in most brain regions of 18- or 30-day-old rats rather than in adults. The decreased activity of this enzyme suggests that during the initial phases of systemic hyperammonemia the brain GGT does not play an important role in the hydrolysis of Gln (as glutaminase) in the developing brain. From the comparison of the action of various NH_4^+ concentrations on the activity of GGT in cortical synaptosomal membranes or in purified kidney enzyme it is evident that ammonia mediates its inhibitory action indirectly, probably through an interaction with membrane phospholipids or through changes in the phosphorylation of membrane proteins.

Table II

Effect of NH_4Cl on γ-glutamyl transpeptidase in cortical synaptosomes of 30-day-old rats in comparison with its action on purified enzyme from rat kidney

The enzyme activity was assayed in Tris-HCl buffer (80 mM, pH 7.4 or 9.0) in the presence or absence of GlyGly (acceptor). Enzyme activity was expressed as percentage of control values for P_2 fraction (3.64 ± 0.17 and 3.19 ± 0.09 nM *p*-nitroaniline · mg^{-1} protein · min^{-1}, at pH 7.4 and 9.0, respectively) and for purified enzyme (262.9 ± 4.3 and 211.1 ± 7.8 nM *p*-nitroaniline · mg^{-1} protein · min^{-1} at pH 7.4 and 9.0, respectively). Values are given as the mean of 3 separate measurements (in triplicates) ± S.E.M.

NH_4Cl (mM)	GlyGly (20 mM)	γ-Glutamyl transpeptidase Relative activity (%)			
		P_2 fraction		Purified enzyme	
		pH 7.4	pH 9.0	pH 7.4	pH 9.0
None	None	8.8	22.6	7.5	18.8
None	Yes	100.0	100.0	100.0	100.0
12.5	Yes	98.6	109.2	105.2	119.8
25.0	Yes	99.6	112.6	108.0	135.1
37.5	Yes	97.0	116.9	108.9	152.1
50.0	Yes	93.1	121.6	108.1	153.8
75.0	Yes	89.6	129.8	106.8	155.3
100.0	Yes	89.4	134.5	98.2	152.3

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