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The γ-Glutamyl Cycle in the Choroid Plexus: Its Possible Function in Amino Acid Transport

(brain/rabbit/enzymes/cerebrospinal fluid/glutathione)

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ABSTRACT **Various anatomic regions of rabbit brain have been examined for activities of the enzymes of the γ-glutamyl cycle. While these enzyme activities were widely distributed in the brain, they are present in much higher concentrations in the choroid plexus than in other parts of the brain. The activities observed are of about the same order of magnitude as found in the kidney. These observations and other considerations suggest that the γ-glutamyl cycle may play a significant role in the transport of amino acids between blood and cerebrospinal fluid.**

There is much evidence that the choroid plexuses of the brain are involved in the secretion of cerebrospinal fluid, and that the major functions of the choroid plexuses are intimately associated with the maintenance of brain homeostasis (1, 2). The epithelium that covers the choroid plexus is similar in many of its structural features to the epithelium of the kidney tubule, and to other cellular structures that participate in transport. The rate of flow of blood in the choroid plexuses exceeds that of the whole brain and that of the kidney (2). The accumulated evidence indicates that the choroid plexus functions in several transport phenomena at the blood–cerebrospinal fluid barrier; indeed, there would seem to be some analogy between the formation of urine by the nephron and the secretion of cerebrospinal fluid by the choroid plexus. The amino acids present in the glomerular filtrate are efficiently reabsorbed in the renal tubule, and relatively small amounts of amino acids normally appear in the urine. That the concentrations of virtually all of the amino acids in the cerebrospinal fluid are substantially lower than those found in the blood (3–5) suggests that an active transport system is operative in the choroid plexus.

Studies in this laboratory on the metabolism of glutathione (6–9) have led to formulation of a cycle of enzyme-catalyzed reactions in which glutathione is converted by transpeptidation with amino acids to form γ-glutamyl amino acids and cysteinylglycine. Cysteinylglycine is hydrolyzed by a peptidase to yield free cysteine and glycine; the γ-glutamyl amino acids are cleaved by γ-glutamyl cyclotransferase to 5-oxoproline and free amino acids. The coupled decyclization of 5-oxoproline to form glutamate and cleavage of ATP to ADP and inorganic phosphate is catalyzed by 5-oxoprolinase. The cysteine, glycine, and glutamate formed in these reactions are converted to glutathione by the successive actions of γ-glutamylcysteine synthetase and glutathione synthetase, thus completing a cycle in which free amino acids are first attached covalently to a glutamyl moiety and subsequently released again as free amino acids. The evidence in support of the hypothesis that this cycle functions in the transport of amino acids across the renal tubule has recently been reviewed (9).

Patients have been found who exhibit an error of metabolism associated with urinary excretion of large quntities of 5-oxoproline, and the available data indicate that these individuals lack 5-oxoprolinase activity (9–11). 5-Oxoprolinuria is associated with high concentrations of 5-oxoproline in blood and cerebrospinal fluid; that the concentration of 5-oxoproline in the cerebrospinal fluid is 6-times greater than that of the blood serum suggests that the brain produces 5-oxoproline. Administration to mice of 2-imidazolidone-4-carboxylate, an analog of 5-oxoproline, leads to decreased utilization of 5-oxoproline, to accumulation of this compound in the tissues (brain, kidney, liver), and to its excretion in the urine (8). Mammalian brain contains 5-oxoprolinase, γ-glutamylcysteine synthetase, γ-glutamyl transpeptidase, and γ-glutamyl cyclotransferase; indeed, highly purified preparations of γ-glutamyl cyclotransferase have been obtained from human and sheep brain (12). Histochemical studies have indicated localization of brain γ-glutamyl transpeptidase activity in the epithelium covering the choroid plexus, and also in the endothelium of brain capillaries (13). Several γ-glutamyl amino acids, including the γ-glutamyl derivatives of glutamine, glycine, alanine, valine, isoleucine, glutamate, and serine, have been found in mammalian brain (14–16).

The considerations reviewed above have led us to examine the choroid plexus for the several enzyme activities of the γ-glutamyl cycle. The studies reported here indicate that the enzymes that catalyze the reactions of the γ-glutamyl cycle are present in the choroid plexus. Of particular interest, we find that these activities are present in much higher concentrations in the choroid plexus than in other regions of the brain. The present findings show that the choroid plexus can catalyze the reactions of the γ-glutamyl cycle at rates of about the same order of magnitude as the kidney, and thus suggest that the γ-glutamyl cycle may play a significant role in the transport of amino acids between blood and cerebrospinal fluid.

EXPERIMENTAL

Materials. The γ-glutamyl amino acids used in these studies were prepared as described (17), by Dr. Ralph A. Stephani and Miss Charlene Michaud of this laboratory. White New Zealand rabbits (3–4 kg) were used in these studies. The brains

Synonyms: pyroglutamic acid, 2-pyrrolidone-5-carboxylic acid, 5-oxopyrrolidine-2-carboxylic acid.

TABLE 1. *Relative γ-glutamyl cycle activities in various regions of the brain*

Brain region	Relative activity: γ-Glutamyl transpeptidase	γ-Glutamyl cyclotransferase	γ-Glutamyl cysteine synthetase	5-Oxoproline utilization ($^{14}CO_2$ formed)
Choroid plexus	[100]	[100]	[100]	[100]
Pons	2.6	16	15	4.6
Thalamus	1.9	21	24	10.0
Caudate nucleus	2.0	28	6.2	9.3
Cerebellum	1.3	49	63	9.3
Cerebral cortex	1.4	18	17	6.5

The activities found for the choroid plexus preparations were (per 100 mg of tissue), respectively, 3800 nmol of *p*-nitroaniline formed (from γ-glutamyl *p*-nitroanilide) per min, 415 nmol of 5-oxoproline formed (from γ-glutamylmethionine) per min, and 4040 nmol of 5-oxoproline formed per hr in the coupled assay system for γ-glutamyl α-aminobutyrate synthetase (17). The value for 5-oxoproline oxidation was 10.8 nmol of $^{14}CO_2$ formed per hr in a system containing the minced tissue (7). The ratio of the activities of γ-glutamyl cyclotransferase towards γ-glutamylmethionine and γ-glutamyl-α-aminobutyrate were 3.7 and 2.0, respectively, for choroid plexus and cerebellum. The enzyme activities given here were, respectively, 44, 13, 11, and 31% of those exhibited by rabbit-kidney preparations.

were dissected immediately after decapitation of the animals, and the various regions of the brain were chilled on ice and used for study within 1 hr. The choroid plexuses of the third and fourth ventricles were combined; about 15–20 mg of tissue was obtained from each brain (total weight, about 8–10 g). The determinations reported here are based on studies of 10–12 animals.

Methods. The determinations of enzyme activity were performed essentially as done earlier in this laboratory. γ-Glutamyl cysteine synthetase activity was determined on tissue extracts prepared by homogenizing the tissue with 5 volumes of a solution (0–4°) containing 0.15 M KCl–5 mM 2-mercaptoethanol–1 mM $MgCl_2$ (17) (25 volumes were used with choroid plexus); the activity was determined by measuring the amount of 5-oxoproline formed in the presence of excess γ-glutamyl cyclotransferase (17). γ-Glutamyl cyclotransferase activity was measured in tissue extracts prepared as described (18), with L-γ-glutamyl-L-methionine and L-γ-glutamyl-L-α-aminobutyrate as substrates. The studies on the utilization of [*U*-^{14}C]5-oxo-L-proline by tissue minces were performed as described (7). 5-Oxoprolinase activity was determined on tissue extracts prepared by homogenization in 50 mM Tris·HCl (pH 7.8) containing 5 mM 5-oxo-L-proline; enzyme activity was determined as described (7).* γ-Glutamyl transpeptidase assays were performed on tissue extracts that were prepared in ice-cold 10 mM Tris·HCl buffer (pH 8.0)–80 mM $MgCl_2$. The tissues were homogenized with 5 volumes of the buffer, except in the studies of the choroid plexus in which 10–15 volumes were used. The homogenates were centrifuged at 12,000 × *g* for 30 min at 5°. The supernatant solutions, which contained less than 5% of the total γ-glutamyl transpeptidase activity of the homogenate, were discarded; the pellets were suspended in 0.1 mM Tris·HCl buffer (pH 8.0)–1% sodium deoxycholate. The suspensions were homogenized in a Potter–Elvehjem homogenizer equipped with a Teflon pestle. The solutions were allowed to stand at 25° for 4 hr and then centrifuged again. The supernatant solutions thus obtained were dialyzed for 18 hr against several volumes of 10 mM Tris·HCl buffer (pH 8.0). γ-Glutamyl transpeptidase activity was determined by following the release of *p*-nitroaniline from L-γ-glutamyl-P-nitroanilide in the presence of 20 mM glycylglycine (19), and also by the following procedure in which glutathione was used as the substrate. The reaction mixtures (final volume, 0.2 ml) contained 0.1 M Tris·HCl buffer (pH 8.5), 5 mM glutathione (adjusted to pH 8.5 by addition of Tris buffer), amino acid (40 mM), and deoxycholate extract of the tissue. Controls in which enzyme or amino acid were separately omitted were run simultaneously. After incubation at 37° for 5–20 min, the reactions were terminated by addition of 0.2 ml of 1.5 N acetic acid; aliquots were analyzed for glutathione as described by Ball (20).

* We are indebted to Mr. Paul Van Der Werf for performing these determinations.

RESULTS

Table 1 gives the relative activities (based on units of enzyme per gram of tissue) of γ-glutamyl transpeptidase, γ-glutamyl cyclotransferase, and γ-glutamyl cysteine synthetase of the choroid plexus and other regions of the brain. The data indicate that the γ-glutamyl transpeptidase activity of choroid plexus is considerably higher than of the other regions of the brain. The γ-glutamyl cyclotransferase and γ-glutamyl cysteine synthetase activities of choroid plexus were also much higher than the corresponding activities of other parts of the brain. It is of interest that substantial amounts of γ-glutamyl cyclotransferase and γ-glutamyl cysteine synthetase activities were found in the cerebellum. Table 1 also includes data on the oxidation of 5-oxo-L-[^{14}C]-proline to $^{14}CO_2$ by tissue slices; the ability of the choroid plexus to catalyze this reaction was at least 10 times greater than that exhibited by the other regions of the brain. These observations were substantiated by direct determination of 5-oxoprolinase activity performed on homogenates; in these studies also, the choroid plexus exhibited by far the highest activity.* Comparison of these enzyme activities with those of the corresponding activities exhibited by kidney preparations indicated that the activities of the choroid plexus are 10–44% of those of the kidney.

TABLE 2. *γ-Glutamyl transpeptidase of choroid plexus and kidney*

Amino acid	Specific activities*: Choroid plexus	Kidney
None	167	155
L-Glutamine	661	693
L-Methionine	348	528
Glycine	275	226
L-Alanine	605	561
L-Phenylalanine	242	242
L-Isoleucine	316	236
L-Arginine	401	201
L-Glutamate	634	545
Glycylglycine	510	1170

* nmol of glutathione utilized per min/per mg of protein.

Glutathione synthetase was also determined, but the determination of this activity in crude tissue preparations is somewhat unreliable because of the presence of γ-glutamyl cyclotransferase and γ-glutamyl transpeptidase. However, when this activity was assayed by the procedure previously used in this laboratory (21), in which the tissue preparation is incubated with γ-glutamyl-α-aminobutyrate, ATP, magnesium ions, and hydroxylamine, the formation of dipeptide hydroxamate was 13.8 μmol/g of choroid plexus per hour; this value, which is undoubtedly minimal, may be compared with 3.5 μmol found for cerebellum. Although detailed studies on cysteinyl glycinase activity were not carried out, the presence of this activity in the choroid plexus and in other regions of the brain was demonstrated by qualitative chromatographic studies, which indicated that cysteinylglycine is rapidly cleaved to its constituent amino acids by these tissue preparations.

The relative activities of rabbit kidney and choroid plexus γ-glutamyl transpeptidase in the presence of several different amino acids are given in Table 2. In general, the findings indicate that the γ-glutamyl transpeptidase activity of the choroid plexus is quite similar to that of the kidney. Both kidney and choroid plexus preparations exhibit a rather broad amino-acid specificity.

DISCUSSION

The findings presented here are consistent with the thesis that the choroid plexus has a γ-glutamyl cycle that may function in the transport of amino acids. This suggestion is also supported by the finding of very high concentrations of 5-oxoproline in the cerebrospinal fluid of patients with the disease 5-oxoprolinuria, and other considerations cited above. It is of interest that previous studies have indicated that the choroid plexus contains considerable amounts of sodium–potassium-stimulated ATPase (22) and carbonic anhydrase (23), enzymes that are also believed to be involved in transport phenomena. Histochemical studies (L. L. Ross, unpublished data) have revealed that the transpeptidase is localized in the apical portions of the epithelial cells of the choroid plexus, which is the site at which transport probably occurs. The γ-glutamyl cycle may function to reabsorb amino acids from an ultrafiltrate of the blood (as it apparently does in renal tubules), or the cycle may function to transport amino acids from the cerebrospinal fluid in the ventricle to the blood. It should be emphasized that we do not propose that the γ-glutamyl cycle mediates all amino-acid transport across the choroid plexus, or for that matter in the brain. There is substantial evidence for several different amino-acid transport systems in brain (24, 25). Furthermore, there are apparently differences in the relative concentrations of the individual amino acids in cerebrospinal fluid as compared to those in blood plasma (3–5), again suggesting that more than one transport system may be operative.

Although the enzymes of the γ-glutamyl cycle are concentrated in the choroid plexus, substantial amounts of these activities are found in other areas of the brain. Thus, we found moderate levels of γ-glutamyl cyclotransferase and γ-glutamyl cysteine synthetase activities in the cerebellum. The possibility must therefore be considered that the enzymes of the γ-glutamyl cycle function elsewhere in the brain. They may, in fact, be localized in certain cell types and not present in others. Preliminary histochemical studies on the distribution of γ-glutamyltranspeptidase activity in mammalian brain (L. L. Ross, unpublished data) are consistent with this interesting possibility, and show that enzyme activity is present in the perikarya of specific neuronal groups within the brain stem. These findings suggest that γ-glutamyl cycle may mediate amino-acid transport in certain brain cells as well as in the choroid plexus.

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