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γ-L-glutamyltaurine

Review Article

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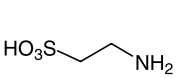
Summary. The discovery of the dipeptide γ-glutamyltaurine (γ-GT; glutaurine, Litoralon) in the parathyroid in 1980 and later in the brain of mammals gave rise to studies on intrinsic and synthetic taurine peptides of this type. It was suggested that γ-glutamyltransferase (GGT; γ-glutamyltranspeptidase) in the brain is responsible for the *in vivo* formation of this unusual dipeptide. γ-GT has been prepared by both synthetic and enzymatic methods. The chemical syntheses included the use of protecting groups and coupling methods. A wide spectrum of analytical and spectroscopic methods was used to confirm the structure of the synthetic compounds and to elucidate the position of the peptide bond. Enzymatic preparation of γ-GT from taurine takes advantage of the selective transpeptidation action of GGT on L-glutamine, glutathione, γ-glutamyl-*p*-nitroanilide or other glutamine donors. Although the functional roles of γ-GT in the brain are only poorly understood, many of its established CNS effects have been reported in the last 25 years. Its effect on emotional arousal and its anti-conflict potencies are synergistic with the anxiolytic drug diazepam. γ-GT exhibits anti-conflict potency, which is exerted by reducing aversion or phobia and/or the anxiety levels. γ-GT also acts as endogenous modulator in excitatory aminoacidergic neurotransmission. It is suggested that such acidic peptides through N-methyl-D-aspartic acid receptors could be part of the neurochemical substrate underlying self-stimulation of the medial prefrontal cortex. Other γ-GT effects in neural systems include: effects on the monoamine concentration in the brain; effects on aggressive behavior in the cat; effects on thyroid hormones in the rat; amelioration of electroshock-induced amnesia; potent and long-lasting antiepileptic action (on intra-amygdaloid injection); affect the glutamatergic system in schizophrenic disorders. Roles for γ-GT in non-neural systems have also been reported, e.g., effects on the metamorphosis of amphibians; on plasma rennin regulation; on radiation protection; on uric acid levels; on human antibody-dependent cell-mediated cytotoxicity (ADCC) and many more.

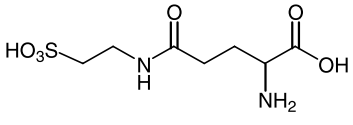
Keywords: Taurine – γ-Glutamyl taurine – Dipeptide – Neural systems – Non-neural systems

1 Introduction

Taurine (β-amino-ethan-sulfonic acid), a naturally occurring, sulfur-containing, conditionally essential amino acid is found at high concentrations in mammalian plasma and tissues. Since taurine plays a number of important roles in mammalian tissues, it has been thoroughly investigated over the past 50 years, but even so its precise biochemical function is not fully understood. It was generally accepted that taurine is not utilized in protein synthesis in the same way as other common α-amino acids. Consequently, studies on its peptidic derivatives were limited, and up to the end of the 1970s there was no information available on the existence of naturally occurring taurine peptides. The discovery of γ-glutamyltaurine (γ-GT, glutaurine, Litoralon) in the parathyroid in 1980 (Furka et al., 1980) and later in the brain of mammals (Marnela et al., 1985; Nakamura et al., 1990) prompted subsequent studies on both intrinsic and synthetic taurine peptides.



Taurine



γ-glutamyltaurine (γ-GT)

Peptides are attractive targets for drug discovery because of their affinities and specificities toward biological receptors: Many peptides have biological activities as substrates, antagonists, inhibitors of enzymes, antigens, regulators of gene expression and more. In recent years, studies on oligopeptides containing taurine residues have received considerable attention in view of their tendency

to adopt preferential secondary structures as well as their stability towards enzyme degradation. It seemed possible that sulfonic acid analogs of amino acids built into peptides might provide a means of inhibiting the parent peptide.

Although the functional role of γ-GT in the brain is only poorly understood, many of its effects in neural systems have been reported over the past 25 years. These studies have covered, *inter alia*, the effects of γ-GT on the monoamine concentration in the brain, on aggressive behavior in cats, on thyroid hormones in rats, and on electroshock-induced amnesia as well as anti-conflict effects. The activities of γ-GT in non-neural systems have also been reported, e.g., effects of γ-GT on the metamorphosis of amphibians and on the concentration and activity of plasma rennin in some mammals. The radiation-protective properties of γ-GT and its positive inotropic effect on the locust heart have also been described.

Seminal work on γ-GT – its structure, properties and activity – has been performed mainly by three groups of researchers: A Hungarian group (Furka, Feuer, Torok, Schulz, Sebestyen, Kapa, Csaba, Kovacs, Gulyas, Cserhalmi, and others); a Finnish group (Lahdesmaki, Marnela, Timonen, Varga and others); and Japanese group (Higashiura, Ienaga, Toyomaki, Kimura, Uemura and others).

2 Isolation of γ-GT from parathyroid and brain

The first report of covalent binding of taurine in some low-molecular-weight acidic peptides came from the group of Reichelt as long ago as 1974 (Reichelt and Edminson, 1974). The isolation of pure γ-L-GT from a protein-free aqueous extract of bovine parathyroid powder was first reported in 1980 (Furka et al., 1980), but a patent had been filed even earlier in January 1977 (Feuer et al., 1977), claiming that: *deproteinized, defatted aqueous extract of the parathyroid gland insoluble in benzene, chloroform and carbon tetrachloride is found to be L-gamma-glutamyl-taurine which has vitamin-A type activity and is generally effective for the treatment of a wide range of mammalian disorders which are directly or indirectly connected with pathological alterations of the aerobiospherical genetical adaptational system (AGAS)*. Later, it was shown that GT was the predominant structure of the taurine-containing peptides present in trichloroacetic acid (TCA) extracts of calf brain synaptosomes and synaptic vesicles (Marnela et al., 1984a, 1985). Electron impact mass spectrometry was used to elucidate the structures of a number of low-molecular-weight acidic peptides containing taurine; these included: GT, N-acetyl-Asp-Glu-taurine, N-acetylaspartyltaurine, N-acetylglutamyltaurine, aspartyltaurine, Ser-Glu-Ser-taurine, and seryltaurine (Marnela et al., 1984b). The structure of GT was later confirmed by fast atom bombardment (FAB) mass spectrometry (Marnela et al., 1984b). This technique could, however, not differentiate between the α- and γ-forms of GT, since both have a molecular weight of 254. Dimer formation, often encountered with peptides, was demonstrated in the negative ion FAB mass spectrum of synaptosomal GT (m/z 507). The following steps were subsequently undertaken to elucidate the position of the peptide bond in GT: The peptide was extracted from calf brain synaptic vesicles and subjected to paper electrophoresis. The product was analyzed further in an automatic amino acid analyzer both prior to and after acid hydrolysis. It was claimed that both the α- and γ-forms were present in approximately equal amounts (Marnela et al., 1987). The retention times of the two configurations are entirely different (Kontron AS70 resin in the Li^+ form and alumina type guard column), i.e., 5 min for the γ-form and 107 min for the α-form. Several years later, the dipeptide was again isolated from bovine brains, and its structure was determined unequivocally as γ-GT by means of a mass spectrometric technique [B/E linked scan sputtered ion mass spectrometry (SIMS), which had been shown to be useful general technique for distinguishing the γ-isomer from the α-isomer of glutamyl dipeptides]. No α-GT isomer could be detected in the bovine brain (Nakamura et al., 1990).

It has been suggested that γ-GT can function as an intracellular storage form of taurine. Indeed, it has been shown that taurine can form small peptides with a number of other amino acids, e.g., N-acetylglutamyltaurine, N-acetylaspartylglutamyltaurine, aspartyltaurine, N-acetylaspartyltaurine, seryltaurine, serylglutamylseryltaurine, N-acetylaspartylphosphoserylseryltaurine, and N-acetylaspartylserylphosphoseryltaurine, as shown by HPLC and electron impact ionization mass spectrometry (Lahdesmaki and Marnela, 1985). Two other low-molecular-weight peptides occurring in calf brain nerve terminals and their subcellular vesicles were purified, and their amino acid compositions and sequences were determined by means of the dansyl chloride method, carboxypeptidase and aminopeptidase. The suggested sequences were: NH_2-alanyl-glycyl-glutamyl-phosphoserine-COOH and N-acetylaspartyl-glutamyl-taurine-SO_3, respectively (Lahdesmaki et al., 1980). Japanese scientists using immunochemical and immunohistochemical methods claimed that γ-GT is localized in neurons (Tomida et al., 1985; Tomida and Kimura, 1987; Ida et al., 1987). They worked with antisera against

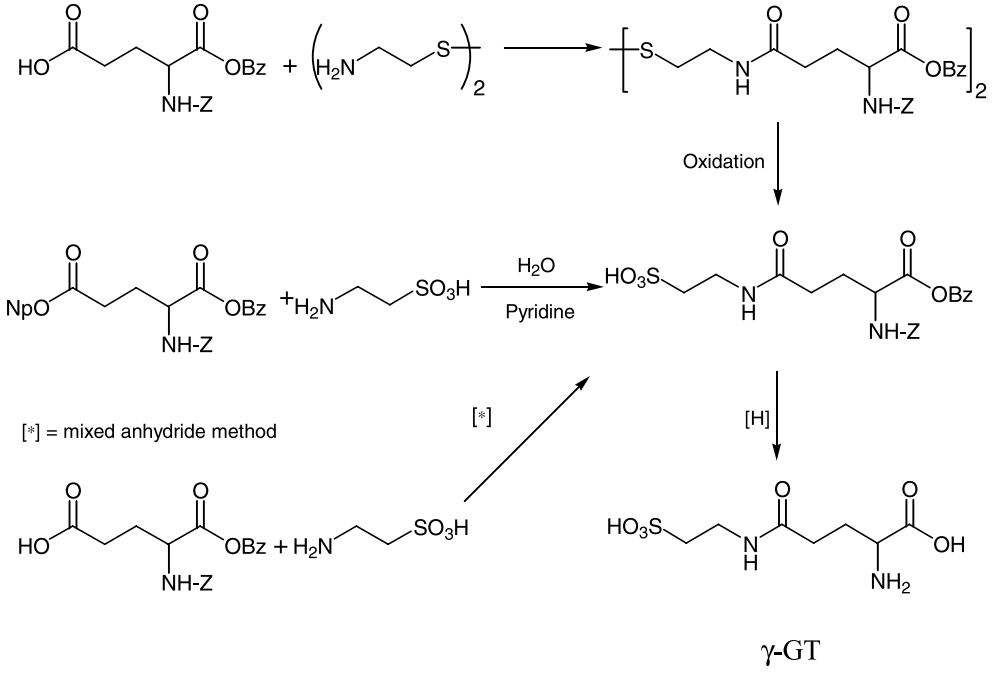
taurine and γ-GT and found that the immunoreactivity of the two (taurine-like and γ-GT-like) differed fundamentally one from the other; i.e., each antiserum exhibited little cross-reactivity with the other antigen. In addition, an immunohistochemical study of the rat brain revealed positive staining in different neuronal cell populations with the two different antisera. The predominant stained peptide was γ-GT, and neither the α-isomer nor any aspartyltaurine could be detected.

It has been suggested that γ-glutamyltransferase (γ-glutamyltranspeptidase; GGT) in the brain is responsible for the *in vivo* formation of γ-GT. Accordingly it is possible that GGT could catalyze the transfer of the γ-glutamyl moiety of glutathione to taurine. Indeed, when partially purified rat brain GGT was incubated at 310 K with taurine in a Tris-HCl buffer (pH 8.2) containing NaCl, $MgCl^2$ and γ-glutamyl-*p*-nitroanilide or glutathione, as the γ-glutamyl donor, the formation of γ-GT was detected. The activity of GGT was highest in the pons-medulla and lowest in the cerebellum and the hippocampus, however, most of the activity was found in the nuclear pellet. Upon further subcellular fractionation, most of the activity was found to reside in the subfraction enriched in micro-vessels. In particular, the luminal surface of the capillary endothelial cells and the pia matar contained an abundance of GGT (Varga et al., 1985). γ-GT was also detected in a protein-free extract of rat brain after intra-ventricular administration of ^{14}C- or ^{3}H-labeled taurine. The work-up comprised a combination of ion-exchange chromatography, electrophoretic separation and thin-layer chromatography. However, γ-GT could not be identified by physicochemical methods (Torok et al., 1981).

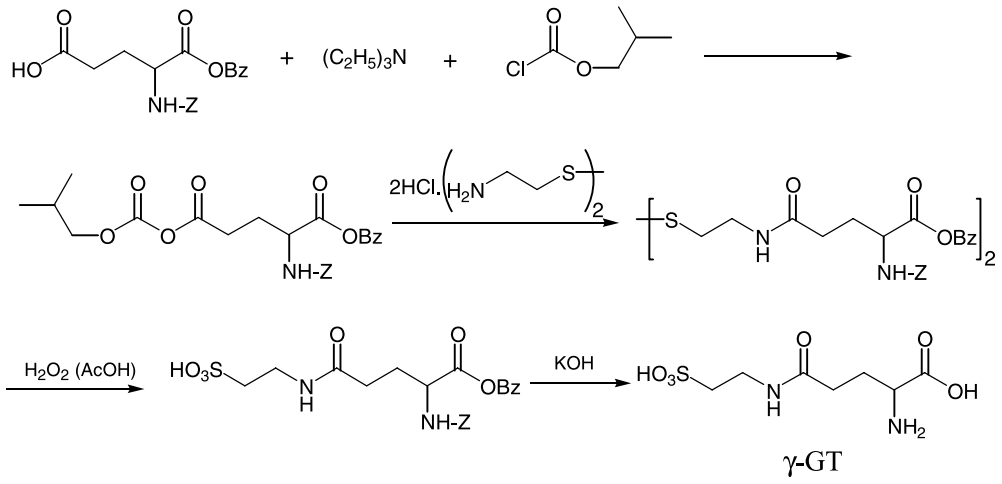
Brain γ-GT is apparently a product of – and not a substrate for – the cellular γ-glutamyl transpeptidation reaction. Thus, taurine can function as a γ-glutamyl acceptor from γ-L-glutamyl-*p*-nitroanilide. The active center of cellular GGT recognizes the γ-glutamyl moiety in substrates, but shows no activity with γ-GT. On the contrary, it is a potent competitive inhibitor of the enzyme (Lahdesmaki et al., 1984).

3 Preparation of γ-L-GT by synthetic methods

γ-GT was synthesized for the first time in 1980 (Sebestyen et al., 1980). The intermediate derivative Z-Glu(Tau)-OBzl was prepared from Z-Glu-OBz in three different ways (Scheme 1): (i) from cystamine, by means of the mixed anhydride method, followed by oxidation, (ii) from taurine, by the active ester procedure via Z-Glu-(ONp)-OBz, and (iii) from taurine, by applying mixed anhydride coupling. The protecting groups were removed by hydrogenolysis. The synthetic substance proved to be identical with the substance isolated by means of the paper electrophoresis technique at two different pH values



Scheme 1



Scheme 2

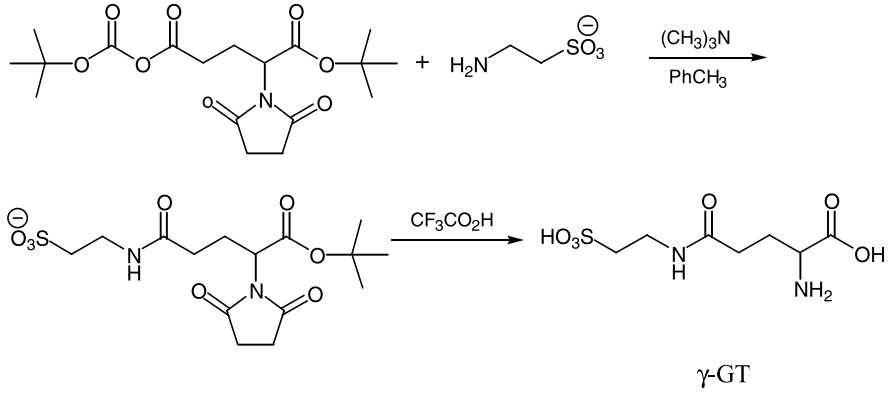
(6.5 and 2.0) and with the substance obtained by descending paper chromatography in BuOH-pyridine-AcOH-water (15:10:3:12).

The method for preparing γ-L-GT from Z-Glu-OBz and cystamine [method (i)] was patented in the United States in 1977 (Feuer et al., 1977) (Scheme 2). It comprises reacting carbobenzyloxy-L-glutaminic acid-α-benzyl ester with triethylamine and isobutylchloroformate and then treating the reaction mixture with cystamine dihydrochloride to recover N,N′-bis-(N-carbobenzyloxy-γ-[α-benzyl]-L-glutamyl)-cystamine. The latter is reacted with hydrogen peroxide in glacial acetic acid to produce carbobenzyloxy-γ-(α-benzyl)-L-glutamyltaurine. That compound is treated with hydrogen bromide in glacial acetic acid to recover γ-(α-benzyl)-L-glutamyltaurine, and this compound is treated with potassium hydroxide solution to yield γ-L-GT.

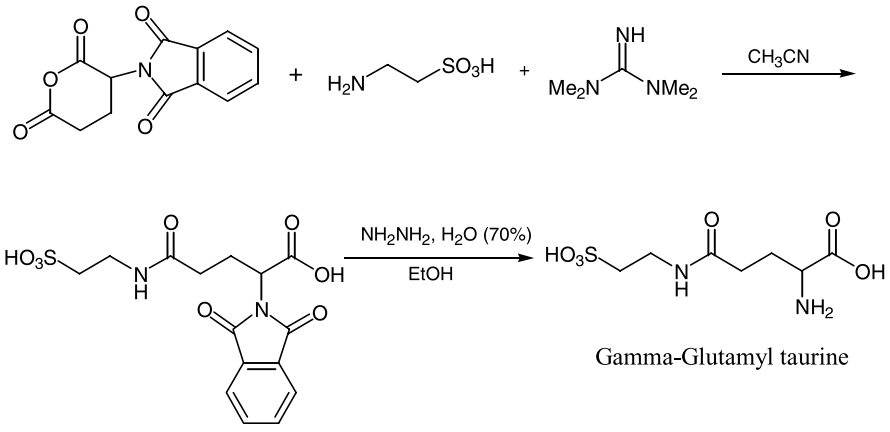
The dipeptide was also prepared by coupling Me_3CO_2C-Glu(ONSu)-$OCMe_3$ (NSu = succinimido) with $H_2NCH_2CH_2SO_3^-$, $Me_3N + CH_3Ph$ and de-blocking the resulting dipeptide with CF_3CO_2H (Felix, 1982) (Scheme 3).

An improved synthesis was suggested in 1987: γ-GT was prepared in 73% yield by coupling N-phthaloyl-L-glutamic anhydride with taurine in MeCN containing tetramethylguanidine, followed by hydrazinolysis with 70% $NH_2NH_2 \cdot H_2O$ in EtOH (Gulyas et al., 1987) (Scheme 4).

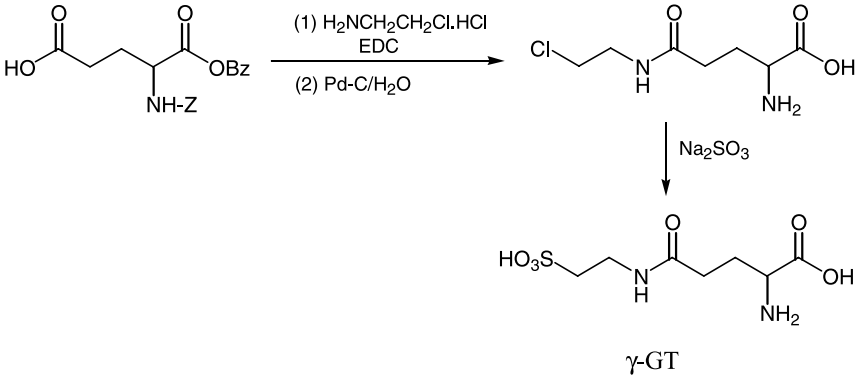
One year later, the active-ester method (HONB + DCC) was employed again for the synthesis of both α-GT and γ-GT. For purposes of comparison, the dipeptide isomers were also prepared by means of a new method using a substitution reaction of a sulfo group for Cl or Br *via* the β-haloethyl amide. When Na_2SO_3 or $(NH_4)_2SO_3$ was used in the substitution, the new method gave pure taurine dipeptides in good yield without racemization (Ienaga et al., 1988a) (Scheme 5). In a study of the mode of linkage at the acidic peptide bond, spattered ion mass spectrometry (SIMS) B/E linked scan mass spectrometry was shown to



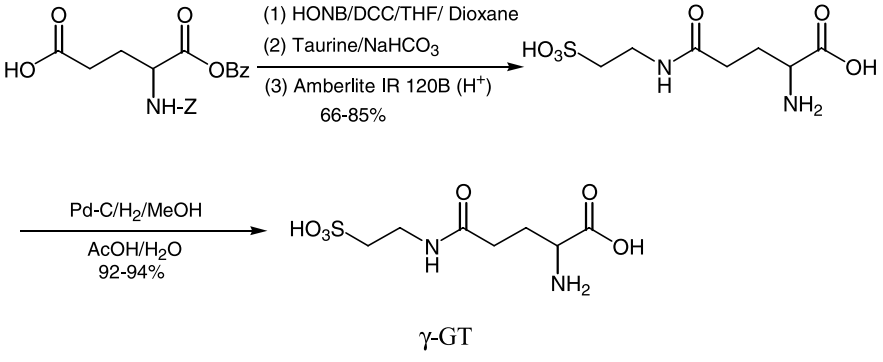
Scheme 3



Scheme 4



Scheme 5



Scheme 6

be the most useful method for distinguishing between the α- and γ-isomers (Ienaga et al., 1988b).

Five years later, it was suggested that, for the synthesis of γ-GT, α-benzyl-γ-L-glutamyltaurine would be a useful intermediate that could be easily purified and crystallized (Scheme 6). This intermediate was prepared from Me_3CO_2C-Glu-OCH_2Ph and taurine by the active-ester method and purified by ion-exchange chromatography and re-crystallization. α-GT can also be synthesized by this method (Higashiura and Ienaga, 1992).

Twelve synthetic derivatives and analogs of γ-L-GT have also been prepared, i.e., γ-L-glutamyl derivatives and β-L-aspartyl derivatives of taurine, nortaurine, N-methyltaurine, taurineamide, taurylmethylamine, taurylpiperidine, β-alanineamide, orthanilic acid, 2-aminoethanephosphonic acid, ethanolamine-O-sulfate, and ethanolamine-O-phosphate.

Most of these compounds were prepared by the active ester or the mixed anhydride method. α,γ-L-Glutamyl-*bis*-taurine and DL-2-amino-8-sulfo-octanoic acid have also been synthesized (Gulyas et al., 1984).

4 Preparation of γ-L-GT by enzymatic methods

As early as 1981, various γ-glutamyl peptides were synthesized using highly purified γ-glutamylcysteine synthetase from *Proteus mirabilis*. The accumulation of each peptide was measured after incubation for long periods. Good formation was observed in the synthesis of peptides from taurine and also from other amino acids, e.g., L-cysteine, L-γ-aminobutyrate, L-serine, L-homoserine, glycine, L-alanine, L-norvaline, L-lysine, L-threonine, and L-valine. Peptide syntheses were confirmed by analyses of the component amino acids after hydrolysis of the peptides. The structure of the glutamyl peptides, especially the peptide linkage at the γ-carbonyl residue of L-glutamate, was determined by mass spectrometry of the N-trifluoroacetyl methyl ester derivatives of the glutamyl peptides (Nakayama et al., 1981).

The formation of γ-GT from L-glutamine and taurine was carried out with GGT (from a wheat bran koji extract) from *Penicillium roqueforti* (IFO 4622). From a reaction mixture comprising 500 mM L-glutamine, 500 mM taurine, 100 mM Tris-HCl buffer (pH 8.5), and 0.6 units/mL of GGT, about 180 mM γ-GT (36%) were formed. The peptide was isolated by ion-exchange column chromatography and identified by IR, ^{1}H-NMR, and mass spectrometric analyses. The suggested reaction system has three advantages: (i) L-glutamine can be used as the γ-glutamyl donor [this compound is cheaper than other γ-glutamyl donors e.g., glutathione, γ-glutamyl-*p*-nitroanilide, etc.]; (ii) the formation of γ-GT does not require complicated operations or reaction systems, because a crude extract from wheat bran koji with *P. roqueforti* can be used in a simple reaction system composed of the two substrates and the extract; and (iii) the peptide can be formed using a harmless microorganism (*P. roqueforti*), which is used in the production of cheese (Tomita et al., 1989).

GGT can also catalyze the transfer of γ-glutamyl moiety of LL-glutathione and of the unnatural isomer DL-glutathione to taurine, forming γ-GT and cysteinyl taurine (Km: LL-glutathione 0.336 mM, DL-glutathione 0.628 mM) (Oikawa et al., 1999). In addition to its ability to synthesize γ-GT, GGT may, under certain conditions, use γ-GT as a γ-glutamyl donor for other transpeptidations. In addition to GGT, γ-glutamyl-cyclotransferase is also supposed to play a major role in the biodegradation of γ-GT. The relative activities of the two enzymes are quite different in various areas of the central nervous system, and their ratio might determine the actual concentration of γ-GT in the different regions of the brain (Torok et al., 1981).

A different enzymatic method for the production of γ-GT from L-glutamine and taurine takes advantage of the selective transpeptidation reaction of GGT of *Escherichia coli* K-12, a periplasmic non-glycosylated enzyme. Therefore, it is much easier to purify GGT from *E. coli* to a homogeneous state. The optimum conditions for the production of γ-GT were: incubation for 1 h at 37°C of 200 mM L-glutamine, 200 mM taurine and 0.2 U/mL *E. coli* GGT, pH 10. The γ-GT so obtained, 45 mM (22.5%), was purified on Dowex 1×8 and C18 columns and identified by NMR spectroscopy and polarimetry (Suzuki et al., 2002). A similar enzymatic method was developed for synthesizing various γ-D-glutamyl compounds. The method, which is both efficient and stereospecific, involves bacterial GGT and D-glutamine as the γ-glutamyl donor. In the synthesis of γ-GT with D-glutamine as the γ-glutamyl donor instead of L-glutamine, byproducts suchas γ-glutamylglutamine and γ-glutamyl-γ-glutamyltaurine were not produced, and the yield of γ-GT dramatically increased from 22.5 to 71%. It was also shown that in the absence of these γ-glutamyl byproducts the purification procedure could be simplified. Other possibilities of synthesizing various γ-D-glutamyl compounds have also been shown (Suzuki et al., 2003).

5 Effects of γ-GT in neural systems

5.1 Anti-conflict effect of γ-GT

The effect of γ-GT on emotional arousal and its anti-conflict potencies, have been investigated. The findings of various studies suggest that γ-GT has a central effect, which correlates with its anti-conflict effect. One of these studies investigated the effects of the dipeptide on operant responses maintained under two types of negative reinforcement (avoidance) schedules (Sidman-type and discriminated avoidances) in the rat. Two types of positive reinforcement schedules and/or conflict schedules (e.g. differential water reinforcement of low rate and multiple fixed ratio punishment schedules) were also examined. It was found that γ-GT induced a prominent increase in the punished response (anti-conflict effect) without eliciting a marked change in the non-punished response. This behavioral change did not appear immediately, but gradually, when doses of more than 1 mg/kg were given.

A maximum level was achieved on days 3–6 and persisted for 7–14 days after administration of γ-GT. The anti-conflict effect of γ-GT was synergistic with that of the anxiolytic drug diazepam (Kuribara and Tadokoro, 1982).

In another study the effects of a single post-trial i.p. administration of γ-GT and some of its analogs on passive avoidance latency were examined in male and female Wistar rats (Schulz et al., 1985). The avoidance latency was decreased by γ-GT and γ-aminobutyryl-ethanolamine phosphate but lengthened by DL-γ-aminoisobutyryl-ethanolamine phosphate. No differences were observed between the responses of immature male and female rats following γ-GT treatment. They also demonstrated intergroup differences in passive avoidance behavior following dipeptide administration in tests of open-field activity of the animals examined immediately after a 24 h retention test. The same researchers also investigated the anti-conflict properties of γ-GT, diazepam, γ-aminobutyryl-ethanolamine phosphate and DL-γ-aminoisobutyrylethanolamine phosphate in a ''time-to-emerge'' (TTE) conflict paradigm in non-deprived rats. In doses between 0.05 and 0.50 mg/kg i.p., diazepam and γ-GT decreased the TTE latency in a dose-dependent manner versus saline- or vehicle-treated controls. The number of irresolute responses was decreased following administration of diazepam and γ-GT and was positively correlated with the TTE latency-decreasing activity of these compounds (Schulz and Feuer, 1986a).

With the aim to postulate the possible site of action of γ-GT in the brain, the effects of γ-GT on the aggressive behavior and sleep-wakefulness cycle were studied in freely moving cats. The dipeptide, even in doses as low as 0.1 μg/kg, significantly shortened the latency of the rat-killing reaction elicited by hypothalamic stimulation. On the other hand, the same doses failed to modify the sleep-wakefulness cycle to any significant degree throughout the study period of 4 h (Kukorelli et al., 1986).

The anti-conflict potency of γ-GT was further supported by studies on the effects of γ-GT on conditioned taste aversion (CTA) in rats. The dipeptide reduced neophobia at a dose of 5.0 mg/kg (i.p.) in a ''1-bottle forced choice paradigm'' for CTA, but did not affect the animals' memory of intoxication following chronic treatment at 0.05–5.00 mg/kg (i.p.). Acute treatment with γ-GT (10–1000 μg/kg, i.p.) did not affect CTA in a ''2-bottle test'', when administered immediately following an unconditioned stimulus (LiCl injection). In contrast, when given 90 min prior to the retention test, injections of γ-GT (50.0 μg/kg) and γ-aminobutyryl ethanolamine phosphate (100 and 500 μg/kg) resulted in a higher intake of saccharin solution by the rats. This effect is comparable to the action of diazepam tested in the same experimental procedure. The results support the hypothesis that these dipeptides exhibit anti-conflict potency, which is exerted by reducing aversion or phobia and/or the anxiety level of the animals in the experimental situation (Schulz and Feuer, 1986b). In contrast to the above-described study, treatment of rats with γ-GT or some of its analogs at doses of 100 or even 500 μg/kg i.p., did not impair spatial memory, regardless of the timing of administration of these compounds during the experimental session (Schulz, 1988).

5.2 γ-GT as a modulator in excitatory aminoacidergic neurotransmission

Folic acid (FA) and 5-formyl-tetrahydrofolic acid (FTHF) have been shown to produce marked stimulation of locomotor activity after bilateral injection into the rat nucleus accumbens. A study was designed to determine whether the hypermotility response produced by the folates is mediated through the activation of excitatory amino acid receptors in the nucleus accumbens (Stephens et al., 1986). Both γ-D-glutamylaminomethylsulfonic acid and γ-D-GT antagonized the stimulation of locomotor activity produced by quisqualic acid, FA and FTHF. However, these dipeptides also inhibited the response to N-methyl-D-aspartic acid (NMDA), suggesting that they are not able to distinguish between quisqualate and NMDA receptors in the nucleus accumbens.

An important component of the neurotransmission of the bulbospinal respiratory drive involves endogenous excitatory amino acids acting as AP4-sensitive sites and other non-NMDA (quisqualate/kainate) receptors. Among a number of antagonists for complete block of the motor output, the potency of γ-GT was found to be quite low (McCrimmon et al., 1989). When the *in vitro* effects of γ-L-GT on different stages of excitatory aminoacidergic neurotransmission were tested with γ-D-GT as the reference compound, it was found that γ-L-GT enhanced the K^+-stimulated release of [^{3}H]glutamate from cerebral cortical slices (25% at 0.1 mM) and slightly inhibited its uptake by crude brain synaptosomal preparations (about 10% at 1 mM). γ-L-GT was also found to be a weak displacer of glutamate and its agonists from their binding sites in brain synaptic membrane preparations, being, however, less selective than γ-D-GT to quisqualate sites. The basal influx of Ca^{2+} into cultured cerebellar granular cells was not affected by 1 mM γ-L-GT, but the glutamate- and its agonist-activated influx was significantly

inhibited in low Mg^{2+} (0.1 mM) and Mg^{2+}-free media. The glutamate-evoked increase in free intracellular Ca^{2+} and the kainate-activated formation of cGMP in cerebellar slices were both markedly inhibited by 0.1 mM γ-L-GT. It was thus proposed that γ-L-GT might act as endogenous modulator in excitatory aminoacidergic neurotransmission (Varga et al., 1990).

γ-D-GT is known to be an antagonist with a high relative affinity for kainate and quisqualate receptors. The effects of intra-ventricular and intra-cortical microinjections of acidic amino acid antagonists on self-stimulation (SS) of the medial prefrontal cortex (MPC) were also investigated in the rat (Cobo and Mora, 1991). Spontaneous motor activity of the animal and SS of the contralateral non-injected MPC were used as controls for the non-specific effects of the drugs. Intra-ventricular microinjections of γ-D-glutamylglycine (an antagonist of NMDA, kainate and quisqualate receptors) or 2-amino-5-phosphonovalerate (a specific antagonist of NMDA receptors) produced a dose-related decrease of SS in the MPC. Spontaneous motor activity of the animal was not significantly affected. Unilateral microinjections of D-glutamylglycine or 2-amino-5-phosphonavalerate into the MPC produced a decrease of SS in the ipsilateral side, while no effects were found on the contralateral MPC. On the contrary, intra-ventricular microinjections of γ-D-GT produced a dose-related decrease of both SS and spontaneous motor activity of the rat. Moreover, intra-cortical microinjections of γ-D-GT had no effect on SS of this cortical area. These results suggest that acidic amino acids through NMDA – but not kainate or quisqualate – receptors could be part of the neurochemical substrate underlying SS of the MPC in the rat.

5.3 Other neural-related activities of γ-GT

Effect on brain transmitters: A highly selective action of γ-GT on brain transmitters was found in certain brain areas, especially in the cerebellum. Thus, the effect of γ-GT was studied on the turnover of dopamine, nor-epinephrine, and serotonin in the hypothalamus, mesencephalon, amygdala, septum, hippocampus, cortex, and cerebellum following intracerebroventricular administration. Dopamine and nor-epinephrine turnover was increased in the cerebellum, and nor-epinephrine turnover was also increased in the hypothalamus. Serotonin turnover was decreased in the mesencephalon. There was no change in catecholamine or serotonin turnover in the amygdala, septum, hippocampus, and cortex (Feuer et al., 1983).

Action in peripheral thyroid hormonal regulation: γ-GT was also examined for its effect on circulating thyroid hormones in the rat. In acute experiments performed over a 24-h period, γ-GT depressed plasma triiodothyronine (T3) levels in a dose-dependent manner, but thyroxine (T4) levels were not significantly affected. In chronic experiments performed over a two-week period, γ-GT significantly increased T3 levels, but as with the acute studies, T4 levels were not significantly altered. Following acute γ-GT administration, TSH concentrations were elevated above control levels. The increased T3 observed following chronic γ-GT administration might be due to a secondary increase in TSH levels. These data support the hypothesis that γ-GT aids in peripheral thyroid hormonal regulation. Observed differences between acute and chronic γ-GT action are thought to result from the effect of γ-GT on the negative feedback inhibiting the action of TSH (Baskin et al., 1987).

Effect on electroconvulsive shock-induced amnesia: The effects of oral γ-GT treatment on electroconvulsive shock-induced amnesia were also studied. Oral treatment with γ-GT (1–20 μg) was ineffective in treating electroshock-induced amnesia, if the treatment was applied before the electroconvulsive shock. However, 50 μg given in the same experimental paradigm could attenuate the amnesia considerably. When the treatment with γ-GT was applied immediately after the electroconvulsive shock, doses as low as 1 and 10 μg were effective in attenuating the amnesia in both 24- and 48 h retestings. However, when γ-GT was given 1 h before the 24- and 48 h retestings, it proved to be ineffective. The data indicate that γ-GT is effective in preventing electroconvulsive shock-induced amnesia by counteracting the effect of the shock on the memory consolidation phase, but that it is less effective in promoting learning, or possibly that it has no effect on retrieval processes. Both glutamine, as a precursor of inhibitory transmitters (e.g. GABA, glutamate), and taurine, which is also an inhibitory transmitter in the brain, can counteract convulsions. It is possible that γ-GT has a similar action and is thereby able to attenuate the action of electroconvulsive shock (Balazs and Telegdy, 1988).

Anti-epileptic effect: γ-GT often appears to have effects analogous to those of taurine itself. The anti-epileptic effect of taurine – stronger than that of γ-aminobutyric acid (GABA) – has been shown in amygdala-kindled animals and in cobalt or penicillin-induced seizures. Similarly, it was found that γ-GT has potent and long-lasting anti-epileptic action. Intra-amygdaloid injections of γ-GT strongly suppressed seizures in amygdala-kindled rats that

had been stimulated with intensities of 10 or 50 μA above the generalized seizure-triggering threshold (mean 82 μA). The suppressive effect of γ-GT persisted for as long as three days. Taurine, in contrast, had relatively weak suppressive effects. Thus, γ-GT seems much more potent than taurine in the suppression of epileptic seizures when injected directly into the kindling site. However, systemic injections of γ-GT (100 mg/kg, i.p.) failed to show any significant suppressive effect on kindled seizures. This finding suggests poor penetration of γ-GT into the brain and hence a probable lack of clinical applicability (Uemura et al., 1992). It was therefore suggested that other taurine dipeptides with better penetration characteristics might be worth exploring (Uemura et al., 1992).

Concentrations of γ-GT under anoxic conditions: It is commonly accepted that GGT, which is an ATP-independent enzyme, is involved in *de novo* dipeptide synthesis in the mammalian brain under anoxic conditions. The transport of amino acids such as glutamate was evaluated in the ischemic rat striatum by determination of the intra- and extracellular concentrations of γ-glutamyl dipeptides (the products of the transpeptidation) and glutathione (the physiological γ-glutamyl donor) (Orwar et al., 1994). An ischemic period (0–30 and 31–60 min) resulted in prominent increases in the respective concentrations of extracellular γ-glutamylglutamate (24- and 67-fold), γ-GT + γ-glutamylglycine (5.8- and 19-fold), and γ-glutamylglutamine (2.6- and 6.8-fold), as revealed by *in-vivo* microdialysis. Furthermore, under anoxic conditions *in vitro* (0–30 and 0–60 min), the respective striatal tissue concentrations were increased for γ-glutamylglutamate (20- and 17-fold), γ-GT (6.7- and 11-fold), γ-glutamylglutamine (1.7- and 1.2-fold), and γ-glutamylglycine (14- and 18-fold), whereas glutathione levels were, decreased on average, by approximately 350 μM.

γ-GT in schizophrenic disorders: By applying rigid bioanalytical techniques and by using drug-naive patients, it is possible to gain in-depth information on the pathophysiology of brain disorders such as schizophrenia. Many such studies support the notion that the glutamatergic system is affected in schizophrenic disorders. γ-GT is probably among the substances that aid in the discrimination between schizophrenic patients and controls. In one such study (Do et al., 1995), the cerebrospinal fluid (CSF) collected from a group of patients with schizophrenia, who either had been drug free for at least one year or were drug naive for psychotropic drugs, and the CSF from control subjects were analyzed (HPLC and gas chromatography-mass spectrometry) for 18 amino acids, N-acetylaspartate, N-acetylaspartylglutamate, and 5-hydroxyindoleacetic acid, derived from serotonin, and homovanillic acid, derived from dopamine. Significant differences were found only for taurine (15% lower in the patients) and isoleucine (7% higher). A number of unidentified substances were detected, one of which proved to be markedly reduced (16%) among the schizophrenic patients. Liquid chromatography-mass spectrometry with continuous flow FAB interface identified this substance as γ-glutamylglutamine. The decreased level of γ-glutamylglutamine may reflect a deficiency either in the GGT system, which is probably involved in glutamate uptake, or in glutamine, an important precursor of releasable glutamate. Although glutamate was non-significantly reduced in the patients, it was one of the five substances (including γ-glutamylglutamine) that best discriminated between the schizophrenic patients and the controls.

6 Effects of γ-GT on non-neural systems

6.1 Radiation protective effect of γ-GT

It is well known that taurine has a radiation protective effect and that ionizing radiation produces hypertaurinuria in mammals, including man. The latter effect is so characteristic that an attempt was made to use it for the early diagnosis of radiation sickness. Since parenteral administration of parathyroid extract significantly prolonged the survival of rats exposed to sublethal irradiation, a hypothesis was put forward that this protective effect is not due to the hypercalcemic effect of the parathyroid hormone but rather to the bioactive material γ-GT (Feure and Ormai, 1978). This hypothesis was later validated, and it was shown that exposure to X-rays produced mild hypoglycemia in rats. The hyperglycemia developed as a result of increased carbohydrate absorption following damage to the intestinal wall and of enhanced degradation of glycogen in liver and muscle tissue. Treatment with a bovine protein-free parathyroid extract (PF-PTE) or γ-GT prevented the development of hyperglycemia induced by X-ray whole body irradiation with 154.8, 219.3 or 258.0 mC/kg. Plasma immunoreactive insulin (IRI) values did not change following treatment. It was suggested that γ-GT acts *via* its antagonistic effect on glucocorticoids or *via* its regenerating effect on the intestinal mucosa (Feuer and Ormai, 1981).

Further investigation of the radiation protective effects in mice of γ-GT, some of its derivatives, and combinations of these compounds with substances of the aminoalkyl-thiol group showed that γ-GT exerts a radiation protective effect in animals irradiated with $LD_{50/30}$ of roentgen rays and ^{60}Co gamma rays (Feuer and Benko,

1981). γ-GT also had a favorable effect when administered after irradiation, but its protective effect was especially marked in the case of prolonged irradiation. Among various combinations of compounds the best results were obtained by simultaneous administration of γ-GT with subminimal doses of AET or cystamine. The mechanism of the protective effect was not clear, but it was suggested that the compounds possess an immunoregulating or immunostimulating effect that counteracts the damaging effect of irradiation on the immunosystem.

Oral topical and parenteral formulations containing γ-GT for treatment of skin injuries and autoimmune diseases caused by heat or light irradiation were devised and patented. The patent covers tablets containing γ-GT 0.01, lactone 83.99, PVP 3.00 g, alcohol 4.00 mL, distilled water 9.00 mL, talc 2.00, starch 10.00, and Mg stearate 1.00 g. These tablets were administered to volunteers in a study of the prevention of dermatitis solaris caused by sunlight (Szocsik et al., 1991).

6.2 Effect of γ-GT on metamorphosis of amphibians

In a study conducted on *Rana dalmatina*, prolonged treatment of the frogs with γ-GT (0.5 μg/mL in tap water), beginning at development stage 28 accelerated the resorption of the tail fin, shortened the body length, and enhanced limb-bud appearance. γ-GT also retarded ossification and reduced spontaneous mortality during developmental stages 28–30 of the experimental animals (Feuer et al., 1978a). In addition, γ-GT antagonized the metamorphosis-enhancing effect of triiodothyronine, significantly delaying the shortening of the body-length of tadpoles. The experimental results imply that γ-GT fulfills the role of a balance hormone (Feuer et al., 1979a).

Another study by the group of Feuer showed that at stage 26 of frog metamorphosis, the number of lysosomes and the acid phosphatase activity in tail-fin mesenchymal cells increased with γ-GT treatment (Feuer et al., 1979b). Macrophage cells, not present in controls, were observed in the experimental group. At stage 27, γ-GT retarded growth slightly but enhanced morphogenesis. At stage 28, accelerated morphogenesis was again observed with γ-GT-treated group, and a cell type with high lysosomal activity appeared in the tail fin margin and median areas. γ-GT also retarded the metamorphosis of *R. arvalis*. From day 13 of treatment, γ-GT moderated the metamorphosis-inhibiting effect of antibiotics acting at the transcription and translation levels (Feuer et al., 1979c). In another experiment, stage 26 larvae of *R. arvalis* were placed for 2 h daily in an aqueous solution of 10 μg/mL of sodium prednisolone succinate or of 10 μg/mL of prednisolone +0.5 μg/mL of γ-GT. Body length and development of the larvae were monitored. Prednisolone enhanced the development of larvae, and this effect was antagonized by γ-GT (Torok et al., 1979). In yet another study, it was shown that γ-GT considerably inhibited the cytochemically detected transcription-inducing effect (appearance of free histone) of prednisolone and triiodothyronine. Such an inhibition also took place if the morphogenetic hormones were given after – and not simultaneously with – γ-GT. When γ-GT was injected alone, it also displayed inductor properties, increasing the number of nuclei containing free histone (Csaba et al., 1979).

Levamisole accelerated the metamorphosis of *R. arvalis* in a way similar to that observed with vitamin A and γ-GT, i.e., it accelerated tail lysis, gut transformation, and intestinal mucosal goblet cell development. At high doses (500–2000 μg/mL) levamisole was teratogenic in the tadpole. Thus, the effects of levamisole on the immune system may be related to its direct vitamin A-like effect or to an indirect lysosomal labilizing property (Feuer et al., 1980a). γ-GT increased the involution of explanted tail tips of *R. arvalis* tadpoles *in vitro*, while it significantly antagonized a similar effect of triiodothyronine. This finding, in accordance with the results of earlier *in vivo* experiments, implies a direct effect of γ-GT on the target tissues (Feuer et al., 1980b).

6.3 Various activities, effects and applications of γ-GT

Effect on rat thymus cultures: γ-GT promoted growth of thymus cultures taken from rats of different age groups. Epithelial growth was prominent, and signs of activation of the macrophage cells were evident. In cell cultures, the nuclei and nucleoli increased in size (Feuer et al., 1978b). γ-GT exerted on rat thymus cultures an effect synergistic with vitamin A and antagonistic to prednisolone. When applied alone, γ-GT increased the vitality and macrophage reaction of thymus cultures (Feuer et al., 1978c).

Effects of γ-GT on plasma renin: γ-GT increased the plasma renin concentration (PRC) and plasma renin activity (PRA) in rats and dogs. In both species, peak values were observed 30 min after i.v. injection of 1 μg/kg of γ-GT. Increase of the dose to 10 or 100 μg/kg decreased γ-GT activity. Lower doses of γ-GT (1 μg/kg) administered i.v. decreased blood pressure in dogs within 15 min. A dose of 10 μg/kg decreased effective renal plasma flow from the 15th minute on. PRA was already enhanced by

the 15th minute after injection and reached a maximum at the 30th minute for all doses applied. Pretreatment with vitamin A (10,000 IU/kg/day i.m. for six days) had no influence on the γ-GT-produced changes of the above-described parameters. These effects of γ-GT are probably intra-renal or are based on action on the renal cortex. Apparently, γ-GT, as a lysosome labilizing substance, acts on the juxtaglomerular cell granules considered to be lysosome analogs (Feuer and Gaal, 1979).

Effect of γ-GT on manifestations of osteolathyrism: The effect of a parathormone-free, gel-filtered bovine parathyroid extract (designated extract P) and of γ-GT, on β-aminopropionitrile (BAPN)-induced osteolathyrism in male rats, was examined (Feuer et al., 1980c). Daily administration of 20 mg BAPN + 0.2 mg extract P/100 g body weight or of 40 mg BAPN + 0.2 μg γ-GT/100 g body weight was found to attenuate the manifestations of osteolathyrism to a significant extent, although they did not prevent their development completely. The significantly decreased Ca, P, chondroitin sulphate and chrondroprotein values due to the administration of BAPN was accompanied by a significant increase of cartilage in response to the extract and of both cartilage and bone in response to γ-GT.

Effect of γ-GT on the pineal gland: Since the putative precursor of γ-GT, the amino acid taurine, is known to affect the synthesis and/or release of melatonin by the pineal gland, it seemed worthwhile to investigate the morphological alterations caused in the gland in response to γ-GT administration. It was found that γ-GT treatment did not interfere with the melatonin-synthesizing function of the rat pineal gland, but that in response to prolonged treatment it did cause a numerical increase and aggregation of the mitochondria in the cell processes and their subsequent degeneration. γ-GT also stimulated autophagy, probably through its general lysosome-activating effect (Feuer et al., 1980d).

Inotropic effect of γ-GT on locust heart: Taurine, γ-GT and some other derivatives of taurine had a positive inotropic effect on isolated locust heart. γ-GT was the most effective compound, increasing the frequency of the spontaneously generated action potentials of the heart at doses of 5×10^{-11}–10^{-8} M. Among the other taurine derivatives, only γ-D-GT was more potent than taurine. At high doses, all the investigated substances were ineffective. The arrhythmic type of potential generation in the locust heart became regular under the influence of γ-GT, whereas taurine and its other derivatives failed to restore the regular firing of heart muscle (Feuer and Rozsa, 1981).

Effect of γ-GT on uric acid level: When γ-GT was administered orally to chicks over one week in doses of 0.5 and 2 μg/100 g, there was a reduction of the serum, hepatic, and renal tissue uric acid levels. In dogs, elevation of the serum uric acid level in response to parathyroidectomy could be prevented by a four-week oral treatment with 0.3 μg/kg of γ-GT. Differences in the uric acid levels in hypo- and hyperparathyroidism can perhaps be attributed to the deficiency or excess of γ-GT produced by the parathyroid (Feuer et al., 1981).

Effect of γ-GT on human antibody-dependent cell-mediated cytotoxicity: The effects of *in vitro* and *in vivo* treatment with γ-GT on human antibody-dependent cell-mediated cytotoxicity (ADCC) were studied in tumor patients and healthy subjects in a xenogeneic test system using chicken erythrocytes as target cells. A marked increase in killer cell activity was observed in tumor patients with originally low cytotoxic capacity, whereas the originally normal ADCC activity of other tumor patients and healthy subjects was not influenced by γ-GT treatment. The changes in cytotoxicity were not accompanied by changes in lymphocyte populations. Incubation of effector cells with γ-GT *in vitro* did not cause a change in ADCC activity in lymphocyte populations. Some similarities were found between the effects of γ-GT treatment on ADCC and that of dialyzable leukocyte extracts (Lang et al., 1981).

Inhibition by γ-GT of micronucleus formation: The ability of γ-GT to prevent the genotoxic action of mitomycin C (MMC) in rat bone marrow cells was examined by means of the micronucleus test. γ-GT (0.83 mg/kg) administered concurrently with MMC (0.75 mg/kg) did not exhibit any protective action. Pretreatment with a single dose (0.83 mg/kg) of γ-GT 24 h before the administration of MMC (0.75 mg/kg) prevented the enhanced micronucleus formation induced by the mutagen. It is possible that the metabolism (and its time course) of γ-GT differs from that of MMC and that the two agents do not thus reach the target cells in the bone marrow at the same time and in the same effective concentrations. It is also possible that γ-GT pretreatment facilitates accumulation of the drug in the body, thereby ensuring the development of an anti-mutagenic concentration in the bone marrow cells. Moreover, this effective concentration may be coupled with a higher poly-ADP-ribose level, which can promote repair of the DNA damage induced by MMC. Further studies are required to elucidate whether γ-GT is itself an anti-mutagenic compound or whether it exerts such effect through its vitamin A-like activity, through enhancement of poly-ADP-ribose production, or

possibly through a quite different mechanism (Toth and Csaba, 1988).

Neurohormonal regulation by γ-GT of cellular magnesium: It has been suggested that in addition to the parathyroid hormones, calcitonin, taurine, insulin and epinephrine, γ-GT may be involved in the hormonal regulation of Mg^{2+} homeostasis (Durlach and Durlach, 1984). A complex neurohormonal system is instrumental in the control of the stability of intracellular magnesium and of the consequences of disturbances in the magnesium status. Exchanges between extracellular compartments and soft tissues elicit secretion of hormones and neurohormones (e.g. adrenalin, insulin, taurine and γ-GT) and finally β-stimulation of the adrenergic receptors. These neurohormonal factors regulating cellular magnesium content must be taken into account if we are to appreciate the significance of the variations of erythrocyte magnesium concentrations in pathological or iatrogenic disturbances such as diabetes mellitus, phaeochromocytoma or stress pathology. Both taurine and γ-GT appear to be important factors in magnesium homeostasis. In magnesium deficiency, taurine and possibly γ-GT, through their membrane stabilizing, Ca-binding and cGMP level-lowering effects, can specifically counterbalance the noxious effects induced in the cell by epinephrine-insulin hypersecretion. Through an eventual ''Mg-sparing'' effect, taurine, and possibly γ-GT, may also act specifically on magnesium homeostasis (Millart et al., 1995).

Manufacture of γ-GT-enriched fish sauce: A technique for manufacturing γ-GT-enriched fish sauce has been developed. A salt-free sauce was produced by mixing alkalase, fermenting fish meat, soy sauce koji, glucose and dried yeast. An enzyme solution was prepared by hydrolyzing wheat gluten and defatted soybeans, in the presence of alkalase and peptidase. The γ-GT-enriched fish sauce was produced by incubating the salt-free sauce and the enzyme solution with glutaminase extracted from bread flour and skimmed milk. The salt-free fish sauce so obtained was found to contain 10–20 times more γ-GT than a comparable salt-containing fish sauce (Maeda, 1999).

7 Conclusions

In the late 1970s, a great deal of excitement accompanied the isolation and identification of γ-GT. From that time until the mid-1990s, intensive work was undertaken on the isolation, structure determination, enzymatic preparation, non-enzymatic syntheses and biologic activities of this dipeptide. It is surprising and unexplainable why interest in this unique dipeptide has declined in the past decade.

Although a variety of effects of γ-GT have been recorded in both neural and non-neural systems, several effects deserve special attention. The most important of these are summarized below.

γ-GT exhibits anti-conflict activity and has an unusual effect on emotional arousal. Effects as the reduction of aversion, of phobia or of anxiety levels, as well as impair of spatial memory, deserve extensive research and more supporting data.

There is a large body of supporting evidence that γ-GT plays a central role in the modulation of excitatory aminoacidergic neurotransmission. This dipeptide, and maybe other sulfonic amino acids and sulfonic peptides, could become part of the neurochemical substrate underlying SS of the MPC.

γ-GT exerts a variety of influences on neural activities: (i) It prevents electroconvulsive-shock-induced amnesia. (ii) It is a potent anti-epileptic agent when injected directly into the kindling site, more potent than taurine. Systemic injection has no effect, and this limits the potential clinical application of γ-GT. Additional research could produce some simple and more lipophelic pro-drugs of γ-GT, which could become powerful anti-epileptic drugs. (iii) The pathophysiology of schizophrenia remains a topic of ongoing interest and γ-GT is probably one of the substances that may be used to discriminate between schizophrenic patients and controls. The reduced concentration of γ-GT (more than 16%) can be utilized as a biomarker in detection of schizophrenia and for evaluating the disease status.

The roles played by γ-GT in non-neural systems are also a source of inspiration for future research. For example, the antagonism by γ-GT of the metamorphosis of amphibians can be envisioned as a future source of insect control. The clinical potential of γ-GT in reducing blood pressure and its inotropic action in the heart deserve serious consideration and are certainly worthy of further experimentation. γ-GT reduces the serum, hepatic and renal tissue uric acid levels. Since a large section of the population suffers from elevated uric acid, γ-GT or one of its analoges could be developed as a novel therapeutic compound. Similarly, the radioactivity protective and anticancer properties of γ-GT as well as its use in the treatment of skin injuries are worthy of in-depth investigation.

All the findings described in this review require mechanistic support, which is currently lacking. It is important therefore to strive and provide genuine insight into the mechanism(s) of γ-GT activities. Involvement of γ-GT in a wide range of activities makes this task difficult and it will be hard to formulate a unified path of action.

We would like to conclude with a wish: it is disheartening to see that the scientific work on this molecule has, by and large, gone into hibernation over the past 10 years. Such a situation is uncalled for regarding a molecule with this much potential. We trust that this review will provide the input to reverse this situation and to motivate intensive research on the chemistry of peptidomimetics and pseudopeptides and a search for new applications. We hope that the use of γ-GT will be promoted by introduction of γ-GT-containing products in the form of tablets and lotions, as therapeutic and nutritional agents.

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