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Short communication

Inactivation of Brain Glutamate Dehydrogenase Isoproteins by MDL 29951

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In addition to the recognition site for glutamate, the N-methyl-D-aspartate (NMDA)-preferring glutamate receptor subtype shows a binding site for glycine. In this paper, we present the effects of 3-(4,6-dichloro-2-carboxymethylamino-5,7-dichloroquinoline-2-carboxylic acid (MDL 29951), a potent inhibitor of glycine binding to the NMDA receptor, on glutamate dehydrogenase (GDH) from bovine brains. The incubation of GDH isoproteins from bovine brains with MDL 29951 resulted in a dose-dependent loss of enzyme activity. Separately or together, 2-oxoglutarate and NADH did not give an efficient protection against the inhibition, indicating that GDH isoproteins saturated with NADH or 2-oxoglutarate are still open to attack by MDL 29951. MDL 29951 was an uncompetitive inhibitor with respect to both 2-oxoglutarate and NADH for GDH isoproteins. These results suggest that the binding site of MDL 29951 is not directly located at the catalytic site, and the inhibition of GDH isoproteins by MDL 29951 is probably due to a steric hindrance, or a conformational change altered upon the interaction of the enzyme with its inhibitor. The inhibitory effects of MDL 29951 on GDH isoproteins were significantly diminished in the presence of ADP. GDH I reacted more sensitively with ADP than GDH II on the inhibition by MDL 29951. Our results suggest a possibility that the two types of GDHs are differently regulated by MDL 29951, depending on the physiological concentrations of ADP.

Keywords: ADP, Enzyme inhibition, Glutamate dehydrogenase isoproteins, MDL 29951

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Introduction

Although glutamate, in the form of its sodium salt, is a widely used food additive, the specific mechanism underlying the effect of ingestion of glutamate on the brain has not been definitively understood. Due to its neurotoxic potentials, it has been suggested that glutamate may be involved in the pathogenesis of human degenerative disorders. In contrast to the extensive investigation of glutamate receptor antagonists as neuroprotectants, there has been relatively little study on the strategies for regulating synaptic glutamate directly.

One enzyme central to the metabolism of glutamate is glutamate dehydrogenase (GDH). GDH (EC 1.4.1.3) is a family of enzymes, catalyzing a reversible deamination of L-glutamate to 2-oxoglutarate (Fisher, 1985). It is differentially distributed in various catalytically active isoforms of the enzyme (Plaitakis *et al.*, 1984; Colon *et al.*, 1986; Abe *et al.*, 1992; Plaitakis *et al.*, 1993). Although the origin of the GDH polymorphism is unknown, the presence of differently sized mRNAs, and multiple gene copies for GDH, has been reported (Amuro *et al.*, 1988; Michaelidis *et al.*, 1993; Shashidharan *et al.*, 1994). We isolated two types of GDH isoproteins from bovine brain (Cho *et al.*, 1995; Cho and Lee, 1996; Choi *et al.*, 1999), and reported the regulatory properties of the GDH isoproteins (Ahn *et al.*, 1999a; Cho *et al.*, 1996; Kim *et al.*, 1997; Cho *et al.*, 1998a; Cho *et al.*, 1998b; Cho and Yoon, 1999; Cho *et al.*, 1999). The importance of the physiological nature of GDH isotypes has attracted considerable interest. For instance, the enzyme isolated from one of the patients with a variant form of multisystem atrophy displayed a marked reduction of one of the GDH isoproteins (Mavrothalassitis *et al.*, 1988; Hussain *et al.*, 1989; Plaitakis *et al.*, 1993). Recently, Cavallaro *et al.* (1997) identified GDH as one of the late memory-related genes in the hippocampus. The existence of the hyperinsulinism-hyperammonemia syndrome (caused by mutations in a human GDH gene) further highlights the

importance of GDH in the regulation of insulin secretion, and indicates that GDH has an important role in regulating hepatic ureagenesis (Fahien *et al.,* 1988; Bryla *et al.,* 1994; Stanley *et al.,* 1998). It is, therefore, essential to have a detailed structural and functional description of the various types of GDH to elucidate the physiological nature of GDH.

From a potential therapeutic point of view, the N-methyl-D-aspartic acid (NMDA) receptor complex has attracted considerable interest (Wood *et al.,* 1990). The NMDA receptor complex possesses several allosteric binding sites that alter the cellular response to glutamic acid. Two identical subunits are proposed to bind glutamic acid, while two other identical subunits bind glycine to a strychnine-insensitive binding site (Johnson and Bigge, 1991). Since glycine is a cotransmitter with glutamate and obligatory for receptor activation, potent antagonists, such as competitive NMDA antagonists, can have potential clinical applications in the NMDA receptor-related disorders. Recently, the pharmacological effects of 3-(4,6-dichloro-2-carboxyindol-3-yl)-propionic acid (MDL 29951) as a novel glycine antagonists of the NMDA receptor have been reported (King *et al.,* 1990). It is a potent antagonist for the strychnine-insensitive glycine binding site of the NMDA receptor (Baron *et al.,* 1992). However, the reaction mechanism of the biochemical and pharmacological actions of MDL 29951 has not yet been understood.

The known glutamate-degrading enzymes, such as glutamine synthetase, GDH, and glutamate pyruvate transaminase, have been shown to have neuroprotective value in models of glutamate excitotoxicity (Matthews *et al.,* 2000). In this study, we investigated the effects of MDL 29951 on the activities of two types of GDH isoproteins from bovine brains at various physiological conditions. To our knowledge, this is the first report showing inhibitory effects of MDL 29951 on GDH isoproteins isolated from brain tissues.

Materials and Methods

Materials NADH, NADPH, NAD^+, $NADP^+$, 2-oxoglutarate, glutamate, ADP, and GTP were purchased from Sigma (St. Louis, USA). MDL 29951 was purchased from Tocris. Bovine brains were obtained from the Majang Slaughterhouse, Seoul, Korea. The GDH isoproteins were purified from bovine brain by the method developed in our laboratory (Cho *et al.,* 1995), and were homogeneous as judged by a Coomassie-stained gradient SDS-polyacrylamide gel electrophoresis. All other chemicals and solvents were of reagent grade or better.

Enzyme assay GDH activity was measured spectrophotometrically in the direction of reductive amination of 2-oxoglutarate by following the decrease in absorbance at 340 nm, as described previously (Ahn *et al.,* 1999b), except no ADP was used unless otherwise indicated. All assays were performed in triplicate, and initial velocity data were correlated with a standard assay mixture containing 50 mM triethanolamine, pH 8.0, 100 mM ammonium acetate, 0.1 mM NADH, and 2.6 mM EDTA at 25°C. GDH concentrations were adjusted to give a measured rate of about 0.04 absorbance units per min. One unit of enzyme was defined as the amount of enzyme required to oxidize 1 μmol of NADH per min at 25°C. GDH activity was also measured in the direction of oxidative deamination of glutamate by following the increase in absorbance at 340 nm, as described previously (Ahn *et al.,* 1999b).

Enzyme kinetic studies Unless otherwise specified, highly purified GDH fractions were used for inhibition studies. The modification was carried out at 25°C by incubating the enzyme with various concentrations of MDL 29951 in 50 mM triethanolamine, pH 8.0. At the indicated time intervals, an aliquot of the incubation mixture was assayed for enzyme activity, as described previously. Controls without an inhibitor were included, and the remaining activities were assayed. For protection experiments, the enzymes were preincubated with substrates prior to the addition of MDL 29951. Aliquots were withdrawn from the mixture to determine the remaining activity. For enzyme kinetic studies, the assays were carried out in the absence, or presence of allosteric effectors by varying the substrate under investigation, while keeping the other substrate and reagents at the optimal concentration. Affinity for the inhibitor binding (K_3) was determined as described by Shemisa and Fahien (1971), and data were analyzed by non-linear regression to obtain K_3.

Quantitative Affinity Chromatography Affinity matrix and quantitative affinity chromatography was prepared by the coupling of MDL 29951 to AH-activated Sepharose 4B (Veronese *et al.,* 1979.) Briefly, affinity chromatographic elutions were performed in the dark with a column of 0.9 × 15 cm bed volume at 4°C, and 0.5 ml fractions were collected at a flow rate of 9 ml/hr. GDH isoproteins (0.15 mg each) in the eluting buffer (20 mM potassium phosphate, pH 8.0 containing 0.1 mM EDTA and 10 mM 2-mercaptoethanol) were applied to the column equilibrated with an eluting buffer containing corresponding concentrations of MDL 29951. The elution position of GDH was determined by assays of fractions for the GDH activity using NADH as described (Cho *et al.,* 1995). The variation of enzyme elution volume with MDL 29951 was plotted according to the following equation (Veronese *et al.,* 1979.)

$$1/(V - V_o) = [L]K_{LM}/K_L(V_o - V_m)[LM] + K_{LM}/(V_o - V_m)[LM] \quad \text{(Eq. 1)},$$

where V = protein elution volume; V_o = volume at which protein elutes in the absence of interaction (determined in a column with thioethyloxy groups replacing MDL 29951); V_m = void volume (determined by Blue Dextran 2000); [L] = concentration of soluble ligand; [LM] = concentration of immobilized ligand; K_{LM} = dissociation constant for the immobilized ligand-protein interaction; K_L = dissociation constant for the soluble ligand-protein interaction. K_L values were calculated as the ratio of ordinate intercept to slope of $1/(V - V_o)$ vs. [L] plots.

Results and Discussion

In contrast to the extensive investigation of glutamate receptor antagonists as neuroprotectants, there has been relatively little study on the strategies for reducing synaptic glutamate directly. The known glutamate-degrading enzymes, such as

glutamine synthetase, GDH, and glutamate pyruvate transaminase, have been shown to have neuroprotective value in models of glutamate excitotoxicity (Matthews *et al.*, 2000). It was suggested that GDH is associated with the pool of glutamate that is released as a neurotransmitter at the nerve endings (Aoki *et al.*, 1987). The precise roles of GDH in the central nervous system, as well as the predominant direction of the reaction it catalyzes, remain unclear. However, given the extensive nature of the glutamatergic pathways in the brain (Young and Fagg, 1990), GDH may play a role in a number of human neurodegenerations. Therefore, the regulation of GDH that is important in adjusting the levels of the neurotransmitter glutamate might be worth examining in the context of experimental and clinical disorders. In this study, we investigated the effects of MDL 29951, a novel glycine antagonist of the NMDA receptor, on the activities of two types of brain GDH isoproteins, an enzyme central to the metabolism of glutamate, at various physiological conditions.

Purified GDH isoproteins from bovine brains were inhibited by MDL 29951 at 25°C. The time course of inhibition in the direction of reductive amination of 2-oxoglutarate is shown in Fig. 1(A) and (B) for GDH I and GDH II, respectively. Since protection experiments are found to be critical for determining the location of the site of the MDL 29951 reaction in GDH, we examined the effect of 2-oxoglutarate and NADH on the inhibition by MDL 29951. The inhibition was not effectively prevented by preincubation of the GDH isoproteins with NADH (Fig. 1), indicating that the GDH isoproteins saturated with NADH are still open to attack by MDL 29951. Similar to the effect of NADH, 2-oxoglutarate also gave only a minor protection against the inhibition caused by MDL 29951 (Fig. 1). A combination of NADH and 2-oxoglutarate in preincubation with GDH isoproteins never gave complete protection against MDL 29951 for both GDH I and GDH II. The inhibition by MDL 29951 in the direction of oxidative deamination of glutamate were also not effectively prevented by preincubation of the GDH isoproteins with 2-oxoglutarate or NADH (data not shown). There were no differences in the sensitivities to inhibition by MDL 29951 between GDH I and GDH II, in both direction of reductive amination of 2-oxoglutarate and oxidative deamination of glutamate. These results suggest a possibility that the microenvironmental structures of the MDL 29951 binding site on GDH isoproteins may be similar to each other. A more detailed analysis of the effect of MDL 29951 on GDH isoproteins showed that MDL 29951 was an uncompetitive inhibitor with respect to both 2-oxoglutarate and NADH (data not shown). These results suggest that the MDL 29951-modified site is not located at the coenzyme or substrate binding site, and the inactivation of GDH by MDL 29951 may be due to a steric hindrance, or a conformational change altered upon the interaction of the enzyme with its inhibitor.

Since it is well documented that GDH is highly regulated by allosteric regulators, it is important to take into account the regulators when considering the inhibitory actions of drugs on GDH. Therefore, we examined the effects of GTP and ADP, well known GDH regulators (McCarthy *et al.*, 1980; Fisher, 1985), on the inhibition by MDL 29951. The data in Fig. 2 shows the effects of allosteric effectors GTP and ADP on the inhibition of GDH isoproteins by MDL 29951. The allosteric inhibitor GTP did not affect inhibition by MDL 29951. As shown in Fig. 2*A*, the affinities for inhibitor binding (K_3) were not significantly changed by the presence of GTP for both GDH isoproteins, indicating that the inhibition by MDL 29951 was complete in the absence or presence of GTP. In contrast to the effects of GTP, the sensitivities of the two isoproteins to inhibition by MDL 29951 in the presence of ADP depended on their affinities for inhibitor binding, as measured by the constant K_3 (Fig. 2B). The inhibitory effects

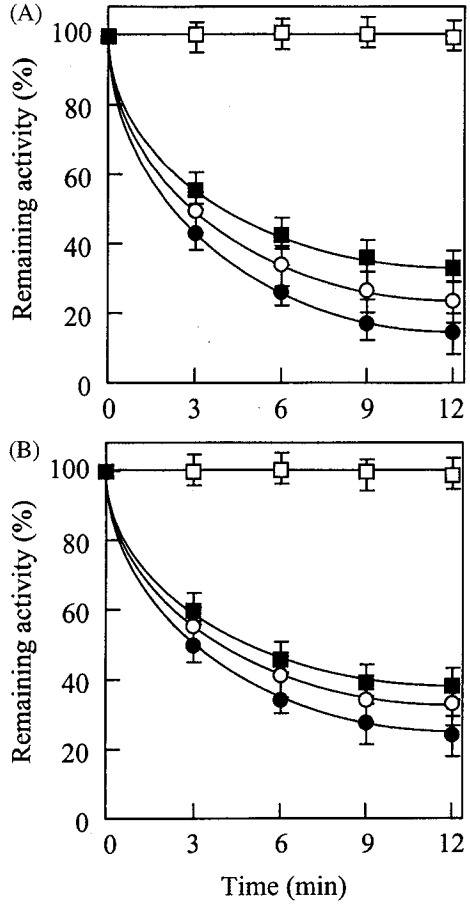

Fig. 1. Inhibition of GDH by MDL 29951. GDH I (A) and GDH II (B) were treated with MDL 29951 in the presence and absence of 2-oxoglutarate or NADH. At indicated times, the remaining activities were assayed by the addition of the standard assay mixture. This diluted the concentrations of MDL 29951, 2-oxoglutarate, and NADH to 0.1 mM, 10 mM, and 1 mM, respectively, during the assay. All data represent mean values ± standard deviation for two separate experiments. □ (GDH only); ● (GDH + MDL 29951), ■ (GDH + 2-oxoglutarate + MDL 29951); ○ (GDH + NADH + MDL 29951).

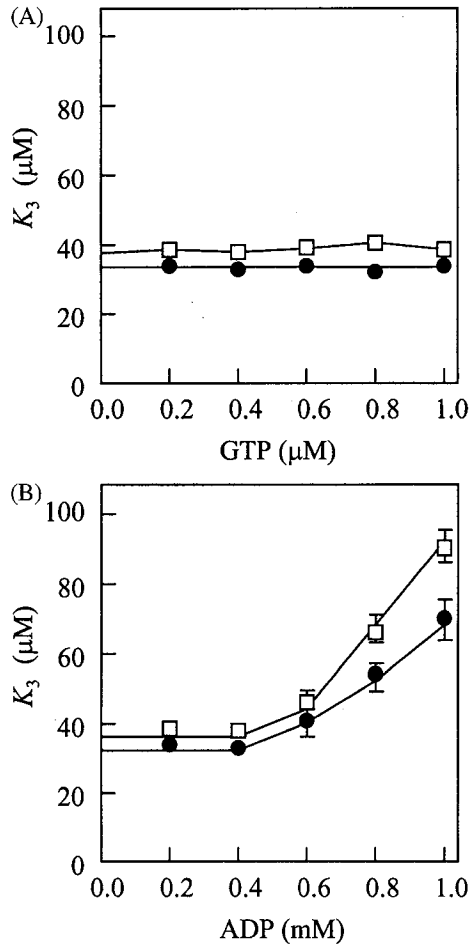

Fig. 2. Effects of ADP or GTP on the inhibition of GDH isoproteins by MDL 29951. The concentration of MDL 29951 was varied while the GTP (A) or ADP (B) concentration was held constant as indicated. K_3 (mM) values were determined by non-linear regression and were plotted against the ADP or GTP concentration as described by Shemisa and Fahien (1971). All data represent mean values ± standard deviation for the two separate experiments. GDH I (□); GDH II (●)

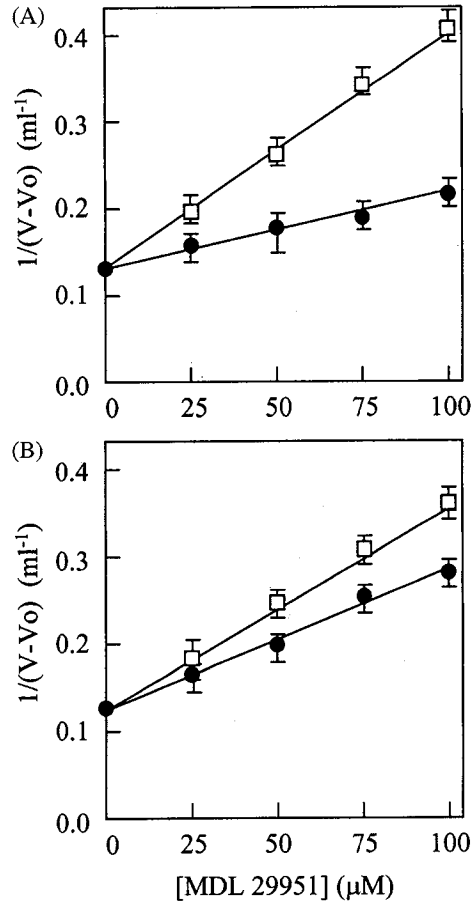

Fig. 3. Influence of ADP on the binding of GDH isoproteins to MDL 29951-Sepharose. $1/(V - Vo)$ against the total soluble MDL 29951 concentration is plotted in the presence of varying amounts of ADP. The protein elution volume (V) was determined from the affinity chromatography elution profiles of GDH isoproteins on MDL 29951-Sepharose in the presence of varying concentrations of soluble MDL 29951 in the standard elution buffer. The concentrations of ADP used are: 0.0 mM (□) and 1.0 mM (●). All data represent mean values ± standard deviation for two separate experiments. (A) GDH I; (B) GDH II

of MDL 29951 on GDH isoproteins were diminished in the presence of ADP. Moreover, the sensitivities to inhibition by MDL 29951 in the presence of high concentrations of ADP were distinct for the two GDH isoproteins. GDH I (K_3 values from 36 μM to 85 μM) was more sensitively affected by high concentrations of ADP than GDH II (K_3 values from 32 μM to 68 μM) on the inhibition by MDL 29951.

In view of the inhibitory effects of MDL 29951 on GDH isoproteins, we undertook the direct characterization of the presumed binding processes by quantitative affinity chromatography on MDL 29951-Sepharose. ADP was added in separate experiments to the eluting buffer at concentrations up to 1.0 mM. No appreciable changes were observed for both GDH isoproteins in their binding affinities for MDL 29951-Sepharose, when a low concentration of ADP (up to 0.5 mM) was added to the eluting buffer (data not shown). On the other hand, a reduction of the elution volume of GDH isoproteins was observed. The binding affinity significantly changed in the presence of a high concentration of ADP (1.0 mM) (Fig. 3.) The influence of ADP at a high concentration on the binding of GDH isoproteins to MDL 29951-Sepharose was significantly distinct for the two isoproteins. GDH I (K_L values from 45 μM to 162 μM) was more sensitively affected by a high concentration of ADP than GDH II (K_L values from 62 μM to 125 μM) on their binding affinities for MDL 29951-Sepharose (Fig. 3.) These results are consistent with those obtained from enzyme inhibition studies (Fig. 2).

Previously, the presence of two human GDH-specific genes encoding highly homologous polypeptides was reported (Shashidharan *et al.*, 1994). The first gene is expressed in all tissues, designated GLUD1 (housekeeping GDH), whereas the second gene is expressed specifically in neural and

testicular tissues, designated GLUD2 (nerve tissue-specific GDH). Recently, it was reported that ADP regulates differently the activities of the GDH isotypes that are proportionally greater for the nerve tissue-specific human GDH than for the housekeeping GDH (Shashidharan *et al.*, 1997; Plaitakis *et al.*, 2000). Similar results were reported from our laboratory, showing that bovine brain GDH I and GDH II are differently regulated by ADP (Cho *et al.*, 1995). The data in this work show that the sensitivity of GDH I to inhibition by MDL 29951 in the presence of ADP was significantly higher than that of GDH II. These observations are consistent with previous reports that there are at least two different GDH activities differing in their relative thermal stability and allosteric regulation characteristics in the brain (Plaitakis *et al.*, 1984; Abe *et al.*, 1992; Cho *et al.*, 1999). Since physiological ADP levels can vary from 0.05 to >1.0 mM, depending on the rate of oxidative phosphorylation, our results suggest the possibility that the two types of GDHs are regulated differently by the actions of MDL29951, depending on the physiological concentrations of ADP; and the different regulatory properties of ADP on GDH isotypes may be of importance for regulating glutamate fluxes in vivo under changing energy demands.

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References

Abe, T., Ishiguro, S. I., Saito, H, Kiyosawa, M. and Tamai, M. (1992) Partially deficient glutamate dehydrogenase activity and attenuated oscillatory potentials in patient with spinocerebellar degeneration. *Invest. Ophthalmol. Vis. Sci.* **33**, 447-452.

Ahn, J.-Y., Choi, S. Y. and Cho, S.-W. (1999a) Identification of lysine residue involved in inactivation of brain glutamate dehydrogenase isoproteins by o-phthalaldehyde. *Biochimie* **81**, 1-7.

Ahn, J.-Y., Cho, E. H., Lee, K. S., Choi, S. Y. and Cho, S.-W. (1999b) Chemical modification of brain glutamate dehydrogenase isoproteins with phenylglyoxal. *J. Biochem. Mol. Biol.* **32**, 515-520.

Amuro, N., Yamaura, M., Goto, Y. and Okazaki, T. (1988) Molecular cloning and nucleotide sequence of the cDNA for human liver glutamate dehydrogenase precursor. *Biochem. Biophys. Res. Commun.* **152**, 1395-1400.

Aoki, C., Milner, T. A., Sheu, K.-F. R., Blass, J. P. and Pickel, V. M. (1987) Regional distribution of astrocytes with intense immunoreactivity for glutamate dehydrogenase in rat brain: implications for neuron-glia interactions in glutamate transmission. *J. Neurosci.* **7**, 2214-2231.

Baron, B. M., Harrison, B. L., McDonald, I. A., Meldrum, B. S., Palfreyman, M. G., Salituro, F. G., Siegel, B. W., Slone, A. L., Turner, J. P. and White, H. S. (1992) Potent indole- and quinoline-containing N-methyl-D-aspartate antagonists acting at the strychnine-insensitive glycine binding site. *J. Pharmacol. Exp. Ther.* **262**, 947-956.

Bryla, J., Michalik, M., Nelson, J. and Erecinska, M. (1994) Regulation of the glutamate dehydrogenase activity in rat islets of Langerhans and its consequence on insulin release. *Metabolism* **43**, 1187-1195.

Cavallaro, S., Meir, N., Yi, CL., Musco, S., Ma, W., Goldberg, J. and Alkon, D. L. (1997) Late memory-related genes in the hippocampus revealed by RNA fingerprinting. *Proc. Natl. Acad. Sci. USA* **94**, 9669-9673.

Cho, S.-W., Lee, J. and Choi, S. Y. (1995) Two soluble forms of glutamate dehydrogenase isoproteins from bovine brain. *Eur. J. Biochem.* **233**, 340-346.

Cho, S.-W., Ahn, J.-Y., Lee, J. and Choi, S. Y. (1996) Identification of a peptide of the guanosine triphosphate binding site within brain glutamate dehydrogenase isoproteins using 8-azidoguanosine triphosphate. *Biochemistry* **35**, 13907-13913.

Cho, S.-W. and Lee, J. E. (1996) Modification of brain glutamate dehydrogenase isoproteins with pyridoxal 5-phosphate. *Biochimie* **78**, 817-821.

Cho, S.-W., Cho, E. H. and Choi, S. Y. (1998a) Activation of two types of brain glutamate dehydrogenase isoproteins by gabapentin. *FEBS Lett.* **426**, 196-200.

Cho, S.-W., Yoon, H.-Y., Ahn, J.-Y., Choi, S. Y. and Kim, T. U. (1998b) Identification of a NAD^+ binding site of brain glutamate dehydrogenase isoproteins by photoaffinity labeling. *J. Biol. Chem.* **272**, 31125-31130.

Cho, S.-W., Cho, E. H., Hwang, S.-H. and Choi, S. Y. (1999) Reactive cysteine residue of bovine brain glutamate dehydrogenase isoproteins. *Mol. Cells* **9**, 91-98.

Cho, S.-W. and Yoon, H.-Y. (1999) Photoaffinity labeling of brain glutamate dehydrogenase isoproteins with an azido-ADP. *J. Biol. Chem.* **274**, 13948-13953.

Choi, S. Y., Hong, J. W., Song, M.-S., Jeon, S. G., Bahn, J. H., Lee, B. Y., Ahn, J.-Y. and Cho, S.-W. (1999) Different antigenic reactivities of bovine brain glutamate dehydrogenase isoproteins. *J. Neurochem.* **72**, 2162-2169.

Colon, A., Plaitakis, A., Perakis, A., Berl, S. and Clarke, D. D. (1986) Purification and characterization of a soluble and a particulate glutamate dehydrogenase from a rat brain. *J. Neurochem.* **46**, 1811-1819.

Fahien, L. A., MacDonald, M. J., Kmiotek, E. H., Mertz, R. J. and Fahien, C. M. (1988) Glutamate dehydrogenase. IV. Studies on enzyme inactivation and coenzyme binding. *J. Biol. Chem.* **263**, 13610-13614.

Fisher, H. F. (1985) L-Glutamate dehydrogenase from bovine liver. *Methods Enzymol.* **113**, 16-27.

Hussain, M. H., Zannis, V. I. and Plaitakis, A. (1989) Characterization of glutamate dehydrogenase isoproteins purified from the cerebellum of normal subjects and patients with degenerative neurological disorders, and from human neoplastic cell lines. *J. Biol. Chem.* **264**, 20730-20735.

Johnson, G. and Bigge, C. F. (1991) Recent advances in excitatory amino acid research; in *Annual Reports in Medical Chemistry*, Bristol, J. A. (ed.), Vol. 26, pp. 11-22, Academic Press, New York.

Kim, S. W., Lee, J., Song, M.-S., Choi, S. Y. and Cho, S.-W. (1997) Essential active-site lysine of brain glutamate dehydrogenase isoproteins. *J. Neurochem.* **69**, 418-422.

King, F. D., Dabbs, S., Bermudez, J. and Sanger, G. J. (1990) 3-(2-Carboxyindol-3-yl) propionic acid derivatives: Antagonists of

the strychnine-insensitive glycine receptor associated with the N-methyl-D-aspartate receptor complex. *J. Med. Chem.* **33**, 2944-2946.

Matthews, C. C., Zielke, H. R., Wollack, J. B. and Fishman, P. S. (2000) Enzymatic degradation protects neurons from glutamate excitotoxicity. *J. Neurochem.* **75**, 1045-1052.

Mavrothalassitis, G., Tzimagiorgis, G., Mitsialis, A., Zannis, V. I., Plaitakis, A., Papamatheakis, J. and Moschonas, N. K. (1988) Isolation and characterization of cDNA clones encoding human liver glutamate dehydrogenase: Evidence for a small gene family. *Proc. Natl. Acad. Sci. USA* **85**, 3494-3498.

McCarthy, A. D., Walker, J. M. and Tipton, K. F. (1980) Purification of glutamate dehydrogenase from ox brain and liver. *Biochem. J.* **191**, 605-611.

Michaelidis, T. M., Tzimagiorgis, G., Moschonas, N. K. and Papamatheakis, J. (1993) The human glutamate dehydrogenase gene family: gene organization and structural characterization. *Genomics* **16**, 150-160.

Plaitakis, A., Berl, S. and Yahr, M. D. (1984) Neurological disorders associated with deficiency of glutamate dehydrogenase in the rat cerebellar cortex. *J. Neurosci.* **62**, 1133-1146.

Plaitakis, A., Flessas, P., Natsiou, A. B. and Shashidharan, P. (1993) Glutamate dehydrogenase deficiency in cerebellar degenerations: clinical, biochemical and molecular genetic aspects. *Can. J. Neurol. Sci. Suppl.* **3**, S109-S116.

Plaitakis, A., Metaxari, M. and Shashidharan, P. (2000) Nerve tissue-specific (GLUD2) and housekeeping (GLUD1) human glutamate dehydrogenases are regulated by distinct allosteric mechanisms: Implications for biologic function. *J. Neurochem.* **75**, 1862-1869.

Shashidharan, P., Michaelidis, T. M., Robakis, N. K., Kresovali, A., Papamatheakis, J. and Plaitakis, A. (1994) Novel human glutamate dehydrogenase expressed in neural and testicular tissues and encoded by an X-linked intronless gene. *J. Biol. Chem.* **269**, 16971-16976.

Shashidharan, P., Clarke, D. D., Ahmed, N., Moschonas, N. and Plaitakis, A. (1997) Nerve tissue-specific human glutamate dehydrogenase that is thermolabile and highly regulated by ADP. *J. Neurochem.* **68**, 1804-1811.

Shemisa, O. A. and Fahien, L. A. (1971) Modification of glutamate dehydrogenase by various drugs which affect behavior. *Mol. Pharmacol.* **7**, 8-25.

Stanley, C. A., Lieu, Y. K., Hsu, B. Y. L., Burlina, A. B., Greenberg, C. R., Hopwood, N. J., Perlman, K., Rich, B. H., Zammarchi, E. and Poncz, M. (1998) Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *New. Eng. J. Med.* **338**, 1353-1357.

Veronese, F. M., Bevilacqua, R. and Chaiken, I. M. (1979) Drug-protein interactions: evaluation of the binding of antipsychotic drugs to glutamate dehydrogenase by quantitative affinity chromatography. *Mol. Pharmacol.* **15**, 313-321.

Wood, P. L., Rao, T. S., Iyengar, S., Lanthorn, T., Monahan, J., Cordi, A., Sun, E., Vazquez, M., Gray, N. and Contreras, P. (1990) A review of the in vitro and in vivo neurochemical characterization of the NMDA/PCP/glycine/ion channel receptor macrocomplex. *Neurochem. Res.* **15**, 217-230.

Young, A. B. and Fagg, G. E. (1990) Excitatory amino acid receptors in the brain; membrane binding and receptor autoradiographic approaches. *Trends Pharmacol. Sci.*, **11**, 126-133.