Activation of two types of brain glutamate dehydrogenase isoproteins by gabapentin

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Abstract **The stimulatory effects of gabapentin on the activities of two types of glutamate dehydrogenase (GDH) isoproteins homogeneously purified from bovine brain have been studied at various conditions. When the effects of different gabapentin concentrations on GDH activities were studied in the direction of reductive amination of 2-oxoglutarate with NADPH as a coenzyme, a marked activation was observed for both isoproteins, whereas both isoproteins showed activation to a lesser extent with NADH as a coenzyme. Stimulatory effects of gabapentin on GDH activities in the direction of the oxidative deamination of glutamate were also observed, but to a much lesser extent than reductive amination. There were big differences between the two GDH isoproteins in their sensitivity to the action of gabapentin. The largest activation was observed with GDH II when NADPH was used as a coenzyme. Half-maximal stimulation was reached at around 1.5 mM. Gabapentin relieved the inhibition of GDH isoproteins by GTP and this resulted in an increase in the apparent activation by gabapentin in the presence of GTP. 2-Oxoglutarate was found to give rise to high substrate inhibition and gabapentin reduced the substrate inhibition in the presence of 0.2 mM NADH. Since there are neurodegenerative disorders in which GDH activity is decreased, the therapeutic modulation of the activity of this enzyme may be clinically useful.**

Key words: Glutamate dehydrogenase isoprotein; Gabapentin; Enzyme activation; Neurodegenerative disorder

1. Introduction

Glutamate is a major excitatory neurotransmitter [1] and is also known to be the immediate precursor in the biosynthesis of γ-aminobutyric acid (GABA), a widely distributed inhibitory neurotransmitter. Due to its neurotoxic potential, glutamate may be involved in the pathogenesis of human degenerative disorders [2]. One enzyme central to the metabolism of glutamate is glutamate dehydrogenase (GDH) (EC 1.4.1.3). GDHs are a family of enzymes which catalyze the reversible deamination of L-glutamate to 2-oxoglutarate using NAD^+, $NADP^+$ or both as coenzymes [3]. Since the pathology of the disorders associated with GDH defects is restricted to the brain, the enzyme may be of particular importance in the biology of the nervous system. The importance of the pathophysiological nature of the GDH-deficient neurological disorders has attracted considerable interest [4]. The enzyme isolated from a patient with a variant form of multisystem atrophy displayed a marked reduction of one of the GDH isoproteins [5,6]. The origin of the GDH polymorphism is not known. The presence of four differently sized mRNAs and multiple gene copies for GDH in the human brain has been reported [6]. A novel cDNA encoded by an X chromosome-linked intronless gene has also been isolated from human retina [7].

It is only in recent years that the three-dimensional structure of GDH from microorganisms has become available [8,9]. There is, however, relatively low identity between microbial and mammalian GDHs. Very recently, crystallization of bovine liver GDH was reported for the first time from mammalian sources [10]. However, remarkably little is known about the detailed structure of mammalian GDH, especially the brain enzymes. We have previously isolated two types of GDH isoproteins (designated GDH I and GDH II) from bovine brain [11] and the regulatory properties of the GDH isoproteins have been reported [12–14]. Our work also led to the finding that GDH is present in bovine brain in 'heat-labile (GDH I)' and 'heat-stable (GDH II)' forms [11]. Similar results were reported by other investigators showing that reduction of GDH activity in patients with neurodegenerative disorders was largely limited to the heat-labile form [15]. It has been reported that the activities of the GDH isotypes differ in their relative resistance to thermal inactivation, detergent extractability, and allosteric regulation characteristics [16,17]. To our knowledge, comparison of the detailed structure and functions of any GDH isoproteins has rarely been reported. It is, therefore, essential to have a detailed structural and functional description of the various types of brain GDH to elucidate the pathophysiological nature of the GDH-deficient neurological disorders.

Gabapentin (1-(aminomethyl)cyclohexaneacetic acid) is a novel anticonvulsant drug that is orally active in various animal models of epilepsy, especially those associated with interference in GABAergic transmission or provoked by excitatory amino acids [18]. Gabapentin is an analogue of GABA that is absorbed rapidly and it readily penetrates the blood-brain barrier [19]. Despite extensive research, the mechanism of action and the therapeutic range of serum concentration of gabapentin remain unclear [20]. Gabapentin has been demonstrated to activate glutamate decarboxylase [21] and enhances the rate of synthesis of GABA in several regions of the brain [22]. A recent study has demonstrated that gabapentin binds to the $\alpha_2\delta$ subunit of a calcium channel [23]. It also reported that gabapentin activates GDH in rat brain synaptosomes [24]. In vitro studies indicate an inhibition of branched amino acid aminotransferase and interaction with the L transport system [25]. Although numerous biochemical

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Abbreviations: GDH, glutamate dehydrogenase

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activities of gabapentin have been demonstrated, these have not led to a clearer understanding of the anticonvulsant mechanism of this drug. At present it is unclear what its most important site of action is and perhaps a novel receptor site will be identified. It is also uncertain whether the primary site of action is at the L system transporter itself, or whether there is some interaction at the intracellular level.

In the present work, we have comparatively examined the effects of gabapentin on two different types of bovine brain GDH isoproteins at various conditions in view of the central role of GDH in cerebral metabolism. The studies provide additional insight into the mechanism of action of gabapentin on GDH activity.

2. Materials and methods

NADH, NADPH, NAD^+, $NADP^+$, 2-oxoglutarate, glutamate, ADP, GTP, and L-leucine were purchased from Sigma Chemical Co. Gabapentin was from Parke-Davis, Division of Warner-Lambert Co. The GDH isoproteins were purified from bovine brain by the method developed in our laboratory [11] and were homogeneous as judged by Coomassie-stained gradient SDS-polyacrylamide gel electrophoresis. All other chemicals and solvents were reagent grade or better.

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 before [11]. All assays were performed in duplicate, 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 less than 0.04 absorbance units per min. The reaction started with the addition of 2-oxoglutarate to a 10 mM final concentration. 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 glutamate oxidation in 50 mM Tris-HCl, pH 9.5 containing 1.4 mM NAD^+, and 2.6 mM EDTA at 25°C. The reaction was started by the addition of glutamate to a final concentration of 25 mM.

Unless otherwise specified, highly purified GDH fractions were used for activation studies. Stimulation studies with gabapentin were performed at various concentrations in assay buffer at 25°C as described in the figure legends. Initial velocities were determined as described above and the data were fitted by a least-squares method. For the determination of the effects of substrate concentration on the activation by gabapentin, the assays were carried out by varying the substrate under investigation while keeping the other substrate and reagents at the optimal concentration indicated above. In some experiments, the effects of GTP on activation by gabapentin were tested by including it in the incubation as described in the figure legends.

Unless otherwise mentioned, each experimental point represents the mean of triplicate determinations from different preparations and the standard deviation is indicated as an error bar in each figure. At some points, error bars were omitted in the figures for clarity purposes.

3. Results and discussion

Gabapentin is a novel anticonvulsant drug, with a mechanism of action apparently dissimilar to that of other antiepileptic agents. In the present work, we have comparatively examined the effects of gabapentin on two different types of bovine brain GDH isoproteins (GDH I and GDH II) in both directions, oxidative deamination of glutamate and glutamate formation, at various conditions. The results showed that regulation of enzyme activity by gabapentin was distinct for the two GDH isoproteins. As shown in Fig. 1, the activities of GDH isoproteins were enhanced 1.8–2.7-fold by addition of gabapentin. When the effects of different gabapentin concentrations on GDH activities were studied in the direction of reductive amination of 2-oxoglutarate with NADPH as a coenzyme, a marked activation was observed for both isoproteins, whereas both isoproteins showed activation to a lesser extent with NADH as a coenzyme as shown in Fig. 1. There were differences between GDH I and GDH II in their sensitivity to the action of gabapentin and half-maximal stimulation was reached at 1.2–1.7 mM. The largest activation was observed with GDH II when NADPH was used as a coenzyme (Fig. 1).

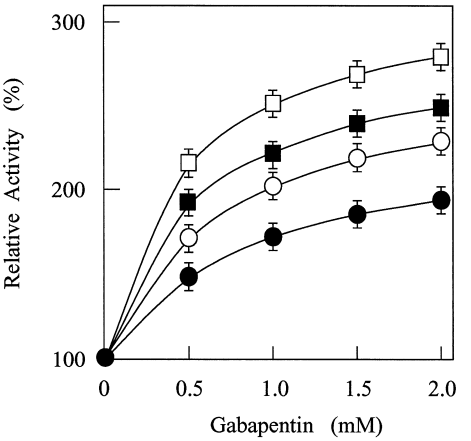

Fig. 1. Activation of GDH isoproteins by gabapentin in the direction of reductive amination. GDH isoproteins were assayed in the direction of reductive amination as described in Section 2 with 0.1 mM NADH or 0.1 mM NADPH. The relative activities are expressed as percentage of each control. ●, GDH I with NADH; ○, GDH I with NADPH; ■, GDH II with NADH; □, GDH II with NADPH.

Stimulatory effects of gabapentin on GDH activities in the direction of the oxidative deamination of glutamate were also observed, but to a much lesser extent than reductive amination as shown in Fig. 2. A slightly higher activation in a dose-dependent manner was observed for GDH I when $NADP^+$

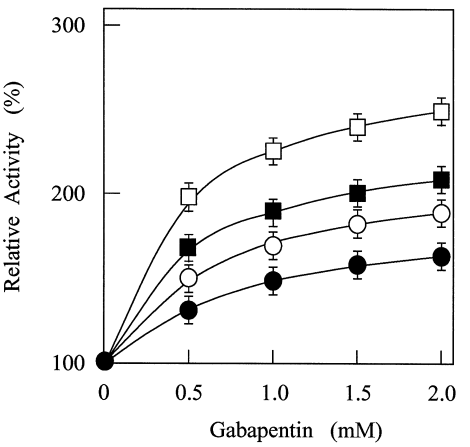

Fig. 2. Activation of GDH isoproteins by gabapentin in the direction of glutamate oxidation. GDH isoproteins were assayed in the direction of glutamate oxidation as described in Section 2 with 1.4 mM NAD^+ or 1.4 mM $NADP^+$. The relative activities are expressed as percentage of each control. ●, GDH I with NAD^+; ○, GDH I with $NADP^+$; ■, GDH II with NAD^+; □, GDH II with $NADP^+$.

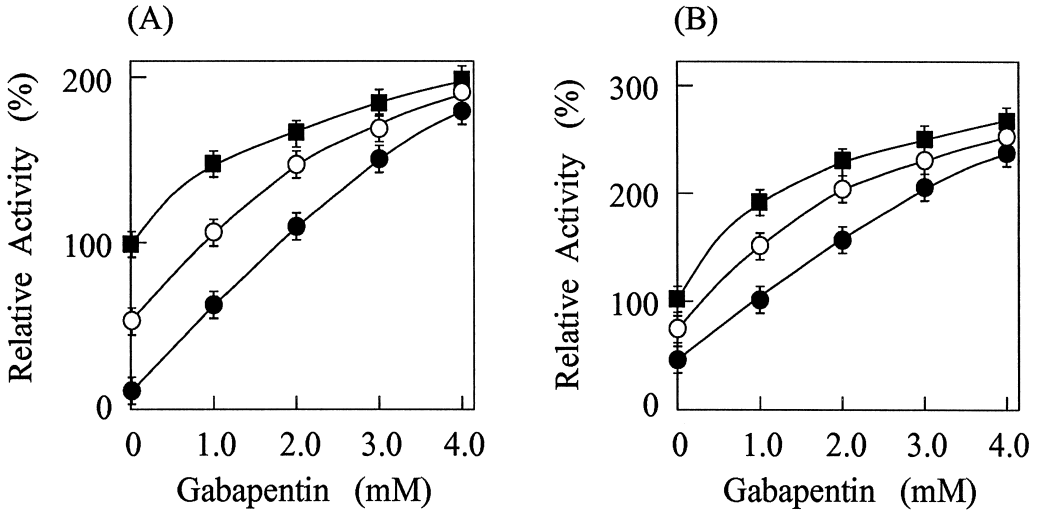

Fig. 3. Effect of GTP on the activation of GDH isoproteins by gabapentin. The enzymes were assayed in the direction of reductive amination as described in Section 2 with 0.1 mM NADH at various concentrations of GTP. The relative activities are expressed as percentage of each control. The GTP concentrations were 0.0 μM (■), 1.0 μM (○), and 2.0 μM (●). A: GDH I. B: GDH II.

was used as a coenzyme (Fig. 2). The effects, however, were reversed in GDH II, showing a higher activation when assayed with NAD^+ (Fig. 2). The stimulatory effects of gabapentin on GDH isoproteins are complex depending on substrate and coenzyme used (Figs. 1 and 2). We do not have a clear explanation for this complexity at this time. Similar results, however, have been obtained by others [26] who reported complex effects of ATP and ADP on GDH depending on pH, substrate and coenzyme used. Very recently, another study [16] showed that two human GDH isoproteins differ in their relative sensitivity to ADP.

The effective concentration of gabapentin used in our study to give half-maximal stimulation is about 1.5 mM. The therapeutic range of serum concentrations of gabapentin has not been established yet [20]. According to recent reports, daily doses of 1200–1400 mg (7–8 mmol) (standard dose) and 3300–3600 mg (19–21 mmol) (high dose) have been frequently used [20,27]. Since gabapentin has a volume of distribution of approximately 58 liters [20], the daily high dose of the drug corresponds to approximately 0.4 mM. Therefore, the effective concentration of gabapentin used in our study is slightly higher than the clinically used concentrations of the drug. Besides, our results are quite consistent with a half-maximal stimulation of GDH in rat brain synaptosomes (1.5 mM) previously reported by Goldlust et al. [24].

GTP is known to be an inhibitor of GDH [28,29] and our previous work also showed that the bovine brain GDH isoproteins were inhibited by GTP to different extents [12]. The concentrations of GTP necessary to give 50% inhibition were between 0.5 and 3.0 μM depending on the GDH isoproteins and the assay conditions [12]. GDH from *Neurospora crassa*, however, is not regulated by GTP. This enzyme contains 48 fewer residues than mammalian GDH, and there is little identity between the 100 residues in the C-terminus [30]. Studies have shown that chemical probes can at least partially desensitize bovine liver GDH to GTP inhibition while not affecting catalytic activity. The amino acids modified by these chemical probes were shown to be a residue in the C-terminus [31]. It seems likely that the regulatory GTP binding domain is located in the C-terminal half of GDH [12,29]. The effects of GTP on activation by gabapentin was studied for both GDH I and GDH II. As shown in Fig. 3A, gabapentin relieved the inhibition of GDH I by GTP and this resulted in an increase in the apparent activation by gabapentin in the presence of GTP. Similar effects, but to different extents, were observed with GDH II as shown in Fig. 3B. Therefore, qualitatively, GTP showed similar effects on the activation of both GDH I and GDH II by gabapentin, however, there were quantitative differences, as shown in Fig. 3. Since inhibition of GDH by GTP and the high substrate inhibition by NADH have been shown to potentiate each other [32], the observed effects of gabapentin on GTP inhibition might be consistent with its effects on NADH inhibition. The in vivo roles of gabapentin on the inhibition of GDH isoproteins by GTP, however, still remain to be resolved, since the magnesium complexes of GTP, which may be the predominant forms, have little inhibitory effect on GDH [28].

Fig. 4A shows the effects of substrate concentration on the activation of GDH I by gabapentin. The results of varying the concentration of 2-oxoglutarate at two different fixed concentrations of NADH (0.1 mM and 0.2 mM) in the presence and absence of 4 mM gabapentin are shown. The higher concentration of 0.2 mM NADH was in the range giving inhibition by this coenzyme. 2-Oxoglutarate was found to give rise to high substrate inhibition both in the absence and in the presence of 4 mM gabapentin. Gabapentin, however, reduced the substrate inhibition in the presence of 0.2 mM NADH. The effects of substrate concentration on the activation of GDH II by gabapentin were similar to those obtained with GDH I, as shown in Fig. 4B. The apparently linear portions of the double-reciprocal plots with varied glutamate concentrations showed similar slopes in the presence and absence of gabapentin as appeared to be true when 2-oxoglutarate was varied (data not shown). Thus, the effects of gabapentin may comprise a direct effect on the enzyme together with a relief of high substrate inhibition.

It has been well documented that ADP and leucine are activators of GDH and they have also been known to reduce high substrate inhibition by NADH [11,26]. Activation of GDH isoproteins by gabapentin is similar to the regulation

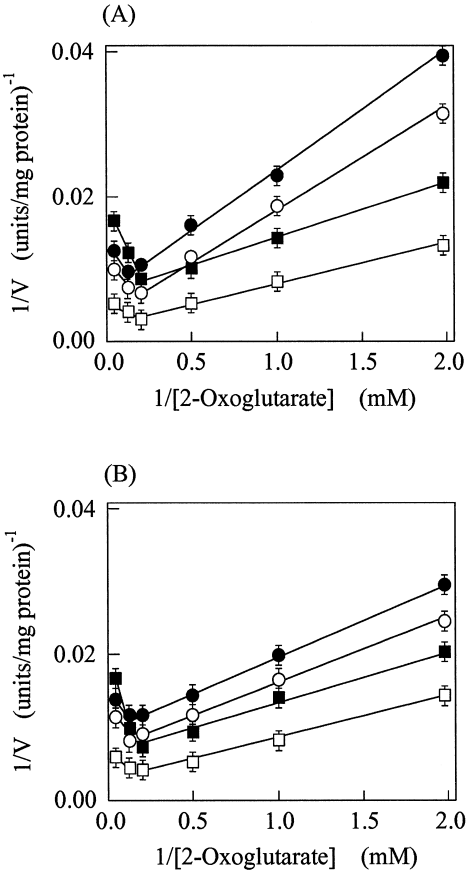

Fig. 4. Effect of substrate concentration on the activation of GDH isoproteins by gabapentin. The enzymes were assayed in the direction of reductive amination at various concentrations of 2-oxoglutarate with 0.1 mM NADH or 0.2 mM NADH. ●, 0.1 mM NADH without gabapentin; ○, 0.1 mM NADH with 4.0 mM gabapentin; ■, 0.2 mM NADH without gabapentin; □, 0.2 mM NADH with 4.0 mM gabapentin. A: GDH I. B: GDH II.

by leucine. It was reported that the GDH from rat synaptosomes is activated by leucine and by its non-metabolizable analogue 2-amino-bicyclo(2,2,1)heptane-2-carboxylic acid [33]. The half-maximal concentration of leucine for decreasing glutamate formation was approximately 1 mM. Although the concentration of leucine in brain ranges from 0.1 to 0.2 mM, it has been suggested that leucine may activate GDH in pathological conditions where leucine concentrations are elevated [34]. In the present work, the activities of the GDH isoproteins obtained at saturating concentrations (10 mM) of gabapentin were not increased further by subsequent addition of either 0.1 mM ADP or 1 mM leucine (data not shown). Activation of GDH provides an example where gabapentin biochemically mimics the action of leucine, although the potential physiological role of gabapentin remains to be resolved. Gabapentin was previously reported to compete with leucine at system L transporters [25] as well as at high affinity binding sites in the brain [35]. If leucine plays a significant role in a neurotransmitter function, the functional similarity between leucine and gabapentin may account for at least part of the pharmacological actions of gabapentin. As suggested by Goldlust et al. [24], studies of the similarities and differences between gabapentin and leucine in various CNS functions may provide more insight into the mechanism of action of gabapentin.

As many proteins have functions distinct from those for which they were originally identified, it will also be of interest to assess the other roles of GDH isoproteins. Recently, Preiss et al. [36] showed that GDH, previously reported to be able to bind RNA, is the cytochrome *c* oxidase transcript binding protein. It has also been reported that a membrane-bound form of GDH is involved in the association of lysosomes to microtubules [37]. Very recently, a novel nerve tissue-specific human GDH, with a thermostability and ADP regulation dissimilar to those of previously reported human GDH, was identified [16]. It remains to be studied whether the distinct properties of the brain GDH isoproteins are essential for the regulation of glutamate metabolism.

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