Effect of γ-Decanolactone on Glutamate Binding in the Rat Cerebral Cortex

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(Accepted June 12, 1997)

Epilepsy is one of the most common neurological disorders. Even though antiepileptic drugs can afford a reasonably satisfactory treatment for 80% of diagnosed patients, chronic intractable epilepsy still affects a significant number of people and more effective and less harmful antiepileptic drugs are needed. Previous studies have shown that γ-decanolactone has dose-dependent sedative effects, including hypnotic, anticonvulsant and hypothermic properties in mice. The present study reports an inhibitory effect of γ-decanolactone on glutamate binding (96.8% with 5 mM) in rat cortex membranes. The non competitive nature of glutamate binding inhibition as a neurochemical correlate of the anticonvulsant activity of γ-decanolactone may be a relevant mode of action for further drug development.

KEY WORDS: Glutamate; binding; anticonvulsant; γ-decanolactone; epilepsy.

INTRODUCTION

Epilepsy, manifested as various types of seizures, is characterized by abnormal neuronal discharges (1). The prevalence of this disease is approximately 1%, it has been estimated that 50 million people worldwide has to cope with this disorder (1,2). Although many are well controlled with available medication, it is estimated that up to 25% of afflicted individuals are unable to discontinue seizure episodes (3).

The etiology and pathophysiology of epilepsy have yet to be clarified. Anomalies in ionic conductances at neuronal membranes, in neuronal connectivity, and with inhibitory or excitatory synaptic function are possibilities under consideration (4).

Glutamate, the major excitatory neurotransmitter of central nervous system (CNS), is essential for integrative and adaptive brain functions and also participate in several acute and chronic brain insults, where an excessive release of glutamate may affect neural cell vitaly (5,6). Glutamatergic receptors may be classified into ionotropic and metabotropic receptors (7,8). Ionotropic receptors are ligand-gated integral ion channels and may be activated by ligands as N-methyl-D-aspartate (NMDA), kainate or alpha-amino-3-hydroxi-5-methyl-4-isoxazole-propionate (AMPA). Metabotropic receptors are linked to G proteins that are coupled to phospholipase C or adenyly cyclase and are activated by ligands as trans-1-amino-cyclopentyl-1,3-dicarboxylate (trans-ACPD), quisquilate, ibotenate and L-2-amino-4-phosphobutyrate (L-AP4) (8). Glutamate and aspartate appear to play important roles in the initiation, spread, and maintenance of the epileptic activity. Evidences in experimental models of epilepsy and in human beings indicate changes in glutamate release and in glutamate receptor function (5). In addition to epileptic activity, the excitotoxicity consequent to excessive glutamatergic ac-
activity appears to be related to a variety of pathologic conditions, including stroke, several neurodegenerative disorders, neuronal injury and death (6).

Despite progress in the understanding of the underlying features of epileptic disorders, the mechanisms of action of antiepileptic drugs remain to be fully elucidated (9). Antiepileptic drugs have been usually classified according to their ability to inactivate sodium channels, and/or block calcium currents and/or enhance GABA-mediated inhibition. Nevertheless, for most experimental seizure models some involvement of excitatory amino acid receptors can be demonstrated by the use of receptors antagonists (1), and it has been long suspected that glutamate neurotransmission is modified by many antiepileptic drugs (9). Antagonism of excitatory amino acids is taught to be involved in the anticonvulsant activity of phenytoin, carbamazepine and benzodiazepine (2). Glutamate antagonism is the basis for the anticonvulsant properties of MK 801 (10) and lamotrigine (11,12).

Our group previously reported that γ-decanolactone, a monoterpene compound, shows hypothermic, hypnotic and anticonvulsant effects in mice (13). The aim of the present study was to determine the interaction of γ-decanolactone with glutamate receptors in membranes of rat cerebral cortex.

EXPERIMENTAL PROCEDURE

Drugs and Reagents. Gama-Decanolactone was purchased from Aldrich Chemical Co. (catalog number D80-4) and solubilized in Tween (80 Polisorbate, 25%). L-[3H]Glutamate was purchased from Amersham. Glutamate was purchased from Merck. All other reagents were of analytical grade.

Animals. Male adult Wistar rats breed in our own animal house were used throughout the study.

Membrane Preparation. Membranes were prepared as described by Souza e Ramirez, 1991 (14). Rats cerebral cortex were homogenized (20:1 vol:weight) in 0.32 M sucrose containing 10 mM Tris/HCl buffer, pH 7.4, and 1 mM MgCl₂. The homogenate was centrifuged twice at 1,000 g for 15 min. and the final pellet discarded. Both supernatants were pooled and centrifuged at 27,000 g for 15 min. The supernatant was discarded and the resulting pellet was lysed (20:1 vol:weight) for 30 min. in 10 mM Tris/HCl buffer, pH 7.4. The lysed pellet was washed three times with lysing buffer (20:1 vol:weight) by centrifuging at 27,000 g for 15 min. Supernatants were discarded and final pellet was used for the experiments. All steps were carried out at 4°C.

Binding of [3H]Glutamate. For measurement of the binding of [3H]glutamate, membranes (100 µg of protein) were incubated at 30°C for 15 min. in 50 mM Tris/HCl buffer, pH 7.4, various glutamate concentrations, in a final volume of 0.5 ml. The reaction was interrupted by cooling the tubes and further centrifugation for 20 min. at 27,000 g. The supernatant was discarded. The walls of the tubes and the surface of the pellets were quickly and carefully rinsed with cold distilled water. The pellets were processed for radioactivity measurement. In order to determine specific binding of [3H]glutamate, each experiment was processed with parallel control tubes containing [3H]glutamate in the presence of 1000 times of non-labeled glutamate. Specific binding was defined as the difference of [3H]glutamate binding between tubes without (total binding) and with (non specific binding) unlabeled glutamate in excess concentration. The effects of γ-decanolactone on [3H]glutamate binding were evaluated by adding increasing concentrations of this compound to the incubation medium. All samples of γ-decanolactone were diluted in 25% of tween. Maximum tween amount used at incubation medium (125 µl tween in 500 µl of incubation medium) was checked for eventual per se effect on binding.

Protein Measurement. Protein was measurement according to method of Lowry et al, 1951 (15).

Statistic Analysis. Results were compared by means of one way ANOVA for inhibition curve and two way ANOVA for competition curve. Maximum number of L-Glutamate binding sites (Bₐₕₚ) and equilibrium dissociation constant (Kd) were calculated by Scatchard plot (16).

RESULTS

Under the incubation conditions used in this study (50 mM Tris/HCl buffer, pH 7.4, 30°C), binding of L-[3H]glutamate to cortex synaptic membranes from control rats is similar to several studies (17). There was no difference in L-[3H]glutamate binding (1000 nM) using either water (29.7 ± 2.0 pmol/mg of protein) or tween at its maximum concentration (24.5 ± 3.0 pmol/mg of protein) (ANOVA, NS), ruling out any per se effect of tween on [3H]glutamate binding. Fig. 1 shows that the binding of L-[3H]glutamate decreases with increasing γ-decanolactone concentrations. The inhibition is dose-dependent, and nearly complete inhibition attained with 5 mM (96.8% inhibition).