Anti-convulsant and adverse effects of the glycineB receptor ligands, d-cycloserine and L-701,324: Comparison with competitive and non-competitive N-methyl-D-aspartate receptor antagonists

Piotr Wlaz*

Department of Pharmacology, Faculty of Veterinary Medicine, Agricultural University, Lublin, Poland

ABSTRACT: In this study, the anticonvulsant and adverse effects of compounds that belong to four different categories of systemically available N-methyl-D-aspartate (NMDA) receptor ligands were compared, namely the competitive antagonist CGP 40116, the noncompetitive antagonist MK-801 (dizocilpine), the glycineB receptor antagonist L-701,324, and the glycineB receptor high-efficacy partial agonist d-cycloserine. The maximal electroshock seizures (MES), which are widely used to detect drug efficacy against generalized tonic-clonic seizures in humans, were produced by transcorneal electrical stimulation. Abolition of tonic hind-limb extension was taken as the end-point. The drug-induced motor and long-term memory deficits were quantified by using the inverted screen test and the step-through passive-avoidance test, respectively. All tested compounds exhibited significant anticonvulsant effect. The rank order of potency for the respective compounds was: MK-801 = CGP 40116 > L-701,324 > d-cycloserine. All of these compounds induced motor impairment at doses close to those found to be anticonvulsant, however, hyperlocomotion and stereotyped behavior occurred only with MK-801. The highest protective indices [PI = TD50 (inverted screen)/ED50 (MES)] were calculated for CGP 40116 and d-cycloserine (2.4 and 2.2, respectively). When tested for memory impairment at one-half the MES ED50, again only MK-801 induced significant memory disruption in the passive avoidance test. In conclusion, these results suggest that glycineB receptor high-efficacy partial agonists and competitive NMDA receptor antagonists may be advantageous to noncompetitive NMDA antagonists and glycineB receptor antagonists as potential antiepileptic drugs.

KEY WORDS: Maximal electroshock seizures, Anticonvulsant action, Neurotoxicity, Epilepsy, Passive avoidance, Mice.

INTRODUCTION

It is now more than 40 years since Hayashi first reported the excitatory effect of glutamic acid on the nervous system [13]. Since then, especially during the last decade, great progress has been made in our understanding of the pharmacology of excitatory amino acid receptors [14]. Glutamate, the principal excitatory amino acid, has been implicated in the pathogenesis of numerous neurological disorders including epilepsy [9]. The N-methyl-D-aspartate (NMDA) receptor is the best characterized receptor subtype via which glutamate exerts its excitatory action. Antagonists of the NMDA receptor, both competitive and noncompetitive, exhibit clear anticonvulsant activity [6,7,28,32,34,39,43]. However, poor anticonvulsant efficiency of these agents to suppress amygdala-kindled seizures in rats together with their propensity to induce serious unwanted side effects [23,24], limit their applicability as anticonvulsants for the treatment of complex partial seizures in humans, which has also been confirmed in clinical conditions [41]. As a consequence of these disappointing results, the researchers’ attention focused on other ways to inhibit the NMDA receptor. One of these alternatives is modulation of the strychnine-insensitive glycine coagonist site (termed glycineB receptor) at the NMDA receptor [17]. Indeed, high-efficacy partial agonists at the glycineB receptor have proven to possess anticonvulsant properties with fewer side effects than in the case of various NMDA antagonists, including low-efficacy agonists/antagonists [31,38,43,44,46]. Therefore, the aim of this study was to directly compare the anticonvulsant and adverse effect potential of compounds that belong to four different categories of the NMDA receptor ligands (i.e., competitive and noncompetitive NMDA antagonists, a glycineB receptor antagonist, and a high-efficacy partial agonist).

MATERIALS AND METHODS

Animals

Male Swiss mice weighing 25–30 g were used throughout this study after at least 1 week of acclimatization. The animals were housed in colony cages under standard laboratory conditions (ambient temperature of approximately 20°C, chow pellets and tap water continuously available). All experiments were performed at the same time of day (between 09.00 h and 12.00 h, with an exception of time course determinations) to minimize circadian
influences. Control and drug experiments were done on the same day to avoid day-to-day variations in convulsive susceptibility. The experimental groups were created randomly and each mouse was used only once.

The experiments were carried out according to ethical guidelines set by the local Animal Research Committee.

Maximal Electroshock-Induced Seizures

Maximal electroshock-induced seizures (MES) were produced by a fixed supramaximal stimulus (50 mA). To evaluate the anticonvulsant potency of the tested drugs, several groups consisting of eight to 10 animals each were injected with varying doses of a given drug, and then challenged with MES. The mice were made to convulse by using saline-soaked transcorneal copper electrodes. Stimulus duration was 0.2 s at a frequency of 50 Hz (sine-wave alternating current). A constant current stimulator (GE-01, COTM, Białystok, Poland) was used, so that changes in impedance of a test object did not result in alterations in the preset current intensity. The tonic hind-limb extension was taken as the end-point. Consequently, abolition of the end-point was considered as a protective action of a drug. The percentage of mice not showing the hind-limb extension (i.e., protected) was noted, and a dose-effect relationship was converted into ED$_{50}$ values with 95% confidence limits by a computer program written on a basis of the method of Litchfield and Wilcoxon [20].

Quantification of Motor Impairment

The drug-induced motor deficit was detected and quantified by using the horizontal (inverted) screen test as described by Yamaguchi and Rogawski [48]. Briefly, untreated drug-treated mice were put individually upon the grid (consisting of parallel steel rods 2 mm in diameter localized 1 cm apart). The screen was then slowly rotated by 180°. An animal that was unable to support its own body wt. for at least 5 s was considered impaired. To evaluate the drug-induced motor impairment, immediately before testing for anticonvulsant effect each animal was subjected to this testing procedure. Based on the percentage of impaired mice, TD$_{50}$ values and their confidence limits for 95% probability were calculated according to Litchfield and Wilcoxon [20].

Dark-Avoiding Acquisition and Retention Testing

The widely accepted experimental paradigm for testing long-term memory, the step-through passive avoidance test, was used. An apparatus that consisted of two compartments (each 10 × 15 × 15 cm), separated by a guillotine door, was used [47]. During the acquisition trial, mice were placed into an illuminated box and then the door was opened. Once the mouse entered the dark compartment (equipped with an electric grid floor), the door was closed and a mouse was punished by an 0.6 mA unscrambled footshock for 2 s. Normally, the naive mouse entered the dark compartment rapidly, usually within 10–30 s. Twenty-four h later (retention testing), each mouse was placed again in the illuminated box. The mouse that avoided the dark box for more than 60 s was considered remembering the task. The percentages of mice successfully passing the task in untreated and treated groups were compared by the Fisher’s exact probability test.

Statistics

Calculation and statistical comparisons of the respective ED$_{50}$ and TD$_{50}$ values (with 95% confidence limits) were done with the help of the computerized method of Litchfield and Wilcoxon [20]. The data from the passive-avoidance test were compared by the Fisher’s exact probability test by InStat program (GraphPad Software, San Diego, CA, USA). In all tests the 0.05 level was used as the criterion for statistical significance.

Drugs

The following drugs were used: D-cycloserine (D-4-amino-3-isoxazolidone; purchased from Sigma, St. Louis, MO, USA), L-701,324 [7-chloro-4-hydroxy-3(3-phenoxy)phenyl-2[H]quinolone; kindly provided by Merck Sharp & Dohme, Harlow, England], MK-801 [(+)-5-methyl-10,11-dihydro-11-dibenzo(c,e)-hepten-5,10-imine hydrogen maleate; kindly provided by Merck and Co., Rahway, NJ, USA], and CGP 40116 [d-(-)-2-amino-4-methyl-5-phosphono-3-pentenoic acid, the active d-stereoisomer of the competitive NMDA antagonist CGP 37849; kindly provided by Novartis Pharma, Basel, Switzerland]. D-cycloserine, MK-801, and CGP 40116 were dissolved in distilled water. L-701,324 was brought into solution first with warmed polyethylene glycol 400 (PEG 400, Sigma), and then diluted with distilled water under continuous stirring (the final concentration of PEG 400 was 10%–20%, depending on the content of L-701,324), and slightly alkalized with NaOH. All drug solutions were prepared freshly and administered intraperitoneally (i.p.) at a volume of 0.1 ml per 10 g b.w. It is noteworthy that PEG 400, in concentrations used, neither alters convulsive threshold nor impairs motor coordination [27]. Pretreatment times for D-cycloserine, L-701,324, MK-801, and CGP 40116 were chosen on a basis of the preliminary experiments (not illustrated), and were 60, 20, 60, and 90 min, respectively.

RESULTS

Anticonvulsant and Neurotoxic Effects

The dose-dependent anticonvulsant and motor impairing effects of D-cycloserine, L-701,324, MK-801, and CGP 40116 are depicted in Fig. 1. The corresponding ED$_{50}$ and TD$_{50}$ values are shown in Table 1. All tested drugs exhibited significant anticonvulsant effect against tonic hind-limb extension. The rank order of potency for the respective compounds was the following: MK-801 > CGP 40116 > L-701,324 > D-cycloserine. All of these chemicals induced motor impairment at doses found to be anti-
convulsant, though to a various extent. The highest therapeutic indices (TI = TD50/ED50) were calculated for CGP 40116 and D-cycloserine (2.4 and 2.2, respectively), while the worst separation between anticonvulsant and adverse effects was found in the case of MK-801 and L-701,324 (TI of 0.7 and 0.9, respectively).

Indeed, CGP 40116 at two highest doses used, that both afforded 100% protection against MES-induced tonic convulsions, produced motor impairment in 50% and 75% of the animals. Similarly, D-cycloserine at a dose that offered full seizure protection introduced measurable neurotoxicity in 50% of the mice. Interestingly, the respective ED50 values for CGP 40116 and D-cycloserine were significantly different (p ≤ 0.05) from TD50 values determined for these compounds. In contrast, MK-801 at a dose that protected 50% of the mice impaired motor function in 75% of the tested animals.

MK-801 dose-dependently enhanced locomotion and produced typical signs of stereotyped behavior (head weaving, stereotyped sniffing, licking, and biting) and hypersensitivity to noise. Similar effects were observed with highest doses of CGP 40116 while lower doses inhibited locomotion. L-701,324 reduced locomotion over the whole range of doses. D-Cycloserine essentially showed no grossly observable behavioral changes except of highest doses of the drug that reduced locomotor activity.

**Time Course for Anticonvulsant and Neurotoxic Effects**

Time course experiments done on animals pretreated i.p. with twice the ED50 of the drugs indicated that peak anticonvulsant activity occurred at different times for the tested compounds as displayed in Fig. 2. The most rapid onset of anticonvulsant action was found for L-701,324; 37.5% and 100% of the animals were protected after 5 and 15 min postinjection, respectively. The most slowly acting drug was CGP 40116, which reached maximal anticonvulsant effect after 60–90 min postinjection. With respect to duration of the anticonvulsant effect, L-701,324 was the compound that anticonvulsant action was also the shortest: 3 h postinjection no seizure protection was observed. MK-801, by contrast, retained its anticonvulsant activity for the longest period of time. The MES challenge 4 h postinjection showed protection in three out of eight animals. The duration of neurotoxic effects was the shortest for D-cycloserine; 2 h after treatment no motor dysfunction was noted. However, at this time still a considerable seizure protection was detected. Interestingly, the peak neurotoxic effect of D-cycloserine was achieved long before the maximum anticonvulsant effect (30–45 min vs. 60 min). Other tested drugs did not show this time dissociation of anticonvulsant and neurotoxic effect; the respective dose–response curves for MES protection and motor impairment ran parallelly.

**Passive Avoidance Behavior**

As shown in Fig. 3, when administered at one-half the ED50 only MK-801 induced significant disruption of memory in the passive avoidance test. Specifically, eight out of 10 animals treated with one-half the ED50 of MK-801 failed to fulfill the criterion. When the drugs were given at doses corresponding to their ED50 values, they produced significant memory deficit in all animals. However, in the case of CGP 40116 still 25% of the animals were able to pass the test.

**DISCUSSION**

Experiments described herein showed that competitive, non-competitive, and two glycine receptor ligands, namely CGP...
coagonist, has created considerable interest that glycine as anticonvulsant agents. The discovery that glycine acts in the most limiting factor in the use of NMDA receptor antagonists and deleterious influences on cognitive performance. This is typed behaviors (often related to psychotic side effects in humans), actions comprise of motor impairment, hyperlocomotion, stereotypy at doses close to those being anticonvulsive. These NMDA receptor-coupled ion channel induce many undesirable effects directly antagonize the NMDA receptor by interacting with the neurotransmitter recognition site or, to a greater extent, with the NMDA receptor-coupled ion channel induce many undesirable effects at doses close to those being anticonvulsive. These actions comprise of motor impairment, hyperlocomotion, stereotyped behaviors (often related to psychotic side effects in humans), and deleterious influences on cognitive performance. This is the most limiting factor in the use of NMDA receptor antagonists as anticonvulsants. The discovery that glycine acts in concert with glutamate at the NMDA receptor, and therefore, is a coagonist, has created considerable interest that glycine receptor ligands may appear to be more interesting as antiepileptic drugs (AEDs). As adverse effects concern, the present direct comparison showed that glycine receptor ligands were not clearly superior to “classical” NMDA antagonists; all NMDA ligands, including D-cycloserine, produced motor impairment and cognitive deficits. The rotarod test indicated that only CGP 40116 and D-cycloserine approached the minimal acceptable level of the therapeutic index. “Typical” NMDA receptor antagonists, both competitive and noncompetitive, produce in laboratory animals a wide spectrum of side effects as pointed out earlier. However, it should be emphasized that even the highest tested doses of L-701,324 and D-cycloserine did not produce such a behavioral syndrome. Our previous studies showed that D-cycloserine, while being effective in increasing electroconvulsive threshold in mice or afterdischarge threshold in amygdala-kindled rats, did not induce pronounced side effects. In contrast, our recent results with the glycine receptor antagonists, L-701,324 and MRZ 2576 suggest that these substances are devoid of selective anticonvulsant effect in this model of temporal lobe epilepsy. It should, however, be noted that stereotyped behavior and hyperlocomotion were not seen with these drugs, which is also confirmed by the present study. The lack of psychotomimetic properties in mice after administration of L-701,324 at doses sevenfold to 15-fold higher than those protecting against MES is most probably connected with the lack of activation of brain dopamine systems, and activation of mesolimbic dopamine systems, as seen with noncompetitive NMDA receptor antagonists, may contribute to the psychotomimetic side effects observed in humans.

To date, it seems that antagonists at the NMDA receptor are not promising candidate drugs to treat epilepsy and despite many years of laboratory testing they have not yet passed this stage of development. Although, as also confirmed by the present observations, they are effective in suppressing seizures in certain animal models of epilepsy or epileptic seizures. As mentioned earlier, they induce motor deficits. This feature is common for all classes of drugs inhibiting the NMDA receptor irrespective if they are competitive, noncompetitive, or glycine receptor antagonists. The whole story has additionally been complicated by the series of studies of Lösch and colleagues who provided evidence that epileptogenesis, as modeled by kindling, increases adverse effect potential of NMDA antagonists and even of some conventional AEDs. These findings were confirmed by the disappointing outcome of a clinical trial with the competitive NMDA receptor antagonist D-CPPene, which was associated with increased incidence and intensity of adverse effects.

The present study demonstrated that D-cycloserine was not less effective against MES than the remaining compounds, which is in line with several previous studies that have shown anticonvulsant effects of D-cycloserine. D-Cycloserine is known to have cognition-improving properties. However, as demonstrated by this study, such an effect disappeared with the increase of dose that is compatible with other findings. Low doses of D-cycloserine (10–20 mg/kg in rodents) enhance cognition in animals while higher doses of the drug inhibit memory processing, which is consistent with the present study. This biphasic effect has been suggested as typical for cognitive enhancers and may have a reflection in the partial agonist nature of D-cycloserine. Interestingly, D-cycloserine, even at low and very low doses that actually selectively potentiate the antielectroshock effects of phentoyin, did not exhibit any proconvulsant effects. Furthermore, as demonstrated previously, low doses of D-cycloserine could even revert the cognitive impairment caused by a conventional AED. This finding should deserve special attention because epilepsy itself, as well as the toxic side effects of AEDs, may trigger a certain degree of cognitive impairment.
In conclusion, despite dose-dependent anticonvulsant action, neither of the examined substances is devoid of motor-impairing and cognition-impairing side effects at anticonvulsant doses against MES. However, the highest protective indices calculated for CGP 40116 and t-cycloserine indicate further need to elucidate whether or not glycine(NMDA) receptor high-efficacy partial agonists and competitive NMDA receptor antagonists are advantageous to non-competitive NMDA receptor antagonists and glycine(NMDA) receptor full antagonists as potential AEDs.

ACKNOWLEDGEMENTS

I wish to thank Prof. Wolfgang Lötscher (Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Hannover, Germany) for his invaluable comments on a previous version of this article. I also thank Merck, Sharp & Dohme (Harlow, England), Merck and Co. (Rahway, NJ, USA) and Novartis Pharma (Basel, Switzerland) for generous gifts of L-701,324, MK-801, and CGP 40116, respectively. This study was supported by a grant from the Agricultural University (Lublin, Poland).

REFERENCES

35. Rao, T. S.; Kim, H. S.; Lehmann, J.; Martin, L. L.; Wood, P. L. Selective activation of dopaminergic pathways in the mesocortex by compounds that act at the phencyclidine (PCP) binding site: Tentative


