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Short communication

Anticonvulsant effects by combined treatment with a glycine$_B$ receptor antagonist and a polyamine site antagonist in amygdala-kindled rats

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Abstract

Antagonists of binding sites within the NMDA receptor complex, i.e., L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(*H*)quinolone), a brain penetrating glycine$_B$ receptor antagonist, and ifenprodil, a polyamine site antagonist, were tested for anticonvulsant properties in fully amygdala-kindled rats, a model of limbic epilepsy. Both drugs were not able to significantly change seizure parameters (focal afterdischarge threshold, seizure severity, and duration of seizure and afterdischarges), when administered intraperitoneally up to doses which produced severe motor impairment. However, the combination of 10 mg/kg ifenprodil and 5 mg/kg L-701,324 had a pronounced anticonvulsant effect on afterdischarge threshold and seizure severity without concomitant increase of adverse effects. These findings support the hypothesis that drugs acting only at one site of the NMDA receptor complex are ineffective, while combinations of such drugs may synergistically act to suppress limbic seizures, thus providing an adequate strategy for the treatment of this type of refractory epilepsy. © 1997 Elsevier Science B.V.

Keywords: Complex partial seizure; Electrical kindling; Epilepsy; NMDA receptor

1. Introduction

Antagonists of the NMDA receptor-mediated excitatory transmission have been intensively studied for possible neuroprotective and anticonvulsant properties (Rogawski, 1992; Löscher and Schmidt, 1994). In respect to their anticonvulsant potency preclinical studies involving a variety of seizure models were promising. However, a recent clinical study revealed that patients with refractory complex partial seizures are more sensitive to the psychopathological adverse effects known to be associated with NMDA receptor antagonists at high doses than control subjects (Sveinbjornsdottir et al., 1993). This disappointing finding was correctly predicted by Löscher and Hönack (1991) from their experiments in amygdala-kindled rats, substantiating that amygdala kindling is a valuable experimental model for the evaluation of anticonvulsant and adverse effects of new compounds in limbic epilepsy (Löscher and Schmidt, 1988).

For the future, it seems to be a useful strategy (1) to concentrate on drugs which only modulate NMDA receptor function having less behavioural side effects than receptor blockers and (2) to use kindled rats in the search of new effective compounds for treatment of refractory seizures. In this respect, two binding sites of the NMDA receptor, i.e., the strychnine-insensitive glycine$_B$ receptor (Johnson and Ascher, 1987; Kemp and Leeson, 1993) and the polyamine binding site (Ransom and Stec, 1988; Johnson, 1996), are possible targets. While application of the glycine$_B$ receptor antagonist 7-chlorokynurenic acid and of the partial agonists D-cycloserine and (+)-HA-966 (*R*-(+)-3-amino-1-hydroxypyrrolid-2-one) revealed equivocal results in amygdala-kindled rats (Morimoto and Sato, 1992; Löscher et al., 1994; Rundfeldt et al., 1994), there are no studies on the effects of polyamine site antagonists in this model available. Here we report the effects on evoked seizures of the new selective glycine$_B$ receptor antagonist L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(*H*)quinolone), which is able to cross the blood–brain barrier (Kulagowski et al., 1994), and of the polyamine site antagonist ifenprodil (Schoemaker et al., 1990; Ransom, 1992), as well as a combination of both drugs, in amygdala-kindled rats.

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2. Materials and methods

2.1. Animals

Female Wistar rats were purchased at a body weight of 200–220 g (Harlan-Winkelmann Versuchstierzucht, Borchen, Germany) and were kept in our animal colony under controlled environmental conditions (24–25°C, 12 h light/dark cycle) with free access to standard laboratory chow (Altromin 1324 standard diet) and tap water. All experiments were done within the same day time in the morning to minimize possible effects of circadian variation.

2.2. Electrode implantation

For implantation of kindling electrodes, the rats were anaesthetized with chloral hydrate (360 mg/kg, i.p.), the skull surface was exposed, and a bipolar electrode was implanted into the right hemisphere aimed at the basolateral amygdala using the following stereotaxic coordinates according to the atlas of Paxinos and Watson (1986): caudal 2.2 mm, lateral 4.8 mm, ventral 8.5 mm (all respective to bregma). The electrode consisted of two twisted Teflon-coated stainless steel wires (250 μm diameter) separated by 0.5 mm at the tip. A screw, which served as grounding electrode, was positioned over the left parietal cortex. Bipolar and ground electrodes were connected to plugs, and the electrode assembly and anchor screws were held in place with dental acrylic cement applied to the exposed skull surface. After surgery, the rats were treated with antibiotics for 1 week to prevent infection.

2.3. Kindling procedure

Electrode stimulation of the amygdala was initiated following a recovery period of 2 weeks after surgery. At the first day of the stimulation period, the threshold for induction of amygdaloid afterdischarges (initial afterdischarge threshold) was determined as described below for the fully kindled state. From the next day on, constant current stimulations (500 μA, 1 ms monophasic pulses, 50 Hz for 1 s) were delivered to the amygdala once daily until at least ten stage-5 seizures were elicited. The severity of seizures was classified according to Racine (1972): 1, immobility, eye closure, twitching of vibrissae, mild facial clonus; 2, head nodding, accompanied by more severe facial clonus; 3, unilateral forelimb clonus; 4, rearing and bilateral forelimb clonus; 5, rearing with loss of balance, falling, and generalized clonic seizure. In these fully kindled rats, the afterdischarge threshold, which is the most sensitive measure of kindled seizure activity, was determined by administering a series of stimulations at intervals of 1 min beginning at 10 μA and increasing in steps of about 20% of the previous current (Freeman and Jarvis, 1981). The afterdischarge threshold was defined as the lowest current intensity producing afterdischarge with a duration of at least 5 s. Determination of afterdischarge threshold was repeated two times at intervals of at least 4 days to prove reproducible thresholds. In addition to seizure severity, seizure duration and afterdischarge duration were measured. Seizure duration was the time period of limbic and/or motor seizures. Limbic seizure activity (stage 1–2) which sometimes occurred after termination of secondarily generalized seizures (stage 3–5) was not included in seizure duration. Afterdischarge duration was the total time of spikes (at least 1 Hz frequency and twice the prestimulation amplitude) in the electroencephalogram (EEG) of the BLA electrode, including the time of stimulation.

2.4. Effects of NMDA antagonists in fully kindled rats

Groups of 6–9 fully kindled rats were used for these experiments. Ifenprodil was tested in doses of 0.4–40 mg/kg i.p., i.e., a dose range previously shown to have anticonvulsant effects in other rodent seizure models (De Sarro and De Sarro, 1992; McAllister, 1992; Zarnowski et al., 1994). L-701,324 was tested in doses of 2–10 mg/kg i.p., i.e., a dose range known to induce anticonvulsant effects in rats (Bristow et al., 1996). Ifenprodil and L-701,324 were administered to kindled rats and afterdischarge threshold was determined 30 min (L-701,324) or 60 min (ifenprodil) after drug injection. For combined treatment, ifenprodil was injected 30 min before L-701,324 and afterdischarge threshold was determined 60 min after ifenprodil.

Shortly before the stimulation, adverse effects of the drugs were judged by a scoring system for ataxia as previously described (Löscher and Hönack, 1990). Briefly, animals were placed in an open field and signs of ataxia in their locomotion activity scored as follows: 1, slight ataxia in hind legs (tottering); 2, more pronounced ataxia with dragging of hind legs; 3, more pronounced dragging of hind legs; 4, marked ataxia with eventual loss of balance during forward locomotion; 5, severe ataxia with frequent loss of balance during forward locomotion; 6, inability to move forward. Additionally, ataxia was judged by the animal's ability to maintain itself on a rotating rod (8 revolutions/min) for 1 min without falling (rotarod test; Löscher and Hönack, 1991).

2.5. Drugs

Ifenprodil was obtained from Research Biochemicals International (via Biotrend, Cologne, Germany). The drug was freshly suspended in isotonic saline containing 1% Tween-80 and injected intraperitoneally in a volume of 3 ml/kg. L-701,324 was kindly provided by Merck, Sharp & Dohme, Harlow, UK. It was freshly dissolved in polyethylene glycol (PEG 400), diluted with water and alkalinized with NaOH (final concentration of PEG 400 about 20%; final pH 9–10) and injected intraperitoneally in a

volume of 2 ml/kg. For control recordings, rats received vehicle injections with the same volume and time interval to afterdischarge threshold determination 2–3 days prior to every individual drug experiment.

2.6. Statistics

Non-parametric statistical analysis (Wilcoxon signed rank test for paired replicates) was performed for afterdischarge threshold and score (seizure severity, ataxia) comparison; paired *t*-test was used for comparison of seizure and afterdischarge durations. An error probability of less than 5% was considered significant.

3. Results

The antagonist of the polyamine site in the NMDA receptor-ion channel, ifenprodil, did not increase the afterdischarge threshold of kindled rats at doses of 0.4, 4, 10 and 40 mg/kg. In contrast, there was a tendency for the afterdischarge threshold to be even lower than in control experiments (Fig. 1A). Seizure severity, and duration of seizure and afterdischarges at afterdischarge threshold current also were not significantly changed, except for the highest dose of ifenprodil where ifenprodil seemed to decrease seizure and afterdischarge duration (Fig. 1B–D). However, at this dose severe ataxia was observed and most of the rats were not able to pass the rotarod test (Fig. 2). Adverse effects of ifenprodil were restricted to motor impairment and did not resemble the phencyclidine-like behavioural effects of competitive and non-competitive NMDA receptor antagonists in kindled rats (Löscher and Hönack, 1991).

The full antagonist of the glycine$_B$ site at the NMDA receptor complex, L-701,324, was also not able to show any anticonvulsant effect in amygdala-kindled rats. Afterdischarge threshold, seizure severity, and duration of seizures and afterdischarges at afterdischarge threshold current were not altered at doses of 2, 5, or 10 mg/kg (Fig. 1). At 5 and 10 mg/kg significant ataxic behaviour was observed which again did not resemble the side effects of classical NMDA receptor antagonists like dizolcipine (Fig. 2).

When testing combinations of both drugs, an anticonvulsant effect on kindled seizures was found. The combination of ifenprodil at 10 mg/kg and of L-701,324 at 5 mg/kg resulted in an almost 4-fold increase of afterdischarge threshold, while generalized seizure activity was suppressed (seizure score < 3; Fig. 1A,B). Under this combined treatment seizure duration and afterdischarge duration at afterdischarge threshold current was cut to about half of the control values, although the higher variability of these parameters prevented statistical significance (Fig. 1C,D). More importantly, the combination of 10 mg/kg ifenprodil and 5 mg/kg L-701,324 did not result in more pronounced adverse effects compared to the individual treatments (Fig. 2). When reducing the dose of ifenprodil

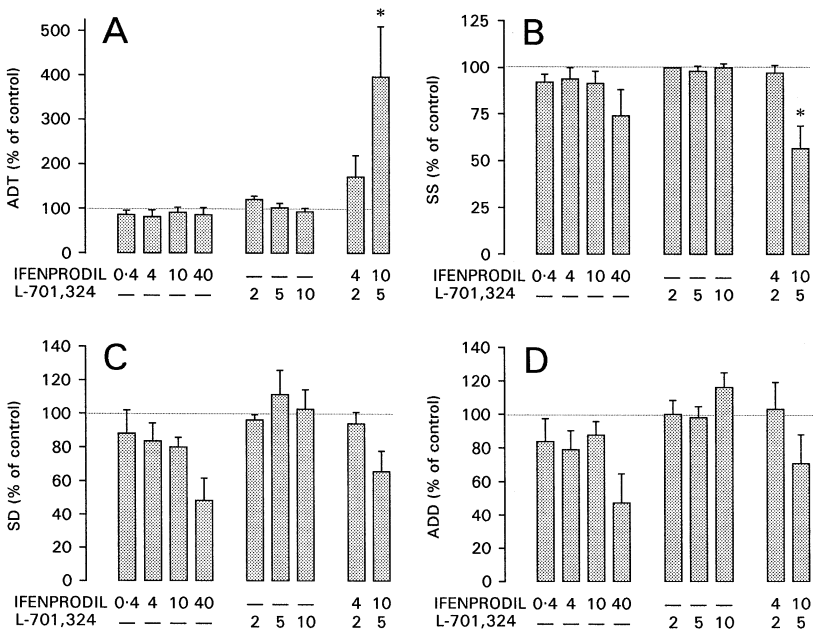

Fig. 1. Effect of ifenprodil, L-701,324, or a combined treatment, on focal seizure threshold and seizure parameters at threshold determined by electrical stimulation of the amygdala in fully kindled rats. The doses of the drugs are shown below each column in mg/kg, i.p. Data are means ± S.E.M. of 6–9 fully kindled rats per experiment (except data for ifenprodil 40 mg/kg, which are from three animals). Control values were determined 2–3 days before each drug experiment in the same group of rats. Drug data are shown as mean (+S.E.M.) percentage of the individual pre-drug values. (A) Afterdischarge threshold (ADT); data are based on mean control values ranging from 26.0–31.9 μA. (B) Seizure severity (SS); data are based on mean control values ranging from score 4.7–5.0. (C) Seizure duration (SD); data are based on mean control values ranging from 51.0–71.7 s. (D) Afterdischarge duration (ADD); data are based on mean control values ranging from 62.3–76.7 s. Statistically significant differences of drug treatment vs. control: $^{*}P < 0.05$.

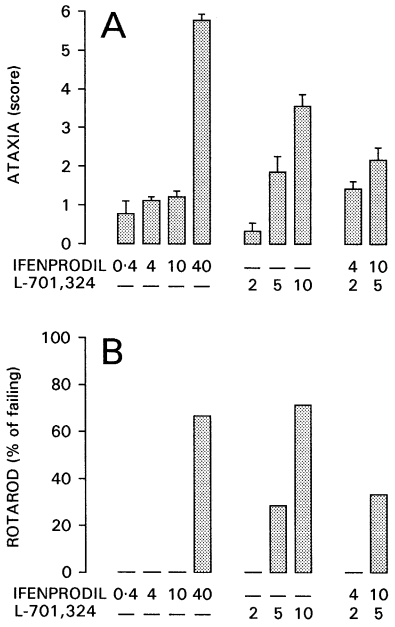

Fig. 2. Ataxia produced by ifenprodil, L-701,324, or combined treatment in amygdala-kindled rats. The doses of the drugs are shown below each column in mg/kg, i.p. Data are means + S.E.M. of 6–9 fully kindled rats per experiment (except data for ifenprodil 40 mg/kg, which are from three animals) and were determined immediately before afterdischarge threshold measurements. Severity of motor impairment was scored by behavioural ratings (A) and also determined in the rotarod test (B). For the rotarod test, the percentage of animals which could not walk on a rotating rod for 1 min without falling is shown. During control experiments no indication of motor impairment was found. The adverse effects of the combined treatments are less than the sum of the respective individual treatments.

to 4 mg/kg and the dose of L-701,324 to 2 mg/kg afterdischarge threshold was elevated only in some rats while the reduction of seizure severity was lost (Fig. 1A,B).

As we have recently shown that application of a low-efficacy partial glycine$_B$ receptor agonist, (+)-HA-966 (*R*-(+)-3-amino-1-hydroxypyrrolid-2-one), induces paroxysmal activity in kindled rats (Wlaź et al., 1994), the EEG of the rats after treatment with ifenprodil and/or L-701,324 was checked carefully. No signs of altered activity was found even at the highest doses used. Moreover, the prolonged postictal immobilization period associated with (+)-HA-966 (Wlaź et al., 1994) was not observed in the present experiments.

4. Discussion

After the disappointing results of the competitive NMDA receptor antagonist D-CPP-ene in a clinical evaluation of its antiepileptic potential (Sveinbjornsdottir et al., 1993), much hope is put into co-agonist and modulatory sites of the NMDA receptor complex as a more effective pharmacological target for treatment of epilepsy. One possible strategy aims to block the strychnine-insensitive glycine site, the glycine$_B$ receptor (Carter, 1992). Recent studies in which drugs acting at this site were tested in amygdala-kindled rats have shown that not the full antagonist 7-chlorokynurenic acid (Morimoto and Sato, 1992) or the low-efficacy partial agonist (+)-HA-966 (Löscher et al., 1994) but the high-efficacy partial agonist D-cycloserine or the full agonist D-serine have anticonvulsant potency (Löscher et al., 1994). These surprising results seemed to substantiate the theory that glycine$_B$ receptors are not saturated at physiological concentrations of glycine and exogenously added agonists can influence the glycine$_B$ receptor activity in vivo (Carter, 1992; Kemp and Leeson, 1993). The present finding that the new selective glycine$_B$ receptor antagonist L-701,324 had no anticonvulsant efficacy in amygdala-kindled rats adds more evidence for the indication that antagonists devoid of agonist activity are ineffective in this model. However, it has to be noted that under certain circumstances, for example after intracerebroventricular administration, glycine$_B$ receptor antagonists and partial agonists may have anticonvulsant activity in kindled rats (Rundfeldt et al., 1994). Therefore, the anticonvulsant efficacy of L-701,324 in kindled rats will be further investigated in much more detail in a subsequent study.

The major aim of this study was to provide the first data of possible effects of a polyamine antagonist on seizure susceptibility in the kindling model. Ifenprodil had no anticonvulsant effects, even at doses causing severe motor deficits, suggesting that polyamine antagonists have no higher anticonvulsant potency than other drugs acting at the NMDA receptor complex in this model (cf., Löscher and Schmidt, 1994). This is in contrast to a number of recent studies reporting anticonvulsant effects by ifenprodil in various seizure models in mice and rats (De Sarro et al., 1992; McAllister, 1992; Doyle and Shaw, 1996; Zarnowski et al., 1994). One reason for the difference in ifenprodil's anticonvulsant efficacy between amygdala kindling and other seizure models may lie in the ambivalence of the effects of polyamines on the NMDA receptors, being both stimulatory and inhibitory due to binding at a high-affinity stimulatory and a low-affinity inhibitory binding site (Marvizón and Baudry, 1994; Johnson, 1996). It is unknown whether polyamine binding at these sites is altered in the chronic epileptic brain, i.e., in fully amygdala-kindled rats. As ifenprodil is a ligand at both the stimulatory and the inhibitory site (Marvizón and Baudry, 1994) and shows selectivity for a NMDA receptor containing NR1/NR2B subunits in vitro (Williams, 1993), the response to administration of ifenprodil and other polyamine antagonists in vivo may be complex, depending on the physiological state and the regional expression of NMDA receptor subtypes. Such a complex modulatory site may serve as a promising target for antiepileptic drug development, provided that more detailed knowledge on the regulation of polyamine binding sites in the normal and

epileptic brain and subtype-selective ligands become available. It has to be noted that ifenprodil also has shown affinity as an antagonist to the 5-HT_3 receptor and to high-voltage activated calcium channels in cultured neurons (Church et al., 1994; McCool and Lovinger, 1995).

Previous studies have shown that combinations of excitatory amino-acid antagonists can synergistically increase the anticonvulsant activity without a similar increase in adverse effects in amygdala-kindled rats (Löscher et al., 1993). Interestingly, the polyamine antagonist eliprodil potentiated the anticonvulsant effects of the competitive NMDA receptor antagonist CGP 37849 in the acute electroshock-induced seizure model (Deren-Wesolek and Maj, 1993). We therefore tried combined treatments of ifenprodil and L-701,324, and co-administration of 10 mg/kg ifenprodil and 4 mg/kg L-701,324 resulted in a pronounced anticonvulsant effect. Although this is in accordance with two other recent studies in which ifenprodil and the $glycine_B$ receptor antagonist 7-chlorokynurenate synergistically blocked NMDA-stimulated polyamine release in the striatum (Voltz et al., 1994) and suppressed prolonged seizure activity after i.c.v. spermine injection (Doyle and Shaw, 1996), it is surprising because both drugs, antagonizing the NMDA receptor complex at two functionally different sites, were ineffective in suppressing kindled seizures when given alone. Unfortunately, the combination dose of ifenprodil and L-701,324 which produced anticonvulsant effects also induced moderate ataxia. However, these adverse effects did not exceed those of the individual drug treatments when no anticonvulsant effect was observed. Therefore, the present study substantiates the previous hypothesis (Löscher et al., 1993) that a combination of compounds intervening at different sites within excitatory amino-acid transmission is superior to a treatment aiming only at one target in order to suppress limbic seizures.

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