ELSEVIER

European Journal of Pharmacology 281 (1995) 327–333

ejp

Influence of combined treatment with NMDA and non-NMDA receptor antagonists on electroconvulsions in mice

Stanisław J. Czuczwar *, Kinga K. Borowicz, Zdzisław Kleinrok, Piotr Tutka, Tomasz Żarnowski, Waldemar A. Turski

Department of Pharmacology and Toxicology, Medical University School, Jaczewskiego 8, 20-090 Lublin, Poland

Received 28 December 1994; revised 25 April 1995; accepted 28 April 1995

Abstract

α-Amino-3-hydroxy-5-methyl-isoxazole-4-propionate/kainate (AMPA/kainate) receptor antagonists (at subthreshold doses against electroconvulsions), 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI 52466 at maximally 5 mg/kg) and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*F*)quinoxaline (NBQX at maximally 20 mg/kg) enhanced the protective effects of NMDA receptor antagonists, MK-801 (dizocilpine) or 2-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid (D-CPP-ene), against electroconvulsions. Similarly, MK-801 or D-CPP-ene reduced the ED_{50} values of both NBQX and GYKI 52466 against maximal electroshock. The adverse effects of D-CPP-ene, evaluated in the chimney and rotorod tests, were potentiated by both GYKI 52466 (2.5 mg/kg) and NBQX (10 mg/kg). Also, D-CPP-ene (0.1 mg/kg) worsened the motor performance of mice pretreated with GYKI 52466 in the rotorod test. Neither MK-801 (0.025 mg/kg) nor D-CPP-ene (0.1 mg/kg) affected the NBQX-induced impairment of motor coordination. Similarly, GYKI 52466 (2.5 mg/kg) or NBQX (10 mg/kg) did not influence the performance of mice treated with MK-801 (0.2 mg/kg). It may be concluded that the blockade of more than one subtype of glutamate receptors leads to a more pronounced anticonvulsive effect when compared with the effect of blockade of an individual receptor subtype. In some cases more efficient seizure protection was not associated with increased adverse effects.

Keywords: AMPA receptor antagonist; NMDA receptor antagonist; Seizure

1. Introduction

It is evident that excitatory amino acids (e.g. glutamate or aspartate) are involved in the generation of seizure activity (Dingledine et al., 1990; Meldrum, 1992). There are also data available indicating a significant increase in plasma excitatory amino acids or elevated extracellular concentrations in some cases of human epilepsy (Huxtable et al., 1983; During, 1991). This may lead to the conclusion that excitatory events mediated by glutamate or other excitatory amino acids in the central nervous system may be associated with the occurrence of epileptic activity in humans.

Excitatory amino acids can influence central neurons via either ionotropic or metabotropic receptors. The former seem to play a pivotal role in the initiation and maintenance of convulsive states in rodents (Czuczwar and Meldrum, 1982; Czuczwar et al., 1985a; Schwarz and Freed, 1986; Steppuhn and Turski, 1993). Further, ionotropic receptors can be subdivided in two main groups –those sensitive to *N*-methyl-D-aspartate (NMDA) and those sensitive to α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate/kainate (AMPA/kainate) (Watkins et al., 1990). These two subpopulations of glutamate receptors differ substantially in terms of electrophysiological properties. Whilst AMPA/kainate receptors produce most of the rapid excitatory post-synaptic potential (fast EPSP), NMDA receptors are responsible for the so-called late EPSP. In addition, the former increase mainly Na^+ conductances and the latter both Ca^{2+} and Na^+ permeability (Wroblewski et al., 1985; Monaghan et al., 1989; Zorumski and Thio, 1992).

* Corresponding author. Tel. and fax +48 (81) 77-89-24.

Elsevier Science B.V.
SSDI 0014-2999(95)00268-5

Interestingly, antagonists of NMDA and AMPA/kainate-mediated excitation possess anticonvulsive activity in various models of experimental epilepsy, including electroconvulsions and chemically or sound-induced seizures (Croucher et al., 1982; Czuczwar and Meldrum, 1982; Czuczwar et al., 1985b, 1994 Turski et al., 1990, 1992; Smith et al., 1991; Meldrum et al., 1992). However, when a single population of ionotropic receptors for excitatory amino acids is blocked, then glutamate can still produce excitatory events through the unblocked one, which may limit the anticonvulsive activity of either NMDA or non-NMDA receptor antagonists applied alone. Consequently, we attempted to study whether a total blockade of ionotropic excitatory transmission would produce a potent protective effect against electroconvulsions in mice. To this end, MK-801 (a non-competitive NMDA receptor blocker; Wong et al., 1986), D-CPP-ene (a competitive NMDA receptor antagonist; Lowe et al., 1990), GYKI 52466 (a non-competitive AMPA/kainate receptor blocker; Donevan and Rogawski, 1993), and NBQX (a competitive AMPA/kainate receptor antagonist; Sheardown et al., 1990) were used.

2. Material and methods

2.1. Animals

The experiments were performed on male Swiss mice, weighing 22–27 g. The animals were kept under standard laboratory conditions on a natural light-dark cycle, with unlimited access to food (Murigran chow pellets; Bacutil, Motycz, Poland) and tap water. The experimental groups consisted of 8–10 mice and were completed by means of a randomized schedule. Each mouse was used only once. The control groups were always tested on the same day as the respective experimental groups. The temperature in the animal room and during testing was always 21 ± 1°C and all the tests were carried out between 10.00 and 13.00 h.

2.2. Drugs

D-CPP-ene (3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid, Sandoz, Berne, Switzerland) and MK-801 (maleate salt; dizocilpine; RBI, Natick, MA, USA) were used as NMDA receptor antagonists. NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*F*)-quinoxaline, Novo Nordisk, Måløv, Denmark) and GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine, Institute for Drug Research, Budapest, Hungary) were used as AMPA/kainate receptor antagonists.

Both NMDA receptor antagonists were dissolved in sterile saline, GYKI 52466 was put into solution in distilled water whilst NBQX was brought into solution in a small quantity of 1 N NaOH and made up with distilled water to the appropriate volume, the final pH being 8.5–9.0. All substances were administered intraperitoneally (i.p.) in a volume of 10 ml/kg body weight, except for MK-801 which was given subcutaneously (s.c.) in a volume of 5 ml/kg. For the combined treatments drugs were given either with an additional antagonist or the vehicle of that antagonist. The doses refer to the free drug forms and the treatment times were: D-CPP-ene 90 min, MK-801 45 min, and both GYKI 52466 and NBQX 15 min prior to the tests. The treatment times for GYKI 52466, MK-801, and NBQX were chosen based upon the time of their peak effects (Chapman et al., 1991; Vezzani et al., 1989). The latency between injection of D-CPP-ene and testing was obtained from Dr. Herrling (personal communication).

2.3. Electroconvulsions

Electroconvulsions were produced by means of an alternating current (0.2 s stimulus duration, 50 Hz) delivered via ear-clip electrodes by a Hugo-Sachs stimulator (Type 221; Freiburg, Germany). The criterion for the occurrence of seizure activity was tonic hindlimb extension.

In order to evaluate the anticonvulsive activity of MK-801, the threshold for electroconvulsions was measured. Maximal electroshock was not used in order to reduce the dosage of this non-competitive NMDA receptor antagonist. At higher dosages MK-801 is reported to exert a clear-cut excitation due to its central sympathomimetic properties (Clineschmidt et al., 1982b). Briefly, at least four groups of mice were challenged with electroshocks of various intensities to yield 10–30%, 30–50%, 50–70% and 70–90% of animals with seizures. Then, an intensity-effect curve was constructed, according to Litchfield and Wilcoxon (1949), from which a CS_{50} (current strength 50 in mA) value was estimated. Each CS_{50} value represents the current intensity necessary to induce tonic hindlimb extension in 50% of mice challenged. The protective action of D-CPP-ene and both non-NMDA receptor antagonists was evaluated as their ED_{50} values (in mg/kg) against maximal electroshock (fixed current intensity of 25 mA). The animals were administered different doses of the investigated drugs to obtain a variable percentage of protection against maximal electroshock, allowing us to construct a dose-effect curve, according to Litchfield and Wilcoxon (1949). Each ED_{50} represents the dose of a drug required to protect 50% of animals against maximal electroshock. In addition, the effects of D-CPP-ene, GYKI 52466, and NBQX upon the threshold for electroconvulsions were also measured to find out their subthreshold doses for the combined treatment.

2.4. Chimney test

The effects of NMDA or non-NMDA receptor antagonists alone or in combination on motor performance were measured with the chimney test of Boissier et al. (1960). Mice had to climb backwards up a plastic Perspex tube (3 cm inner diameter, 25 cm length). The animals unable to perform the test within 60 s were considered to display motor impairment. An ED_{50} value for an excitatory amino acid antagonist alone or combined with another excitatory amino acid antagonist was subsequently calculated.

2.5. Rotorod test

Motor coordination was assessed with the use of the rotorod test (Dunham and Miya, 1957). Each mouse was placed on a 2.5 cm diameter rod rotating at 3 revs./min. The animal's coordination was considered to be impaired if it fell from the rotorod during the 2 min observation period. Respective ED_{50} values were calculated on the basis of dose-response curves.

2.6. Statistics

The CS_{50} and ED_{50} values and their statistical evaluation were calculated by fitting the data by probit analysis according to Litchfield and Wilcoxon (1949), with the use of a computer.

3. Results

3.1. Influence of either NMDA or AMPA / kainate receptor antagonists on the threshold for electroconvulsions

D-CPP-ene (0.1 mg/kg), MK-801 (0.05 mg/kg), and GYKI 52466 (5 mg/kg) were devoid of any anticonvulsive activity against electroconvulsions. D-CPP-ene (0.2 and 0.5 mg/kg), MK-801 (0.1 and 0.2 mg/kg) and GYKI 52466 (10 mg/kg) produced a clear-cut increase in the convulsive threshold. However, NBQX (up to 20 mg/kg) remained ineffective in this test (Table 1).

3.2. Effects of either GYKI 52466 or NBQX on the protective action of MK-801 against electroconvulsions

For the combinations with MK-801 (0.2 mg/kg) GYKI 52466 and NBQX were used in subthreshold doses. It should be stressed that the combined treatment of MK-801 (0.2 mg/kg) and GYKI 52466 (1.25–5 mg/kg) resulted in an elevation of the convulsive threshold. Similarly, NBQX (10 and 20 mg/kg) enhanced the protection offered by MK-801. GYKI 52466 at 0.625 mg/kg and NBQX at 2.5 mg/kg did not modify the effect of MK-801 upon the threshold for electroconvulsions (Table 2).

Table 1
Influence of NMDA and non-NMDA receptor antagonists on the threshold for tonic electroconvulsions

Treatment (mg/kg)	Electroconvulsive threshold (mA)	
Vehicle	5.9	(5.2– 6.8)
D-CPP-ene (0.1)	6.2	(5.5– 7.1)
D-CPP-ene (0.2)	7.4 [a]	(6.6– 8.2)
D-CPP-ene (0.5)	9.1 [a]	(8.2–10.0)
Vehicle	6.1	(5.4– 6.9)
MK-801 (0.05)	6.2	(5.5– 7.1)
MK-801 (0.1)	8.1 [a]	(7.4– 8.9)
MK-801 (0.2)	9.6 [a]	(8.5–10.8)
Vehicle	6.5	(5.9– 7.3)
GYKI 52466 (5)	6.3	(5.5– 7.2)
GYKI 52466 (10)	16.0 [a]	(10.3–24.9)
Vehicle	6.1	(5.3– 7.0)
NBQX (10)	6.4	(5.6– 7.4)
NBQX (20)	6.5	(6.0– 7.0)

Drugs were given i.p.: D-CPP-ene 90 min, GYKI 52466 and NBQX 15 min before testing; MK-801 given s.c. 45 min before testing. The data are CS_{50} values with 95% confidence limits in parentheses, calculated and compared according to Litchfield and Wilcoxon (1949). [a] P at least < 0.05 vs. respective vehicle-treated group.

3.3. Influence of GYKI 52466 or NBQX on the anticonvulsive action of D-CPP-ene against maximal electroshock-induced seizures

GYKI 52466 (2.5 and 5 mg/kg) considerably reduced the ED_{50} value for D-CPP-ene against maximal electroshock. NBQX (10 and 20 mg/kg) exerted a similar effect. Neither GYKI 52466 (1.25 mg/kg) nor NBQX at 2.5 mg/kg affected the anticonvulsive efficacy of D-CPP-ene (Table 3).

3.4. Influence of NMDA receptor antagonists on the protective action of GYKI 52466 against maximal electroshock

D-CPP-ene at the subthreshold dose of 0.1 mg/kg potentiated the protection afforded by GYKI 52466

Table 2
Effects of either GYKI 52466 or NBQX upon the protective action of MK-801 (0.2 mg/kg) against tonic electroconvulsions in mice

Treatment (mg/kg)	Electroconvulsive threshold (mA)	
MK-801 (0.2) + vehicle	9.6	(8.5–10.8)
MK-801 (0.2) + GYKI 52466		
(0.625)	10.7	(10.0– 11.4)
(1.25)	12.8 [a]	(12.1– 13.6)
(2.5)	34.0 [a]	(28.5– 40.6)
(5)	108 [a]	(94.0–124.0)
MK-801 (0.2) + vehicle	9.9	(8.8– 11.2)
MK-801 (0.2) + NBQX		
(2.5)	10.3	(9.4– 11.3)
(10)	34.3 [a]	(32.2– 36.4)
(20)	42.6 [a]	(31.4– 52.8)

For explanations see Table 1. [a] P at least < 0.001 vs. respective control (i.e. MK-801-treated) group.

Table 3
Influence of either GYKI 52466 or NBQX on the protective action of D-CPP-ene against maximal electroshock-induced seizures

Treatment (mg/kg)	ED_{50} of D-CPP-ene (mg/kg)	
D-CPP-ene + vehicle	1.5	(1.1–2.1)
D-CPP-ene + GYKI 52466		
(1.25)	1.1	(0.9–1.4)
(2.5)	0.9 [a]	(0.7–1.1)
(5)	0.2 [a]	(0.1–0.3)
D-CPP-ene + vehicle	2.1	(1.5–2.8)
D-CPP-ene + NBQX		
(2.5)	1.7	(1.4–2.1)
(10)	0.8 [a]	(0.5–1.3)
(20)	0.5 [a]	(0.3–0.8)

Table data are ED_{50} values (in mg/kg, with 95% confidence limits in parentheses), calculated and statistically compared according to the method of Litchfield and Wilcoxon (1949). The original method was modified by computer construction of the dose-effect curve. At least 32 animals were used to calculate each ED_{50} value. The criterion for seizure activity was tonic hindlimb extension. See Table 1 for treatment times and other details. [a] P at least < 0.05 vs. respective vehicle-treated group.

against maximal electroshock-induced convulsions. MK-801 (when given in subthreshold doses of 0.025 and 0.05 mg/kg) also reduced the ED_{50} value for GYKI 52466. D-CPP-ene and MK-801 at lower doses of 0.05 and 0.0125 mg/kg, respectively, did not affect the anticonvulsive activity of GYKI 52466 (Table 4).

3.5. Effects of NMDA receptor antagonists on the anticonvulsive activity of NBQX against maximal electroshock

D-CPP-ene (0.1 mg/kg) and MK-801 (0.025 and 0.05 mg/kg) enhanced the protective action of NBQX against maximal electroshock-induced convulsions, which was reflected by the decreases in its ED_{50} value. When administered in lower doses, D-CPP-ene (0.05 mg/kg) and MK-801 (0.125 mg/kg) remained ineffective in this respect (Table 5).

Table 4
NMDA receptor antagonist-induced enhancement of the anticonvulsive activity of GYKI 52466 against maximal electroshock

Treatment (mg/kg)	ED_{50} of GYKI 52466 (mg/kg)	
GYKI 52466 + vehicle	12.3	(10.6–14.1)
GYKI 52466 + MK-801		
(0.0125)	11.3	(10.0–12.8)
(0.025)	8.2 [a]	(6.7– 9.9)
(0.05)	6.4 [a]	(4.9– 8.3)
GYKI 52466 + D-CPP-ene		
(0.05)	9.9	(8.0–12.1)
(0.1)	8.1 [a]	(6.0–10.9)

The data are ED_{50} values (in mg/kg) with 95% confidence limits in parentheses. For further explanations see Tables 1 and 3. [a] P at least < 0.05 vs. vehicle-treated group.

3.6. Influence of either NMDA or AMPA / kainate receptor antagonists given alone or in combination upon the performance of mice in the chimney and rotorod tests

MK-801 (0.2 mg/kg) did not affect the motor performance of mice in the chimney test and this was also

Table 5
Effects of NMDA receptor antagonists on the protective action of NBQX against maximal electroshock

Treatment (mg/kg)	ED_{50} of NBQX (mg/kg)	
NBQX + vehicle	48.4	(36.7–63.9)
NBQX + MK-801		
(0.0125)	35.7	(28.6–44.6)
(0.025)	20.7 [a]	(15.1–28.5)
(0.05)	15.4 [a]	(10.5–22.7)
NBQX + D-CPP-ene		
(0.05)	43.0	(33.6–55.1)
(0.1)	27.6 [a]	(19.1–39.8)

Table data are ED_{50} values (in mg/kg) with 95% confidence limits in parentheses. For further details refer to Tables 1 and 3. [a] P at least < 0.05 vs. vehicle-treated group.

Table 6
Influence of NMDA or AMPA/kainate antagonists alone or in combination upon the motor performance of mice

Treatment (mg/kg)	Chimney test		Rotorod test	
MK-801 + vehicle	> 0.2		0.10	(0.08– 0.13)
MK-801 + GYKI 52466 (2.5)	> 0.2		0.14	(0.10– 0.18)
MK-801 + NBQX (10)	> 0.2		0.09	(0.06– 0.15)
D-CPP-ene + vehicle	2.1	(1.2– 3.6)	1.4	(1.0 – 2.1)
D-CPP-ene + GYKI 52466 (2.5)	0.4 [a]	(0.3– 0.6)	0.2 [a]	(0.08– 0.5)
D-CPP-ene + NBQX (10)	0.4 [a]	(0.3– 0.6)	0.5 [a]	(0.36– 0.7)
GYKI 52466 + vehicle	12.0	(8.9–16.1)	11.9	(8.1 –17.6)
GYKI 52466 + MK-801 (0.025)	14.6	(11.7–18.2)	10.8	(8.4 –14.0)
GYKI 52466 + D-CPP-ene (0.1)	11.0	(7.5–16.0)	6.8 [a]	(5.4 – 8.5)
NBQX + vehicle	31.2	(27.4–35.4)	12.1	(6.8 –21.8)
NBQX + MK-801 (0.025)	34.7	(30.8–39.2)	13.9	(8.3 –23.3)
NBQX + D-CPP-ene (0.1)	30.5	(23.5–39.7)	13.4	(7.9 –22.7)

Table data are ED_{50} values (in mg/kg). In the chimney test mice had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length). Motor impairment was indicated when the animals were unable to perform the task within 60 s. Further, the motor coordination of the animal was considered impaired if it fell from the rotorod (2.5 cm diameter, 3 revs./min) within 2 min. For other details see Tables 1 and 3. [a] P at least < 0.05 vs. respective control group.

true for the combined treatment of MK-801 (0.2 mg/kg) with either GYKI 52466 (2.5 mg/kg) or NBQX (10 mg/kg). Further, these AMPA/kainate receptor antagonists in the same dose range did not change the ED_{50} for MK-801 in the rotorod test. However, the performance of mice treated with D-CPP-ene in combination with GYKI 52466 (2.5 mg/kg) or NBQX (10 mg/kg) was considerably poorer in both tests (Table 6).

MK-801 (0.025 mg/kg) did not produce any significant changes in the adverse effects of GYKI 52466, whilst D-CPP-ene (0.1 mg/kg) worsened the performance of GYKI 52466-treated animals only in the rotorod test. However, neither MK-801 (0.025 mg/kg) nor D-CPP-ene (0.1 mg/kg) influenced the NBQX-induced impairment of motor coordination (Table 6).

4. Discussion

The results shown from these experiments clearly indicate that NMDA receptor antagonists (in subthreshold doses) considerably potentiated the protective activity of AMPA/kainate receptor antagonists against electroconvulsions and vice versa. Thus, a blockade of glutamate-mediated excitation, at more than one subtype of its ionotropic receptors, leads to a pronounced anticonvulsive effect, with no further increase in the adverse effects in some cases. As regards MK-801, its potent central sympathomimetic activity (Clineschmidt et al., 1982b) may contribute to the observed effects. In fact, Clineschmidt et al. (1982a) provided evidence for the blockade of MK-801's anti-electroshock activity in rodents by haloperidol and prazosin. Similarly, prazosin impaired the protective activity of MK-801 against quinolinic acid-induced convulsions in rats (Vezzani et al., 1989). A distinct correlation between lowering of central monoamine levels and a decrease in the convulsive threshold for both electro-and chemoconvulsions was also found in mice and rats by Kilian and Frey (1973). MK-801, when given alone, may be effective against maximal electroshock-induced seizures in mice (Palmer et al., 1992). It is noteworthy, however, that the combination of this non-competitive NMDA receptor antagonist with either NBQX or particularly GYKI 52466 resulted in an elevation of the electroconvulsive threshold far above the current intensity used for the induction of maximal electroshock-induced seizures.

Generally, when D-CPP-ene was used to block NMDA receptors, addition of an AMPA/kainate receptor antagonist resulted in the enhancement of D-CPP-ene-induced adverse effects. This is consistent with our previous data showing that combinations of D-CPP-ene with antiepileptic drugs produced a considerable impairment of motor performance and long-term memory in mice (Żarnowski et al., 1994). Similarly, D-CPP-ene added to standard antiepileptic therapy produced a series of unwanted actions which seem to disqualify its use in humans for the treatment of epilepsy (Sveinbjornsdottir et al., 1993). MK-801 also produced considerable adverse effects in humans, so it seems to possess only an experimental value to delineate the importance of NMDA-mediated excitation in experimental epilepsy (Rogawski, 1992). In addition, NMDA receptor antagonists were shown to induce a sequence of phencyclidine-like behavioral disturbances in epileptic subjects (kindled rats) within the anticonvulsive dose range (Löscher and Hönack, 1991a). However, these effects were not observed in non-kindled controls, which suggests that kindling-induced epileptogenesis may actually reduce the therapeutic index of NMDA receptor antagonists (Löscher and Hönack, 1991a,b). Interestingly, NBQX was devoid of phencyclidine-like side effects in kindled rats, which may indicate that AMPA/kainate receptor antagonists may possess a better clinical potential than NMDA receptor antagonists (Löscher et al., 1993). Further, it was demonstrated that NMDA receptor antagonists, which per se display very weak anticonvulsive properties at even large doses in the kindling model of epilepsy, synergistically enhanced the protective effect of NBQX against amygdala kindling in rats (Löscher et al., 1993). Consequently, a new strategy for the treatment of epilepsy was postulated by these authors. Our results indicate that the interaction between NMDA and AMPA/kainate receptor antagonists may occur in both directions against electroconvulsions –i.e. NMDA receptor blockers enhance the protective effects of AMPA/kainate receptor antagonists and, additionally, AMPA/kainate receptor antagonists potentiate the anticonvulsive action of NMDA receptor antagonists. It is unlikely that the observed effects may be due to pharmacokinetic interactions since in many cases several fold increases in the convulsive threshold or decreases in the respective ED_{50} values were noted with the combined treatment. It should be additionally accentuated that a second excitatory amino acid antagonist, added to a combination, was always used in subconvulsive doses. It can be assumed, therefore, that a simultaneous blockade of more than one type of glutamate receptor leads to pronounced anticonvulsive effects, which extends the observations of Löscher et al. (1993) to electroconvulsions, a well recognized model of generalized tonic-clonic seizures in humans. According to Peeters et al. (1994), all glutamate ionotropic receptor subtypes may be involved in nonconvulsive epilepsy, evaluated in their WAG/Rij rat inbred strain. In this context, the new strategy for the treatment of human epilepsy (Löscher et al., 1993) would be confirmed and completed with an additional seizure model. However, a recent study by Foutz et al. (1994) provides

evidence of respiratory arrest produced by the combined blockade of NMDA and non-NMDA receptors in cats. For this purpose, MK-801 was administered in a dose of 0.1 mg/kg and NBQX at 40 mg/kg. Interestingly, no such effects were observed when the excitatory amino acid receptor antagonists (MK-801 or NBQX) were given separately. It is, thus, of pivotal importance to find out whether such unwanted effects appear in primates upon the combined blockade of receptors for excitatory amino acids. If this is the case, then the idea of the combined treatment of NMDA and non-NMDA receptor antagonists to control human epilepsy may prove premature. It is probable that the combined treatment of conventional antiepileptics with some NMDA receptor antagonists (Czechowska et al., 1993; Pietrasiewicz et al., 1993) or AMPA/kainate receptor blockers (Żarnowski et al., 1993) could provide an alternative and safer strategy for clinical purposes.

Acknowledgements

The authors express their thanks to Dr. I. Tarnawa (Institute for Drug Research, Budapest, Hungary) for his generous supply of GYKI 52466. The generous gift of D-CPP-ene from Dr. P.L. Herrling (Sandoz, Basel, Switzerland) is also greatly appreciated.

In addition, the authors are grateful to Lublin Medical University School for a research grant, No. Dz. St. 14/94.

References

Boissier, J.R., J. Tardy and J.C. Diverres, 1960, Une nouvelle méthode simple pour explorer l'action 'tranquilisante': le test de la chiminée, Med. Exp. (Basel) 3, 81.

Chapman, A.G., S.E. Smith and B.S. Meldrum, 1991, The anticonvulsant effect of the non-NMDA antagonists, NBQX and GYKI 52466, in mice, Epilepsy Res. 9, 92.

Clineschmidt, B.V., G.E. Martin and P.R. Bunting, 1982a, Anticonvulsant activity of (±)-5-methyl-10,11-dihydro-5*H*-dibenzo-(*a*,*d*) cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic and apparent anxiolytic properties, Drug Dev. Res. 2, 123.

Clineschmidt, B.V., G.E. Martin, P.R. Bunting and M.L. Papp, 1982b, Central sympathomimetic activity of (±)-5-methyl-10,11-dihydro-5*H*-dibenzo-(*a*,*d*) cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic and apparent anxiolytic properties, Drug Dev. Res. 2, 135.

Croucher, M.J., J.F. Collins and B.S. Meldrum, 1982, Anticonvulsant action of excitatory amino acid antagonists, Science 216, 899.

Czechowska, G., M. Dziki, T. Pietrasiewicz, Z. Kleinrok, W.A. Turski and S.J. Czuczwar, 1993, Competitive antagonists of NMDA receptor: CGP 37849 and CGP 39551 enhance the anticonvulsant activity of valproate against electroconvulsions in mice, Eur. J. Pharmacol. 232, 59.

Czuczwar, S.J. and B. Meldrum, 1982, Protection against chemically induced seizures by 2-amino-7-phosphonoheptanoic, Eur. J. Pharmacol. 83, 335.

Czuczwar, S.J., H.-H. Frey and W. Löscher, 1985a, Antagonism of *N*-methyl-D,L-aspartic acid-induced convulsions by anti-epileptic drugs and other agents, Eur. J. Pharmacol. 108, 273.

Czuczwar, S.J., E.A. Cavalheiro, L. Turski, W.A. Turski and Z. Kleinrok, 1985b, Phosphonic analogues of excitatory amino acids raise the threshold for maximal electroconvulsions in mice, Neurosci. Res. 3, 86.

Czuczwar, S.J., M. Gasior, W.A. Turski and Z. Kleinrok, 1994, Influence of a Ca^{2+} channel agonist, BAY k-8644, on the anticonvulsant activity of NMDA and non-NMDA receptor antagonists, Eur. J. Pharmacol. 264, 103.

Dingledine, R., C.J. McBain and J.O. McNamara, 1990, Excitatory amino acid receptors in epilepsy, Trends Pharmacol. Sci. 11, 334.

Donevan, S.D. and M.A. Rogawski, 1993, GYKI 52466, a 2,3-benzo diazepine, is a highly selective, noncompetitive antagonist of AMPA/kainate receptor responses, Neuron 10, 51.

Dunham, N.W. and T.S. Miya, 1957, A note on a simple approach for determining neurological deficits in rats and mice, J. Am. Pharmacol. Assoc. 46, 208.

During, M.J., 1991, In vivo neurochemistry of conscious human brain: intrahippocampal microdialysis in epilepsy, in: Microdialysis in the Neurosciences, eds. T.E. Robinson and J.B. Justice Jr. (Elsevier, Amsterdam) p. 425.

Foutz, A.S., O. Pierrefiche and M. Denavit-Saubie, 1994, Combined blockade of NMDA and non-NMDA receptors produces respiratory arrest in the adult cat, NeuroReport 5, 481.

Huxtable, R.J., H. Laird, S.E. Lippincott and P. Wolson, 1983, Epilepsy and the concentration of plasma amino acids in humans, Neurochem. Int. 5, 125.

Kilian, M. and H.-H. Frey, 1973, Central monoamines and convulsive thresholds in mice and rats, Neuropharmacology 12, 681.

Litchfield, J.T. and F. Wilcoxon, 1949, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther. 96, 99.

Lowe, D.A., H.C. Neijt and B. Aebischer, 1990, D-CPP-ene (SDZ EAA 494) a potent and competitive *N*-methyl-D-aspartate (NMDA) antagonist: effect on spontaneous activity and NMDA-induced depolarizations in the rat neocortical slice preparation, compared with other CPP derivatives and MK-801, Neurosci. Lett. 113, 315.

Löscher, W. and D. Hönack, 1991a, Anticonvulsant and behavioral effects of two novel competitive *N*-methyl-D-aspartate antagonists, CGP 37849 and CGP 39551, in the kindling model of epilepsy. Comparison with MK-801 and carbamazepine, J. Pharmacol. Exp. Ther. 256, 432.

Löscher, W. and D. Hönack, 1991b, Responses to NMDA receptor antagonists altered by epileptogenesis, Trends Pharmacol. Sci. 12, 52.

Löscher, W., C. Rundfeldt and D. Hönack, 1993, Low doses of NMDA receptor antagonists synergistically increase the anticonvulsant effect of the AMPA receptor antagonist NBQX in the kindling model of epilepsy, Eur. J. Neurosci. 5, 1545.

Meldrum, B.S., 1992, Excitatory amino acids in epilepsy and potential novel therapies, Epilepsy Res. 12, 189.

Meldrum, B.S., M.D. Craggs, N. Dürmüller, S.E. Smith and A.G. Chapman, 1992, The effects of AMPA receptor antagonists on kindled seizures and on reflex epilepsy in rodents and primates, in: Molecular Neurobiology of Epilepsy (Epilepsy Res. Suppl. 9), eds. J. Engel Jr., L. Wasterlain, E.A. Cavalheiro, U. Heinemann and G. Avanzini (Elsevier, Amsterdam) p. 307.

Monaghan, D.T., R.J. Bridges and C.W. Cotman, 1989, The excitatory amino-acid receptors: their classes, pharmacology and distinct properties in the function of the central nervous system, Annu. Rev. Pharmacol. Toxicol. 29, 365.

Palmer, G.C., E.W. Harris, R. Ray, M.L. Stagnitto and R.J. Schmiesing, 1992, Classification of compounds for prevention of NMDLA-induced seizures/mortality, or maximal electroshock and pentylenetetrazol seizures in mice and antagonism of MK801 binding in vitro, Arch. Int. Pharmacodyn. 317, 16.

Peeters, B.W.M.M., G.M.J. Ramakers, B.A. Ellenbroek, J.M.H. Vossen and A.M.L. Coenen, Interactions between NMDA and nonNMDA receptors in nonconvulsive epilepsy in the WAG/Rij inbred strain, 1994, Brain Res. Bull. 33, 715.

Pietrasiewicz, T., G. Czechowska, M. Dziki, W.A. Turski, Z. Kleinrok and S.J. Czuczwar, 1993, Competitive NMDA receptors antagonists enhance the antielectroshock activity of various antiepileptics, Eur. J. Pharmacol. 250, 1.

Rogawski, M.A., 1992, The NMDA receptor, NMDA antagonists and epilepsy therapy, Drugs 44, 279.

Schwarz, S.S. and W.J. Freed, 1986, Inhibition of quisqualate-induced seizures by glutamic acid diethyl ester and anti-epileptic drugs, J. Neural Transm. 67, 191.

Sheardown, M.J., E.O. Nielsen, A.J. Hansen, P. Jacobsen and T. Honoré, 1990, 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)-quinoxaline: a neuroprotectant for cerebral ischemia, Science 247, 571.

Smith, S.E., N. Dürmüller and B.S. Meldrum, 1991, The non-*N*-methyl-D-aspartate receptor antagonists, GYKI 52466 and NBQX are anticonvulsant in two animal models of reflex epilepsy, Eur. J. Pharmacol. 201, 179.

Steppuhn, K.G. and L. Turski, 1993, Modulation of the seizure threshold for excitatory amino acids in mice by antiepileptic drugs and chemoconvulsants, J. Pharmacol. Exp. Ther. 265, 1063.

Sveinbjornsdottir, S., J.W.A.S. Sander, D. Upton, P.J. Thompson, P.N. Patsalos, D. Hirt, M. Emre, D. Lowe and J.S. Duncan, 1993, The excitatory amino acid antagonist D-CPP-ene (SDZ EAA-494) in patients with epilepsy, Epilepsy Res. 16, 65.

Turski, W.A., E. Urbańska, M. Dziki, J. Parada-Turska and C. Ikonomidou, 1990, Excitatory amino acid antagonists protect mice against seizures induced by bicuculline, Brain Res. 514, 131.

Turski, L., P. Jacobsen, T. Honoré and D.N. Stephens, 1992, Relief of experimental spasticity and anxiolytic/anticonvulsant actions of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*F*)quinoxaline, J. Pharmacol. Exp. Ther. 260, 742.

Vezzani, A., R. Serafini, M.A. Stasi, S. Caccia, I. Conti, R.V. Tridico and R. Samanin, 1989, Kinetics of MK-801 and its effect on quinolinic acid-induced seizures and neurotoxicity in rats, J. Pharmacol. Exp. Ther. 249, 278.

Watkins, J.C., P. Krogsgaard-Larsen and T. Honorè, 1990, Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists, Trends Pharmacol. Sci. 11, 25.

Wong, E.H.F., J.A. Kemp, T. Priestley, A.R. Knight, G.N. Woodruff and L.L. Iversen, 1986, The anticonvulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist, Proc. Natl. Acad. Sci. USA 83, 7104.

Wroblewski, J.T., F. Nicoletti and E. Costa, 1985, Different coupling of excitatory amino acid receptors with Ca^{2+} channels in primary cultures of cerebellar granule cells, Neuropharmacology 32, 895.

Zorumski, C.F. and L.L. Thio, 1992, Properties of vertebrate glutamate receptors: calcium mobilization and desensitization, Prog. Neurobiol. 39, 295.

Żarnowski, T., Z. Kleinrok, W.A. Turski and S.J. Czuczwar, 1993, 2,3-Dihydroxy-6-nitro-7-sulfamoylobenzo(*F*)quinoxaline enhances the protective activity of common antiepileptic drugs against maximal electroshock-induced seizures in mice, Neuropharmacology 32, 895.

Żarnowski, T., Z. Kleinrok, W.A. Turski and S.J. Czuczwar, 1994, The competitive NMDA antagonist, D-CPP-ene, potentiates the anticonvulsant activity of conventional antiepileptics against maximal electroshock-induced seizures in mice, Neuropharmacology 33, 619.