Physiol. Res. 43: 313–320, 1994

Effects of MK-801 (Dizocilpine) and Ketamine on Strychnine-Induced Convulsions in Rats: Comparison With Benzodiazepines and Standard Anticonvulsants

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Received January 26, 1994
Accepted June 20, 1994

Summary

The effects of two non-competitive NMDA antagonists – MK-801 and ketamine – were studied in a model of generalized seizures elicited by s.c. injection of strychnine (2 or 3 mg/kg) in adult rats. The animals were observed in isolation for 30 min after strychnine administration. Pretreatment with MK-801 (0.5 or 2 mg/kg i.p.) suppressed the tonic, but not the clonic phase of generalized seizures following both doses of strychnine. A similar action of ketamine (20 or 40 mg/kg i.p.) was indicated but it did not attain statistical significance. Strychnine-induced lethality was not changed significantly. A comparison with antiepileptic drugs demonstrated that only phenobarbital (10–80 mg/kg i.p.) was clearly effective against strychnine-induced seizures; carbamazepine (25 or 50 mg/kg i.p.) and partly phenytoin (30 or 60 mg/kg i.p.) were able to suppress the incidence of the tonic phase. Primidone (40 or 80 mg/kg i.p.) as well as the benzodiazepines bretazenil (0.1 or 1 mg/kg i.p.) and midazolam (two lower doses of 0.5 and 1 mg/kg i.p.) were without significant effect. The 2 mg/kg dose of midazolam was partly effective. Only phenobarbital, carbamazepine and the highest dose of midazolam prevented strychnine-induced lethality.

Key words

Motor seizures – Strychnine – Rats – NMDA antagonists – Antiepileptic drugs

Introduction

Strychnine is a commonly used convulsant drug inducing tonic-clonic seizures with a marked tonic phase (Woodbury 1980). There are two different aspects when comparing this with seizures induced by other convulsants: the predominant tonic phase, especially the extension of hindlimbs (Vida 1977, Woodbury 1980, Swinyard *et al.* 1989) and the resistance of strychnine-induced seizures against antiepileptic drugs (Swinyard and Woodhead 1982, Löscher 1986). An advantage of this model is the known mechanism of action of strychnine. Strychnine acts as an antagonist at glycine receptors connected with the chloride ionophore (Young and Snyder 1973) in a manner similar to $GABA_A$ receptors in a supramolecular complex (for review see Duman *et al.* 1987). Glycine was demonstrated to be the main inhibitory transmitter in the spinal cord and brainstem of higher vertebrates (Iversen and Bloom 1972). Strychnine is able to block chloride conductance induced by glycine and thus to reduce the amplitude of inhibitory postsynaptic potentials (Tribble *et al.* 1983).

Antagonists of excitatory amino acids, in particular N-methyl-D-aspartate antagonists represent a promising drugs for the treatment of epilepsies (Clineschmidt *et al.* 1982, Croucher *et al.* 1982, Chapman 1991) and some of them are tested in patients e.g. MK-801 (Troupin *et al.* 1986) or dextromethorphan (Fisher *et al.* 1990). The strychnine seizures, which might represent a model of human refractory epilepsy because of their refractoriness towards most antiepileptics (Löscher 1986) were used in the present study for testing two non-competitive NMDA antagonists – MK-801 and ketamine (Lodge 1990) in comparison with classical anticonvulsant drugs and two benzodiazepines.

Materials and Methods

Experiments were performed in adult male Wistar rats weighing 200–290 g. The following drugs were used: strychnine (Strychnin^R Spofa, 0.1 % solution) in doses of 2 or 3 mg/kg, MK-801 (dizocilpine, Research Institute for Pharmacy and Biochemistry, aqueous solution) in doses of 0.5 or 2 mg/kg, ketamine (Narkamon^R Spofa, 5 % water solution) in doses of 20 or 40 mg/kg, carbamazepine (Sigma, solved in a mixture of ethanol, propylenglycol, and water in a ratio 2:5:3) in doses of 25 or 50 mg/kg, phenytoin (Epanutin^R Parke and Davis, 5 % commercial solution; tricomponent solvent as for carbamazepine) in doses of 30 or 60 mg/kg, phenobarbital (Na^+ salt, Spofa, aqueous solution) in doses of 10, 20, 40 or 80 mg/kg, primidone (Arzneimittelwerke Dresden, dissolved in dimethylsulfoxide) in doses of 40 or 80 mg/kg, midazolam (Dormicum$_R$ Roche, 0.5 % water solution) in doses of 0.5, 1 or 2 mg/kg, and bretazenil (Ro-16 6028 Roche, in water with Tween 80) in doses of 0.1 or 1 mg/kg. The doses were chosen on the basis of our data from experiments with pentylenetetrazol-induced seizures (MK-801 – Velíšek *et al.* 1991; ketamine – Velíšek *et al.* 1989; carbamazepine – Kubová and Mareš 1993; phenytoin – Staňková *et al.* 1992; phenobarbital and primidone – Kubová and Mareš 1991; midazolam – Kubová and Mareš 1992a; bretazenil – Kubová *et al.* 1993) performed in the same strain of rats under identical conditions. Higher doses were not used because pilot experiments demonstrated their side effects.

Three control groups were formed: rats which received an injection of strychnine only and animals pretreated with either tricomponent solvent or dimethylsulfoxide in a volume of 1 ml/kg.

All solutions prepared in our laboratory (i.e. MK-801, carbamazepine, phenobarbital, primidone and bretazenil) had such a concentration of the active drug that the volume of 1 ml/kg was injected. Strychnine was injected subcutaneously in doses of 2 and/or 3 mg/kg, i.e. a submaximal and a supramaximal one, from the point of motor seizures. The other drugs were administered intraperitoneally either 60 min (phenytoin, phenobarbital, primidone), 30 min (carbamazepine), 10 min (MK-801, ketamine, Ro 16-6028) or immediately (midazolam) before strychnine injection. Intervals were chosen according to data on pharmacokinetics of individual drugs (for review see respective chapters in Levy *et al.* 1989) and they were the same as in pentylenetetrazol experiments mentioned above.

After strychnine administration, the animals were put into transparent plastic boxes (22 x 38 x 23 cm) and were observed in isolation for 30 min. The animals' behaviour, the incidence, pattern, latency and duration of epileptic manifestations and the incidence of lethality were evaluated. The number of animals in each experimental group (mostly eight) is given in Tables 1 and 2.

Statistical evaluation of results

The significance of differences between drug-treated groups and the corresponding controls for the incidence of seizures and of fatal outcome was determined by means of Fischer's exact test (a quadripolar table). Latencies (means ± S.E.M) were evaluated by ANOVA (one-way analysis of variance followed by a Tukey's test). The level of statistical significance was set at 5 %.

Results

Strychnine dose-dependently induced generalized tonic-clonic convulsions (major seizures, MS) with an initial, predominant tonic phase preceded by jerks restricted at the beginning to the facial muscles and later involving whole body. Seizures were accompanied by high mortality.

In control experiments strychnine in a dose of 2 mg/kg produced MS in 70 % of the animals with 40 % mortality. The dose of 3 mg/kg increased the incidence of MS to 100 % and of fatal outcome to 80 %. Tonic hindlimb extension (the most severe phase of seizures) was observed in all control animals exhibiting seizures.

Strychnine 2 mg/kg (Table 1)

Incidence

Both doses of MK-801 selectively blocked the tonic phase without influencing the incidence of MS. Similar effects of ketamine did not reach the statistical significance; in addition, the tonic phase seen in one case only after the 40 mg/kg dose of ketamine lasted 3 s (vs. 10.7±1.5 s under control conditions). Higher dose of ketamine also abolished lethality. The suppression of the tonic phase was observed with both doses of carbamazepine and with the 30-mg/kg dose of phenytoin; the effect of the higher dose of phenytoin did not reach statistical significance. Phenobarbital nearly abolished strychnine seizures (only one rat after the 20 mg/kg dose convulsed) and fully blocked the strychnine-induced lethality. Primidone did not exhibit any effect; the same was true for two lower doses of midazolam. The 2 mg/kg dose of midazolam tended to suppress major seizures and abolished their tonic phase. In addition, this dose of midazolam also led to the impairment of behaviour when given alone – rats stayed motionless nearly the whole 30 min observation period. A tendency to shortening of the tonic phase was the only change seen after pretreatment with the higher dose of bretazenil.

Table 1
Effects of non-competitive NMDA antagonists and antiepileptic drugs on seizures and lethality induced by strychnine (2 mg/kg s.c.) in rats

Drug	Incidence			Latency (s)	
Dose	MS	TP	EX	MS	EX
Control					
–	7/10	7/10	4/10	742±76	1022±96
S	7/8	7/8	6/8	739±90	917±110
DMSO	8/8	4/8	7/8	407±58	972±103
MK-801					
0.5	8/8	0/8*	5/8	404±70*	740±104*
2	8/8	0/8*	6/8	366±69*	1068±219
Ketamine					
20	8/8	5/8	7/8	448±75	1051±193
40	8/8	1/8	0/8	474±166	–
Carbamazepine					
25	2/8	0/8*	1/8	–	–
50	2/8	0/8*	1/8	–	–
Phenytoin					
30	4/9	0/9*	1/9	509±88	–
60	6/8	3/8	1/8	375±41*	–
Phenobarbital					
10	0/8*	0/8*	0/8	–	–
20	1/8	1/8	0/8	–	–
40	0/8*	0/8*	0/8	–	–
Primidone					
40	4/8	4/8	2/8	700±293	–
80	5/8	5/8	3/8	705±137	–
Midazolam					
0.5	7/8	7/8	5/8	775±11	821±101
1	3/8	3/8	1/8	–	–
2	1/8	0/8*	0/8	–	–
Bretazenil					
0.1	6/8	6/8	5/8	978±124	1277±175
1	6/8	6/8	4/8	708±177	526±15*

MS: major seizures; TP: tonic phase; EX: exitus; S: solvent for carbamazepine and phenytoin; DMSO: dimethylsulfoxide used as a solvent for primidone
**Significant difference compared to appropriate controls ($p<0.05$; Fischer's exact test for incidence; ANOVA for latencies)*

Table 2

Effects of non-competitive NMDA antagonists and antiepileptic drugs on seizures and lethality induced by strychnine (3 mg/kg s.c.) in rats

Drug	Incidence			Latency (s)	
Dose	MS	TP	EX	MS	EX
Control					
–	10/10	10/10	8/10	540 ± 139	927 ± 60
S	8/11	8/11	6/11	732 ± 144	1097 ± 185
DMSO	8/8	8/8	8/8	454 ± 22	1156 ± 95
MK-801					
0.5	8/8	5/5	7/8	188 ± 15*	481 ± 89*
2	8/8	0/8*	7/8	186 ± 45*	473 ± 89*
Ketamine					
20	8/8	5/8	7/8	281 ± 31	649 ± 161
40	8/8	2/8	8/8	320 ± 32	827 ± 148
Carbamazepine					
25	6/6	6/6	2/6	673 ± 124	–
50	5/8	0/8*	2/8	497 ± 92	–
Phenytoin					
30	8/8	8/8	7/8	342 ± 65	701 ± 265
60	8/8	8/8	7/8	279 ± 28	1094 ± 201
Phenobarbital					
10	5/8	5/8	4/8	820 ± 124	1169 ± 82
20	8/10	8/10	3/10	809 ± 77	–
40	9/10	9/10	1/10	822 ± 125	–
80	0/8*	0/8*	0/8*	–	–
Primidone					
40	6/8	6/8	4/8	623 ± 113	793 ± 195
80	8/8	8/8	7/8	562 ± 98	992 ± 140
Midazolam					
0.5	7/7	7/7	6/7	744 ± 178	1021 ± 194
1	6/8	6/8	5/8	790 ± 146	996 ± 209
2	4/8	4/8	0/8*	709 ± 131	–
Bretazenil					
0.1	6/8	6/8	4/8	953 ± 133	1323 ± 182
1	8/8	6/8	6/8	348 ± 73	493 ± 107

For further details see Table 1.

Latencies

In spite of the suppression of the tonic phase, both tested doses of MK-801 significantly shortened the latencies of MS. A similar effect was obtained with the higher dose of phenytoin. Latencies of fatal outcome were shortened by a lower dose of MK-801 and higher dose of bretazenil.

Some automatisms were observed in the animals in which MS were suppressed; the most frequent ones were chewing, washing and scratching. These behavioral manifestations were identical with those observed under control conditions (animals receiving only strychnine) before the seizures started and their quantity and/or quality were not influenced in any way by the drugs tested.

Strychnine 3 mg/kg (Table 2)

Incidence

The higher dose of MK-801 was able to decrease the incidence of the tonic phase of MS significantly. The changes seen after the lower dose of MK-801 and both doses of ketamine did not attain the level of significance. Pretreatment with both doses of ketamine shortened the tonic phase from the average of 21.0±2.7 s under control conditions to 4.4±0.6 s after the lower dose and 4 s (average of two values only) after the 40 mg/kg dose. The higher dose of carbamazepine abolished the tonic phase of seizures, both doses of this drug tended to suppress the lethality. Phenytoin was without effect. Phenobarbital in the highest dose used (80 mg/kg) abolished seizures induced by this dose of strychnine. In addition, it suppressed lethality in a dose-dependent manner. Primidone and both benzodiazepines did not affect the incidence of seizures, whereas the fatal outcome was prevented by the highest dose of midazolam.

Latencies

Latencies of MS as well as of the fatal outcome were not changed by pretreatment with any drug used except of MK-801 which shortened these latencies.

Discussion

Our results demonstrated marked resistance of strychnine-induced seizures against antiepileptic drugs. Classical antiepileptic drugs in doses suppressing pentylenetetrazol-induced seizures (primidone – Kubová and Mareš 1991, benzodiazepines – Kubová and Mareš 1992a,b, Kubová *et al.* 1993) or their tonic phase (phenytoin – Staňková *et al.* 1992) were unable to influence seizures elicited by strychnine significantly. The antiepileptic drugs tested exhibited surprising discrepancy between their generally accepted mechanisms of action and their ability to affect these convulsions. Among the drugs influencing the GABAergic system only phenobarbital was able to reduce the incidence of tonic-clonic seizures and the mortality induced by strychnine.

On the contrary, both benzodiazepines tested – midazolam (in two lower doses) and bretazenil – did not exhibit any effect against these convulsions. The highest dose of midazolam was effective against both the tonic phase and lethality, but it induced side effects. Both phenobarbital and benzodiazepines interact with the $GABA_A$-receptor supramolecular complex (Haefely 1985). The inability of benzodiazepines to reduce strychnine-induced seizures is in agreement with previous results with clonazepam (Swinyard and Woodhead 1982; Kubová *et al.* 1990). The GABAergic system might also be affected by primidone, which is metabolized to phenobarbital (Baumel *et al.* 1973), but no antistrychnine effects were found with this drug at the doses and interval tested. The diametrally different results with primidone and phenobarbital indicate that no phenobarbital is present in primidone-pretreated rats untill 90 min, a conclusion supported by the inability to find metabolites of primidone up to 2 h after intraperitoneal administration (Löscher and Honack 1989).

On the other hand, it is puzzling that MK-801 and carbamazepine, which do not share a common mechanism of action, display extensive conformity in the pattern of their antistrychnine activity. Ketamine and phenytoin tended to demonstrate similar activity when combined with lower dose of strychnine. MK-801 like ketamine is a non-competitive antagonist of NMDA receptors (for review see Rogawski and Porter 1990, Foster 1991), whereas the anticonvulsant action of phenytoin might be due to its action on neuronal membrane – use-dependent inhibition of sodium channels, inhibition of calcium channels, an increase in activity of sodium-potassium ATPase (for review see DeLorenzo, 1989). Carbamazepine is thought to have a similar action: it clearly diminishes neuronal excitability (for review see MacDonald 1989). All these drugs affected specifically the tonic phase without influencing the incidence of clonic seizures and/or mortality. Specific blockade of the tonic phase by phenytoin and carbamazepine was found in other models of generalized tonic-clonic seizures, e.g. in maximal electroshock and chemically induced convulsions (Swinyard and Woodhead 1982, Swinyard *et al.* 1989). MK-801 and ketamine abolished generalized tonic-clonic seizures induced by metrazol (Velíšek *et al.* 1989, 1991) as well as by bicuculline and picrotoxin (Velíšková *et al.* 1990). On the basis of all these findings it might be concluded that NMDA receptors play a role in the generation of generalized tonic-clonic seizures, but the exact efficacy of NMDA receptor antagonists against the tonic and clonic phases remains to be analyzed. A rather complicated role of the NMDA system even in the generation of generalized tonic-clonic seizures is suggested by our finding that

the latency for clonic seizures, which are still present after the pretreatment with MK-801, is significantly shortened. This finding is in agreement with the data of O'Neill and Bolger (1989) who mentioned only clonic seizures. The dose of strychnine used by these authors was probably too low for inducing tonic seizures and this render a direct comparison with our results more difficult.

A direct interaction of NMDA antagonists with strychnine at the spinal cord level is rather unlikely because NMDA receptors in the spinal cord are restricted only to substantia gelatinosa (Cotman *et al.* 1987). The effects more likely result from an action on supraspinal mechanisms modulating motoneurone activity. Basal ganglia have to be taken into account in this connection.

No correlation was found between clinical efficacy and antistrychnine activity for the antiepileptic drugs tested by us. Thus the "classical" antiepileptic drugs (phenobarbital, phenytoin and carbamazepine) as well as benzodiazepines show qualitative conformity regarding their clinical usefulness for the therapy of generalized tonic-clonic seizures, but exhibit substantial differences in our experiments. It is in agreement with experimental evidence of qualitative differences in the activity of these antiepileptic drugs against strychnine-induced and other epileptic seizures in rodents (for review see Swinyard and Woodhead 1982, Swinyard *et al.* 1989).

The observed differences in antistrychnine activities might be due to the different affinities of the drugs to the glycine-receptor mediating convulsive action of strychnine. Unfortunately, this problem was not yet studied in detail. However, a study of the affinity of phenobarbital to the chloride channel of glycine receptors analogous to the chloride channel of $GABA_A$ receptors might explain the exclusive activity of this drug in the strychnine model. Both chloride channels connected with $GABA_A$ and glycine receptors belong to the superfamily of neurotransmitter-gated ion channels (Grenningloh *et al.* 1987, Pribilla *et al.* 1992). There are some data demonstrating the same effect of drugs on both types of chloride channels (De Deyn *et al.* 1991) as well as data demonstrating differences between these channels (Rienitz *et al.* 1987).

Among the chemically induced seizures, the strychnine-induced convulsions remarkably differ from other models with respect to their susceptibility to antiepileptic drugs (Löscher 1986) which hardly mirrors the clinically documented antiepileptic activity of these drugs. These seizures cannot be recommended for routine testing of anticonvulsant activity of potential antiepileptic drugs but their detailed exploration might provide new insights into the mechanism of action of classical anticonvulsants. In addition, these seizures may be used for analysis of generation of complete tonic seizures (i.e. including tonic hindlimb extension) which are reliably induced by strychnine under control conditions.

Acknowledgement

The authors thank Dr. J. Vorlíček for his kind and helpful statistical advice.

This work was supported by grants No. 71106 and 71157 of the Academy of Sciences of the Czech Republic.

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