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Convulsions induced by centrally administered NMDA in mice: effects of NMDA antagonists, benzodiazepines, minor tranquilizers and anticonvulsants

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1 Convulsions were induced reproducibly by intracerebroventricular injection of N-methyl-D-aspartic acid (NMDA) to conscious mice.

2 Competitive (carboxypiperazine-propylphosphonic acid, CPP; 2-amino-7-phosphonoheptanoic acid, AP7) and non-competitive (MK801; phencyclidine, PCP; thienylcyclohexylpiperidine, TCP; dextrorphan; dextromethorphan) NMDA antagonists prevented NMDA-induced convulsions.

3 Benzodiazepine receptor agonists and partial agonists (triazolam, diazepam, clonazepam, Ro 16-6028), classical anticonvulsants (diphenylhydantoin, phenobarbitone, sodium valproate) and meprobamate were also found to prevent NMDA-induced convulsions.

4 Flumazenil (a benzodiazepine receptor antagonist) and the GABA agonists THIP and muscimol (up to subtoxic doses) were without effect.

5 Flumazenil reversed the anticonvulsant action of diazepam, but not that of MK801.

6 Results obtained in this model differ somewhat from those described in a seizure model with systemic administration of NMDA. An explanation for this discrepancy is offered.

7 This model is a simple test for assessing the *in vivo* activity of NMDA antagonists and also expands the battery of chemically-induced seizure models for characterizing anticonvulsants not acting at NMDA receptors.

Introduction

N-methyl-D-aspartate (NMDA) receptors have been implicated in a number of physiological and pathological phenomena such as learning, regulation of synaptic growth (see Cotman & Iversen, 1987), certain forms of epilepsy (Meldrum, 1985), excitotoxicity (see Rothman & Olney, 1987) and hypoxic/ischaemic brain insults (see Choi, 1988).

In order to study compounds potentially active at the NMDA receptor level, tests based on neurological and behavioural changes have been developed by use of systemic (i.p.) administration of high doses of NMDA (Czuczwar & Meldrum, 1982; Leander *et al.*, 1988). However, the effects induced by such injections were not very reproducible. Lower doses (e.g. 125 mg kg^{-1}) produced only scratching in some mice but tonic seizures and death in others. At higher doses (150, 175 mg kg^{-1}), more animals convulsed and died, but some only exhibited tail biting. As no i.p. dose of NMDA was found consistently to produce clonic convulsions, lethality was selected as the most reliable end-point following an injection of a high dose of NMDA (200 mg kg^{-1}) (Leander *et al.*, 1988). However, even the development of these symptoms was highly variable and the time of occurrence varied from 1 to 15 min. In view of these shortcomings (high dose of NMDA required, inconsistent behavioural effects, variable time course), we were interested in evaluating another *in vivo* method for testing compounds for antagonism of NMDA-induced convulsions. In the present study results were obtained by freehand injection of NMDA into the lateral brain ventricle of conscious mice. A very low dose of NMDA (1 nmol in 1 μl) yielded very reproducible effects which developed in less than 1 min. Such a methodology has already been used for the detection of anticonvulsant properties of two

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κ-opioid agonists and two anticonvulsants, but other known or putative anticonvulsant drugs have not been studied (Vonvoigtlander *et al.*, 1987).

Methods

Animals

Female SPF-Fü albino mice (18–22 g) were used. The animals were housed in groups and had unlimited access to food and water. They were transferred from the colony room to the testing room 2 h before the experiment, which was routinely performed between 09 h 00 min and 15 h 00 min. Groups of eight mice were used per drug dose.

Experimental protocol

Seizures were induced by injection of NMDA (1 nmol in 1 μl) into a lateral ventricle of conscious mice by use of a procedure similar to that described by Clark *et al.* (1968). This dose of NMDA was selected as the minimal dose that reliably induced the appearance of tonic convulsions in at least 90% of treated animals. Immediately after injection, animals were placed in plexiglas boxes (12 × 12 × 15 cm) and were observed for a period of 5 min. Typically, seizure activity consisted of wild running, clonic convulsions, tonic seizures and, in about 20–40% of the animals, death. The episode typically began a few seconds after injection and evolved to its maximal intensity in less than 1 min. The number of mice per group exhibiting tonic convulsions was recorded.

Test drugs were dissolved in phosphate buffer and administered in a volume of 1% body weight. Poorly water soluble drugs were administered as a suspension. For each drug, the route (i.p., p.o. or s.c.) and time-point of injection before NMDA administration were selected as corresponding to its peak activity as previously determined in other behavioural tests, such as prevention of convulsions induced by pentylenetetrazol, maximal electroshock or auditory stimulation.

Data analysis

Data were analysed by means of the Fisher exact probability test (Siegel, 1956). All results are expressed as percentages of control values and the significance level was predetermined at 0.01.

Drugs

The drugs used were: N-methyl-D-aspartate (NMDA, Sigma), MK801 (5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine, Merck, Sharp & Dohme), phencyclidine (Duphar), phenobarbitone (Siegfried), diphenylhydantoin (Fluka), meprobamate (Lederle), sodium valproate (Aldrich), THIP (tetrahydroisoxazolopyridin, Sandoz). All other chemicals were synthesized at F. Hoffmann-La Roche (Basle, Switzerland, or Nutley, U.S.A.).

Results

In all experiments, NMDA-induced tonic seizures appeared to be more sensitive than clonic convulsions to inhibition by the test compounds. This confirms our experience with other convulsants (3-mercaptopropionic acid, pentylenetetrazol) and their prevention by benzodiazepines (Pieri, unpublished observation). Consequently, and for more clarity, only results based on tonic convulsions are presented (with the exception of the experiment shown in Figure 3). For each drug tested vs NMDA, we determined time to peak activity and optimal route of administration in preliminary experiments. Therefore, we felt confident that we were in fact comparing like with like and thus justified in presenting an estimate of the order of potency.

Effects of competitive and non-competitive NMDA antagonists

Prevention of NMDA-induced tonic convulsions by selected competitive and non-competitive NMDA antagonists is represented in Figure 1. The order of potency of these compounds was MK801 > phencyclidine > thienylcyclohexylpiperidine (TCP) = carboxypiperazine-propylphosphonic acid (CPP) > dextrorphan = dextromethorphan > 2-amino-7-phosphonoheptanoic acid (AP7).

Phencyclidine and TCP at the highest doses tested (10 mg kg^{-1}) induced tremors in all animals. MK801 (1–10 mg kg^{-1}) also induced tremors and ataxia, and dextromethorphan (100 mg kg^{-1}) tremors and death.

Effects of benzodiazepine receptor ligands and GABA agonists

Figure 2 shows the inhibition of NMDA-induced tonic convulsions by selective benzodiazepine receptor (BZR) ligands and γ-aminobutyric acid (GABA) agonists, as compared to MK801.

Triazolam and clonazepam were considerably more potent than MK801. Diazepam and Ro 16-6028 (a BZR partial agonist, Martin *et al.*, 1988) were about equipotent and slighly less effective than MK801. Flumazenil (a BZR antagonist, Hunkeler *et*

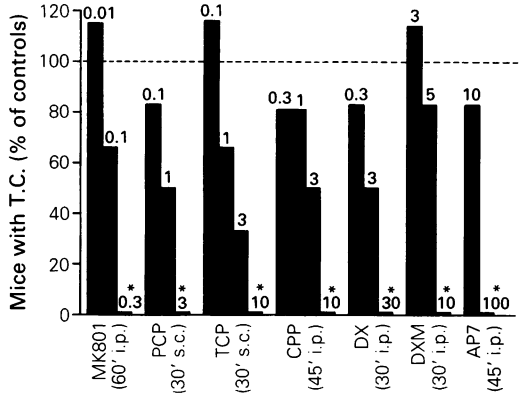

Figure 1 Prevention by competitive and non-competitive N-methyl-D-aspartate (NMDA) antagonists of tonic convulsions (T.C.) induced by i.c.v. NMDA (1 nmol in 1 μl). Results are expressed as % of control values and significant effects ($P < 0.01$) are marked by asterisks. The different routes and times of administration before NMDA injection are shown below the name of each compound and the different doses (in $mg\,kg^{-1}$) used for each drug are shown above the corresponding columns. PCP = phencyclidine; TCP = thienylcyclohexylpiperidine; CPP = carboxypiperazine-propyl-phosphonic acid; DX = dextrorphan; DXM = dextromethorphan; AP7 = 2-amino-7-phosphonoheptanoic acid.

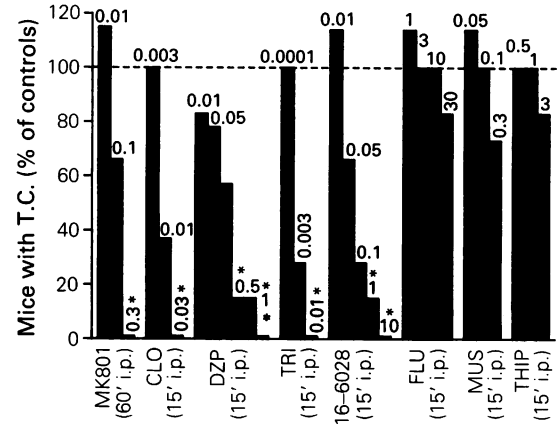

Figure 2 The effects of benzodiazepine receptor ligands and γ-aminobutyric acid (GABA) agonists on tonic convulsions (T.C.) induced by i.c.v. N-methyl-D-aspartate (NMDA, 1 nmol in 1 μl). Results are expressed as % of control values and significant differences ($P < 0.01$) are marked by asterisks. The different times of administration are shown below the name of each compound and the different doses (in $mg\,kg^{-1}$) used for each drug are shown above the corresponding columns. CLO = clonazepam; DZP = diazepam; TRI = triazolam; FLU = flumazenil; MUS = muscimol; THIP = tetrahydroisoxazolopyridin.

al., 1981), and the GABA agonists, muscimol and THIP, were devoid of anticonvulsant activity. The two latter compounds produced pronounced hypomotility and reduced reactivity to the environment at the highest doses tested (1 and 10 $mg\,kg^{-1}$). Flumazenil totally reversed the effects of diazepam but did not reduce those of MK801 (Figure 3).

Effects of non BZR ligands: classical anticonvulsants and a minor tranquilizer

Effects of these compounds are shown in Figure 4. Diphenylhydantoin was the most potent, followed by phenobarbitone, meprobamate and sodium valproate.

Discussion

In the present study we have used intracerebroventricular injection of NMDA in conscious mice as an *in vivo* test for detection of anticonvulsant drugs. This technique offers the following advantages: it is a simple and very rapid method, which requires a minute amount of NMDA (1 nmol in 1 μl) to be injected and produces reliable neurological and behavioural effects which develop in a very short

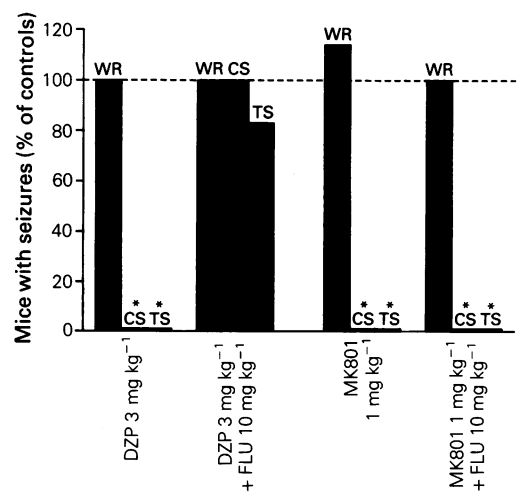

Figure 3 Effects of flumazenil (FLU) on prevention by diazepam (DZP) and MK801 of wild running (WR), clonic (CS) and tonic (TS) seizures induced by i.c.v. N-methyl-D-aspartate (NMDA) (1 nmol in 1 μl). Results are expressed as % of control values and significant differences ($P < 0.01$) are marked by asterisks.

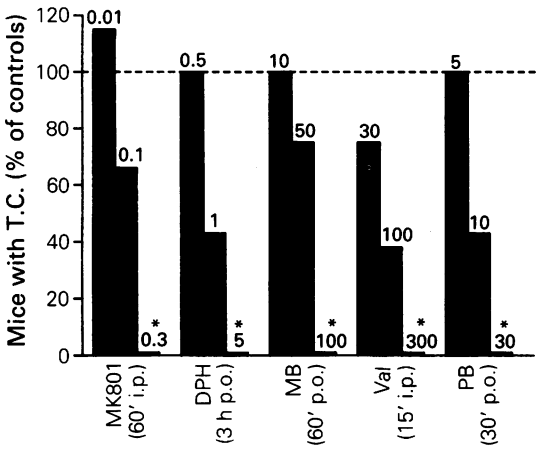

Figure 4 The effect of classical anticonvulsants and minor tranquilizers on tonic convulsions (T.C.) induced by i.c.v. N-methyl-D-aspartate (NMDA, 1 nmol in 1 μl). Results are expressed as % of control values and significant differences ($P < 0.01$) are marked by asterisks. The different routes and times of administration before NMDA injection are shown below the name of each compound and the different doses (in mg kg^{-1}) used for each drug are shown above the corresponding columns. DPH = diphenylhydantoin; MB = meprobamate; Val = sodium valproate ; PB = phenobarbitone.

period of time (wild running, clonic and tonic convulsions usually developing within 10 to 15 s, followed by death in about 20% of animals).

All competitive and non-competitive NMDA antagonists tested were active in this test, together with BZR full or partial agonists (triazolam, diazepam, clonazepam, Ro 16-6028) as well as another minor tranquilizer (meprobamate) and various classical anticonvulsants (diphenylhydantoin, sodium valproate, phenobarbitone). Therefore, this approach seems to be particularly suitable for the early detection of compounds with anticonvulsant activity.

In another study in which the same technique was used, phenytoin and phenobarbitone were found to be ineffective antagonists of the convulsions induced by i.c.v. NMDA (VonVoigtlander *et al.*, 1987). However, it should be noted that in this instance the two drugs were administered by routes and at times before NMDA injection differing from those described in our protocol. We estimated the optimal route and time to peak activity for all drugs tested, and this careful methodology is probably one of the grounds for our positive results with phenytoin and phenobarbitone. Furthermore, this discrepancy could also be explained by the fact that different endpoints were chosen for assessing convulsions (tonic versus clonic convulsions). Indeed, it is well known that diphenylhydantoin, whilst blocking tonic convulsions in experimental animals, is able to unmask the clonic component of convulsions (as in maximal electroconvulsive shocks experiments). It is therefore not surprising that in our experiment (with tonic seizures as end-point), tonic seizures were effectively blocked by phenytoin, whereas in the study of Von-Voigtlander *et al.* (1987) (with clonic convulsions as end-point), the clonic convulsions were not affected.

Previous studies have used intraperitoneal administration of high doses of NMDA to induce convulsions (Czuczwar *et al.*, 1985; Ferkany *et al.*, 1988; Leander *et al.*, 1988). In these studies, benzodiazepine receptor ligands, such as diazepam, and classical anticonvulsants, such as phenobarbitone or diphenylhydantoin, were either totally ineffective or only weakly active. In the course of our study, we repeated these experiments and found results similar to those formed in the literature (data not shown). Thus, it appears that the route of administration (i.p. versus i.c.v.) of NMDA is an important factor in determining the activity of various compounds in this test. In other words, with i.p. NMDA injection, only competitive and non-competitive NMDA antagonists will be detected, whereas i.c.v. NMDA administration allows the detection of a wider range of anticonvulsant compounds. A possible explanation for this unexpected finding might be that the minute amount of i.c.v. NMDA does not diffuse far away from the site of injection, whereas NMDA reaches numerous sites within the CNS after the high systemic dose. Therefore, i.c.v. injection of NMDA is likely to induce primary epileptic activity in a restricted area, which may be more sensitive to NMDA antagonists as well as to compounds acting in a different manner. The prevention of NMDA-induced seizures by benzodiazepine receptor agonists was mediated by the benzodiazepine receptor and not by the NMDA receptor, as shown by the specific blockade with flumazenil (a specific benzodiazepine receptor antagonist).

Our results with dextrorphan are in good agreement with those of Ferkany *et al.* (1988), who found a similar anticonvulsant activity in i.p. NMDA-induced convulsions. Several morphinans have been shown to act as non-competitive NMDA antagonists at the same site as phencyclidine and MK801 (see Kemp *et al.*, 1987).

The observed prevention of tonic convulsions induced by centrally administered NMDA by a variety of compounds with greatly differing mechanisms of anticonvulsant activity was not surprising. Seizure activity, in general, is reduced by agents that antagonise the effect of chemical convulsants at their receptors (in the present model competitive and non-competitive NMDA antagonists), as well as by agents that reduce paroxysmal neuronal activity by inhibiting various cation channels (diphenyl-

hydantoin, phenobarbitone, sodium valproate) or enhancing the synaptic inhibitory action of GABA (benzodiazepine receptor agonists, phenobarbitone, sodium valproate). The mechanism of action of meprobamate is still unknown. No significant protective effect was obtained with $GABA_A$-receptor agonists in a tolerated dose-range, probably because they indiscriminately stimulate $GABA_A$-receptors on excitatory as well as inhibitory neurones.

In summary, the present model of tonic convulsions induced by i.c.v. NMDA is very sensitive to competitive and non-competitive NMDA antagonists and also to various classes of classical anticonvulsants. The model is a convenient test for assessing the *in vivo* activity of agents that interact with the NMDA receptor *in vitro* and it expands the battery of chemically-induced seizure models for the characterization of potential anticonvulsants.

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