ELSEVIER

European Journal of Pharmacology 353 (1998) 191–206

ejp

Evidence for a unique profile of levetiracetam in rodent models of seizures and epilepsy

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Received 4 December 1997; revised 18 May 1998; accepted 26 May 1998

Abstract

The protective and adverse effect potentials of levetiracetam ((*S*)-α-ethyl-2-*oxo*-pyrrolidine acetamide) in rodent models of seizures and epilepsy were compared with the profile of several currently prescribed and newly developed antiepileptic drugs. Levetiracetam was devoid of anticonvulsant activity in the acute maximal electroshock seizure test and in the maximal pentylenetetrazol seizure test in mice (up to 540 mg/kg, i.p.) but exhibited potent protection against generalised epileptic seizures in electrically and pentylenetetrazol-kindled mice (ED_{50} values = 7 and 36 mg/kg, respectively, i.p.). This differs markedly from established and most new antiepileptic drugs which induce significant protection in both the acute seizure tests and the kindling models. Furthermore, levetiracetam was devoid of anticonvulsant activity in several maximal chemoconvulsive seizure tests although an interesting exception was the potent protection observed against secondarily generalised activity from focal seizures induced by pilocarpine in mice (ED_{50} value = 7 mg/kg, i.p.), pilocarpine and kainic acid in rats (minimum active dose = 17 and 54 mg/kg, respectively, i.p.). The protection afforded by levetiracetam on the threshold for methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM)-induced seizures persisted after chronic administration (17–170 mg/kg, i.p., twice daily/14 days) and levetiracetam did not lower the seizure threshold for the proconvulsant action of the inverse benzodiazepine receptor agonist, *N*-methyl-β-carboline-3-carboxamide (FG 7142). The main metabolite of levetiracetam (ucb L057; (*S*)-α-ethyl-2-*oxo*-1-pyrrolidine acetic acid) was found to be inactive in sound-sensitive mice after acute administration of doses up to 548 mg/kg, i.p. Levetiracetam induced only minor behavioural alterations in both normal and amygdala-kindled rats (54–1700 mg/kg, i.p.) resulting in an unusually high safety margin between rotarod impairment and seizure suppression of 148 in corneally kindled mice and 235 in Genetic Absence Epilepsy Rats from Strasbourg. In comparison, existing antiepileptic drugs have ratios between 2 and 17 in the corneally kindled mouse model. These studies reveal a unique profile of levetiracetam in rodent models. Characteristics are a general lack of anticonvulsant activity against maximal, acute seizures and selective protection with a very high safety margin in genetic and kindled animals and against chemoconvulsants producing partial epileptic seizures. This activity differs markedly from that of the established and newly introduced antiepileptic drugs and appears to derive from the parent compound since its major metabolite was inactive in all models studied. Together these results therefore suggest that levetiracetam may offer an effective, broad-spectrum treatment of epileptic seizures in patients, with a minimum of adverse effects.

Keywords: Seizure; Epilepsy; Levetiracetam; Ucb L059; Anticonvulsant; Antiepileptic

1. Introduction

Antiepileptic drug research has for several decades focused on identifying new drug candidates based on their anticonvulsant activity against single acute seizures induced by maximal electrical or chemical stimulation in rodents. The most widely used models have been the maximal electroshock seizure test and the pentylenetetrazol seizure test. All established antiepileptic drugs have anticonvulsant activity in at least one of these two models and the activity profile is assumed to predict clinical efficacy in man (Löscher and Schmidt, 1994). Recently, this approach has been made questionable by experimental observations showing that the new antiepileptic drug candidate, levetiracetam (ucb L059; (*S*)-α-ethyl-2-*oxo*-pyrrolidine acetamide), is devoid of anticonvulsant activity in the acute maximal electroshock seizure and pentylenetetrazol models despite offering potent protection against epileptic seizures in a variety of animal models of chronic epilepsy (Löscher and Hönack, 1993).

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0014-2999/98/$19.00

Levetiracetam was originally discovered by random screening to have potent ability to protect against all phases of seizure activity induced by an acoustic stimulus in sound-sensitive mice (Gower et al., 1992). Results with other genetic animal models of epilepsy revealed protection against postural stimulation-induced seizures in epilepsy-like mice (De Deyn et al., 1992), against seizures induced by acoustic stimulation in sound-sensitive rats and against spontaneous spike-and-wave discharges in rats from the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) (Gower et al., 1995). Testing in kindling models has now also shown marked protection against seizures in fully amygdala kindled rats (Löscher and Hönack, 1993). This potent suppression of seizure activity in genetic and kindled animals contrasts with levetiracetam's lack of anticonvulsant activity in the acute maximal electroshock seizure and pentylenetetrazol models. Indeed, levetiracetam has only been reported to protect against single acute seizures induced by submaximal bolus doses of chemoconvulsants (Gower et al., 1992) and shows only weak anticonvulsant activity in threshold tests involving acute electrical or chemical stimulation (Löscher and Hönack, 1993). These reports suggest that levetiracetam differs from currently known antiepileptic drugs by a relative selective suppression of seizures in animal models of chronic epilepsy.

Again, in contrast to that of all known antiepileptic drugs, the preclinical profile of levetiracetam also reveals a very high safety margin characterised by a remarkable separation of the doses required to induce seizure suppression and those provoking adverse effects. Thus, systemic administration of levetiracetam to normal, non-epileptic rodents is only associated with mild sedation and muscle relaxation appearing at doses 50–100 fold higher than those inducing seizure suppression in genetic and kindled animals (Gower et al., 1992; Löscher and Hönack, 1993). However, epileptogenesis appears to render the brain more susceptible to certain adverse effects of antiepileptic drugs and animals with chronic brain dysfunction associated with epileptogenesis, such as kindled animals, are known to show exacerbated adverse effects with antiepileptic drugs (see Löscher and Hönack, 1991; Hönack and Löscher, 1995) compared to normal animals.

In the present study, we have examined further to what extent levetiracetam affords selective protection in animal models of epilepsy vs. seizure models and compared this with the profile of other known antiepileptic drugs. Seizure suppression observed after chronic administration and the induction of hyperexcitability on cessation of chronic treatment were evaluated in order to assess whether levetiracetam induces tolerance and dependence. Possible seizure protection with the main metabolite (ucb L057; (*S*)-α-ethyl-2-*oxo*-1-pyrrolidine acetic acid) was also studied in order to define the extent to which the protection obtained with levetiracetam derives from the parent compound. Finally, the adverse effect potential of levetiracetam was determined in genetic and kindled animals in order to assess more precisely the extent to which its safety margin may provide a real clinical advantage over existing antiepileptics.

2. Materials and methods

2.1. Animals

Male NMRI mice (IFFA Credo, Belgium) weighing 20–30 g and male Sprague–Dawley rats (IFFA Credo, Belgium) weighing 200–300 g were used in all experiments involving non-genetic models. Male and female genetically sound susceptible mice (Animal Husbandry Unit, UCB, Belgium) aged 5 weeks, weighing 15–25 g were used in the experiments with ucb L057 and flumazenil, respectively. Male Wistar rats of the Genetic Absence Epilepsy Rats from Strasbourg strain (GAERS) aged 6 months, weighing 360–380 g were used in the study comparing levetiracetam, valproate and ethosuximide. The animals were kept on a 12/12-h light/dark cycle with lights on at 0600 h and were housed at a temperature maintained at 20–21°C and at humidity of about 40%. The mice were housed in groups of 10 per cage ($38 \times 26 \times 14$ cm) and the rats, in groups of 8 per cage ($45 \times 42 \times 21$ cm). All animals had free access to standard pellet food and water before random assignment to experimental groups consisting of 10 (mice) or 8 (rats) animals.

2.2. Seizure testing after acute administration

2.2.1. Route of administration and pretreatment time for the compounds

All acute testing was performed after intraperitoneal (i.p.) administration. Depending on their reported selectivity against maximal electroshock or s.c. pentylenetetrazol seizures in mice, an optimal pretreatment time was initially determined in this species for the antiepileptic drugs in one of these tests (data not shown). The optimal pretreatment time for levetiracetam in mice was chosen from pharmacodynamic results obtained in previous studies involving audiogenic seizure-prone and kindled mice except for the shorter pretreatment time used in experiments involving combined administration with flumazenil. Testing with ucb L057 involved two pretreatment times, the first being similar to that for levetiracetam and the other at the time of maximum plasma levels (T_{max}) determined in an associated pharmacokinetic study (data not shown). The pretreatment time for levetiracetam in rats was chosen from previous pharmacodynamic results with this species. The pretreatment times for the other compounds used in rat studies was obtained from reports showing anticonvulsant activity in this species.

2.2.2. Maximal electroshock seizures in mice

Maximal electroshock seizures in mice were induced by a stimulator (WITT IndustrieElektronik, Berlin, Germany) using a current of 50 mA delivered with a pulse frequency of 50 Hz for 0.2 s through corneal electrodes (Matagne and Klitgaard, 1998). Initial testing with these stimulation parameters showed a tonic hindlimb extension in 100% of the vehicle-treated animals ($n = 10$) from a current of 14 mA. The mice were observed for 10 s and the incidence of tonic hindlimb extension was noted.

2.2.3. Chemoconvulsive seizure models in mice

Initial experiments identified dose–effect curves for all bolus-administered chemoconvulsants in vehicle-treated mice ($n = 10$) and from these a convulsive dose inducing clonic convulsions of all four extremities in 97% (CD_{97}) of the animals was identified. The following chemoconvulsants were included in this study: pentylenetetrazol (89 mg/kg, s.c.), bicuculline (3 mg/kg, s.c.), picrotoxin (4.3 mg/kg, s.c.), methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) (15 mg/kg, i.p.), 3-mercapto-propionic acid (31.5 mg/kg, i.p.), pilocarpine (373 mg/kg, i.p.), 4-aminopyridine (10.2 mg/kg, s.c.) and caffeine (283 mg/kg, i.p.). In these tests, the mice were placed individually in Perspex cages and observed for the presence of clonic convulsions in all four extremities for 60 min following administration of the chemoconvulsant. A 30-min observation time was used for DMCM and 3-mercapto-propionic acid convulsions.

Selective ionotropic excitatory amino acid receptor agonists were microperfused into the lateral brain ventricle of unrestrained mice with a speed of infusion of 5 μl/min (Basic Microprocessor Syringe Pump, Harvard Apparatus), according to the method of Steppuhn and Turski (1993). Briefly, a cannula was inserted through the skull of the animal in a position 1 mm posterior and 1 mm lateral to the bregma. Placement and depth of the cannula were fixed by a plate positioned 4.3 mm from the end of the cannula. *N*-methyl-D-aspartate (NMDA) (1 nmol/min), kainic acid (3 nmol/min) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) (3 nmol/min) were infused at a concentration inducing clonic convulsions in 100% of saline-treated animals not later than 60 s after the initiation of infusion. The maximum infusion time was 150 s.

2.2.4. Pilocarpine-induced seizures in rats

The rats were pretreated with methylscopolamine (1 mg/kg, s.c.), 30 min before administration of pilocarpine. The dose of pilocarpine selected (375 mg/kg, i.p.) was a CD_{97} dose for the induction of secondarily generalised seizures (stage 3, 4 and 5 seizures, see below) in 97% of the animals. After administration of pilocarpine, the rats were observed over 120 min and the convulsive behaviour was scored according to Racine (1972) where 0 = no reaction; 1 = stereotype mouthing, eye blinking and/or mild facial clonus; 2 = head nodding and/or severe facial clonus; 3 = myoclonic jerks in the forelimbs; 4 = clonic convulsions in the forelimbs with rearing and 5 = generalised clonic convulsions associated with loss of balance.

2.2.5. Kainic acid-induced seizures in rats

The rats were given kainic acid (13.2 mg/kg, s.c.) at a CD_{97} dose for the induction of stage 4 seizures (see below). After administration of kainic acid the rats were observed for 180 min and the convulsive behaviour was classified using a modified Racine scale (Racine, 1972) where 3 = slight rearing with forelimbs clonus; 4 = rearing, back fully extended and severe forelimb clonus and 5 = rearing, severe forelimb clonus and falling backwards.

2.2.6. Genetic models in mice and rats

Sound-susceptible mice were first subjected to a preselection test and only mice in which a tonic convulsion was provoked by an acoustic stimulus were retained. Audiogenic seizures were induced the following day by a 90-dB, 10 to 20-kHz acoustic stimulus for 30 s and the incidence of wild running, clonic and tonic convulsions was recorded.

Rats from the GAERS strain, bred at the Centre de Neurochimie, INSERM, Strasbourg, were anesthetized with sodium pentobarbital (40 mg/kg, i.p. adm.) and implanted with four platinum electrodes into the left and right frontal cortex and left and right occipital cortex for recording of the electroencephalogram (EEG). Cortical, spontaneous spike-and-wave discharges were recorded bilaterally from the rats placed in individual Plexiglass boxes. The rats were continuously observed and prevented from falling asleep by gently sensory stimulation. After a 20-min habituation period, the rats were injected with either saline or a drug, and the electroencephalogram recorded continuously over consecutive 20-min intervals up to 120 min. The cumulative duration of the spike-and-wave discharges was calculated.

2.2.7. Kindling models in mice

Corneal kindling in mice was produced according to the method recently described (Matagne and Klitgaard, 1998). Kindling was induced by twice daily stimulations for 12 days with a current intensity of 3 mA for a duration of 3 s. Before each stimulation with corneal electrodes, a drop of saline containing Unicaine (0.4%) was placed on the eyes to ensure good conductivity and to induce slight anaesthesia. After 2 days without stimulation, the animals were re-stimulated twice daily and defined as kindled after the appearance of four consecutive generalised seizures (clonic convulsions in the forelimbs with or without rearing, falling and loss of balance). The kindled animals were pretreated with saline in the morning, then stimulated and observed for convulsive behaviour. The same procedure was repeated in the afternoon after pretreatment with a compound. The convulsive behaviour was observed for 1 min after stimulation with the mice placed in a plastic cylinder

and was scored according to a slightly modified Racine scale (Racine, 1972). The generalized seizure as described above was used as the end point.

Pentylenetetrazol kindling in mice was induced by daily s.c. administration of 55 mg/kg of pentylenetetrazol, 5 days per week for 2 weeks. After the appearance of three consecutive generalised seizures during the second week (clonic convulsions in the forelimbs with or without rearing, falling and loss of balance) and a generalised seizure 2 days after the last injection, the mice were defined as kindled. The kindled animals were pretreated with saline, injected with pentylenetetrazol and observed for convulsive behaviour. The same procedure was repeated the following day after pretreatment with a compound. The convulsive behaviour was observed for 30 min after administration of pentylenetetrazol with the mice placed individually in Perspex cages. A generalised seizure scored as described above was used as the end point.

2.2.8. Amygdala kindling in rats

The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p. adm.) and implanted with a bipolar stimulation/recording electrode into the right basolateral amygdala with the following coordinates: AP-2.3, L-4.8, V-8.5 (Paxinos and Watson, 1982). The electrode consisted of two twisted Teflon-coated stainless steel wires. An electrode in the left occipital cortex served as the indifferent reference electrode. Bipolar, reference 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 a postoperative period of at least 2 weeks, the rats were stimulated once daily, 5 days per week, with 500 μA monophasic square wave pulses, 50 Hz for 1 s (Löscher et al., 1986). Kindling was defined as the appearance of at least 10 consecutive stage 4 or 5 seizures according to the scale of Racine (Racine, 1972).

2.3. Seizure testing after chronic administration

2.3.1. Threshold for DMCM-induced seizures in mice

The mice were injected i.p. twice daily for 14 days with saline or different doses of a drug. The doses of the different drugs used were identified in a series of initial experiments ($n = 15$) as the dose required to elevate the threshold for DMCM-induced clonic convulsions in all four extremities to a similar extent. The seizure test was performed 60 min after i.p. administration of saline or a compound on day 15. The threshold for the induction of clonic convulsions in all four extremities was determined by infusing a concentration of 0.5 mg/ml DMCM into the tail vein of unrestrained freely moving mice at a rate of 0.25 ml/min with an infusion pump (Basic Microprocessor Syringe Pump, Harvard Apparatus) and a maximum duration of infusion of 2 min.

2.3.2. Proconvulsant activity induced by the inverse benzodiazepine receptor agonist, FG 7142, in mice

The mice were injected i.p. twice daily for 14 days with saline or different doses of a drug. The doses used were identified in a series of preliminary experiments ($n = 15$) as the dose required to elevate the threshold for DMCM-induced clonic convulsions in all four extremities to a similar extent. Two days later, the mice were given FG 7142 (40 mg/kg, i.p.) and placed individually in Perspex cages where they were observed for 30 min for the occurrence of clonic convulsions in all four extremities.

2.4. Adverse effects

2.4.1. Behavioural alterations in rats

Antiepileptic drug-induced effects on several behavioural parameters were evaluated qualitatively in non-implanted, sex-, age- and weight-matched rats and in amygdala-kindled rats. Particular attention was paid to potential psychomimetic, sedative and ataxic properties of the drugs. One group of amygdala-kindled rats was observed together with a group of non-kindled rats in all experiments by the same experimenter in order to allow a direct comparison between the different antiepileptic drugs-induced behavioural alterations in normal and kindled animals. The doses of antiepileptic drugs were selected on the basis of the protection obtained against secondarily generalised seizures induced by pilocarpine or against fully amygdala-kindled seizures. The behavioural parameters were selected from the Irwin test for rats (Irwin, 1968) and from a similar study recently reported by Hönack and Löscher (1995).

Wet-dog shakes and stereotyped behaviours, such as head weaving, circling, reciprocal forepaw treading and stereotyped sniffing, licking, grooming and gnawing were scored using a ranked intensity scale on which 0 = absent, 1 = intermittent, 2 = continuous and 3 = intense. Spontaneous activity, body, abdominal and limb tone, grip strength, impaired gait, Straub tail, piloerection and tremors were scored according to the rating scale of the Irwin procedure. The behaviour was graded by a well-trained experimenter according to the intensity of the alterations observed from 0 (normal) to 8 (intense). For some parameters such as spontaneous activity and abdominal tone, the normal value is 4 with scoring from 4 to 0 for a decrease and from 4 to 8 for an increase, with 0 and 8 meaning a marked alteration in this behaviour. All these parameters were determined at 15, 30, 60, 120 and 180 min after dosing. Baseline evaluation of the behaviour of each rat was done before each drug injection and demonstrated that none of the selected behavioural parameters was affected in kindled and non-kindled animals in the absence of drugs. Ataxia was also evaluated at 30, 60 and 120 min after dosing by placing the rats for a 1-min period in an open-field cage ($100 \times 100 \times 50$ cm) and scored during

this period according to a published scale (Hönack and Löscher, 1995), where 1 = slight ataxia in hindlimbs; 2 = more pronounced ataxia with dragging of hindlimbs; 3 = further increase of ataxia and more pronounced dragging of hindlimbs; 4 = marked ataxia, animals lose balance during forward locomotion; 5 = very marked ataxia with frequent loss of balance during forward locomotion; 6 = permanent loss of righting reflex but animals still attempt to move forward. To allow a comparative evaluation of drug-induced effects between kindled and non-kindled animals, the scores of all behavioural parameters were added together for the different periods of observations.

2.4.2. Rotarod performance of mice

Adverse effects on motor function were assessed in corneally kindled mice in a rotarod test (Ugo Basile) using a rod with a diameter of 3 cm rotating at a constant speed of 6 rpm. The mice were pretrained and only animals able to remain on the rod for at least 60 s in three consecutive trials were retained. The following day the mice were pretreated with saline or a compound and the number of animals per group unable to remain on the rod for at least 60 s was recorded.

2.4.3. Rotarod performance of rats

Adverse effects on motor function were assessed in normal, amygdala-kindled and GAERS rats in a rotarod test (Ugo Basile) using a rod with a diameter of either 10 or 6 cm (GAERS) rotating at a constant speed of 6 rpm. The rats were pretrained and only animals able to remain on the rod for at least 60 s in three consecutive trials were retained. The following day, the rats were pretreated with saline or a compound and the number of animals unable to remain on the rod after two subsequent 1-min attempts was recorded.

2.5. Drugs

Levetiracetam (ucb L059), its main metabolite, ucb L057, and gabapentin were synthesized in the chemical laboratories of UCB. Pentylenetetrazol (Acros Organics), picrotoxin (Janssen Chimica), pilocarpine hydrochloride, 4-aminopyridine, 3-mercapto-propionic acid, *N*-methyl-D-aspartate (NMDA), kainic acid, scopolamine, sodium valproate and ethosuximide (all Sigma), methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), α-amino-3-hydroxy-5-methylisoxazole-4-propionate hydrobromide (AMPA) and (5*S*,10*R*)-(-)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801) (all RBI), caffeine (Bios), sodium phenobarbital (Federa) and vigabatrin (Hoechst Marion Merrell Dow) were all dissolved in 0.9% saline. Bicuculline (Sigma) was dissolved in glacial acetic acid in 0.9% saline. Carbamazepine (Sigma), phenytoin (Bios), clonazepam, diazepam and flumazenil (all Hoffman-La Roche), *N*-methyl-β-carboline-3-carboxamide (FG 7142, RBI), lamotrigine (Glaxo Wellcome) and topiramate (R.W. Johnson Pharmaceutical Research Institute) were all suspended in 0.9% saline containing 0.1% Tween 80.

2.6. Statistical analysis

An effective dose protecting 50% of the animals (ED_{50}) against the convulsive end point and a toxic dose impairing the rotarod performance of 50% of the animals (TD_{50}) and their associated confidence intervals were calculated using a log-probit analysis. A Fischer test was applied for determining a minor protective effect, defined as a dose inducing protection in a significant proportion of the animals against the appearance of the convulsive end point parameter. A Wilcoxon signed ranked test for paired replicates was applied for determining the minimum active dose capable of suppressing the spike-and-wave discharges in rats of the GAERS strain. A Kruskal–Wallis one-way analysis of variance by ranks followed by multiple comparisons between treatments (Siegel and Castellan, 1988) was applied for determining the significance of differences between treated and control groups in the threshold for DMCM-induced clonic convulsions in mice and in the seizure severity induced by pilocarpine in rats. These data are presented as means ± S.E.M. The statistical significance of differences between kindled and non-kindled behavioural scores was determined using a Mann–Whitney *U*-test. In all experiments, differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Maximal electroshock seizures and s.c. pentylenetetrazol-induced seizures *vs.* corneal electroshock and pentylenetetrazol-kindled seizures in mice

Pretreatment with levetiracetam was completely devoid of any anticonvulsant effect against tonic hindlimb extension induced by a supramaximal (50 mA) current in the maximal electroshock seizure test and against clonic convulsions induced by a maximal (CD_{97}) dose of s.c. administered pentylenetetrazol (ED_{50} values > 540 mg/kg, i.p.). In contrast, potent protection appeared against both corneal electroshock and pentylenetetrazol-kindled seizures (ED_{50} values = 7 and 36 mg/kg, i.p.) (Table 1). This selective effect of levetiracetam in the kindling models and the absence of effect in the seizure tests were distinct from the anticonvulsant profiles of the other antiepileptic drugs. These drugs all generated an anticonvulsive ED_{50} value, or protected a significant proportion of the animals tested (vigabatrin) in at least one of the seizure tests and nearly all of the agents had an equipotent action in the seizure tests and the kindling models (Table 1).

Table 1
Supramaximal electroshock (MES) and maximal s.c. pentylenetetrazol (PTZ) seizures vs. corneal electroshock and pentylenetetrazol kindled seizures in mice: effect of levetiracetam, established and new antiepileptic drugs

Antiepileptic drugs	MES	s.c. PTZ	Electroshock kindling	PTZ kindling
	ED_{50} (mg/kg, i.p.)			
Levetiracetam	> 540	> 540	7 (2–10)	36 (15–96)
Valproate	188 (154–216)	106 (64–170)	66 (52–83)	147 (116–189)
Phenobarbital	12 (9–16)	11 (7–18)	12 (8–17)	5 (3–7)
Clonazepam	> 3	0.02 (0.01–0.04)	0.03 (0.02–0.05)	0.03 (0.02–0.04)
Ethosuximide	> 452	126 (78–196)	> 254	117 (99–161)
Carbamazepine	6 (5–9)	> 42	6 (4–10)	17 (8–28)
Phenytoin	6 (4–10)	> 45[a]	6 (1–16)	38 (22–171)
Vigabatrin	> 2325	> 2325[a]	210[b] (84–5958)	291 (88–541)
Lamotrigine	3 (2–4)	> 82	4 (2–7)	> 82
Gabapentin	90 (63–132)	> 665	55 (30–95)	> 665
Topiramate	12 (8–21)	> 340	> 109	> 340

Maximal electroshock seizures were induced by a supramaximal current of 50 mA for 0.2 s using corneal electrodes and the protective effect against tonic hindlimb extension was assessed ($n = 10$). Maximal pentylenetetrazol seizures were induced by s.c. administration of pentylenetetrazol at a dose of 89 mg/kg, i.e., a dose inducing clonic convulsions in 97% of saline-treated animals (CD_{97}) and the protective effect against clonic convulsions in all four extremities was assessed ($n = 10$). Corneal electroshock kindling and pentylenetretazol kindling were induced by a current of 3 mA for 3 s and 55 mg/kg s.c. of pentylenetetrazol, respectively. The protective effect against generalised kindled seizures (clonic convulsions in the forelimbs, i.e., a stage 3 seizure) was assessed in both models ($n = 8–11$). Values given are ED_{50} values, i.e., the doses protecting 50% of the animals, with associated 95% confidence intervals. All compounds were administered at their optimal pretreatment times, i.e., 15 min for valproate and carbamazepine, 30 min for ethosuximide, 45 min for phenobarbital, 60 min for levetiracetam, clonazepam, lamotrigine and topiramate, 120 min for phenytoin and gabapentin and 18 h for vigabatrin.
[a] Protection against clonic convulsions in a significant proportion of the animals ($P < 0.05$).
[b] Truncated, non-linear dose–response relationship with reduction of antiepileptic activity at 1292 mg/kg.

3.2. Seizures induced by ionotropic excitatory amino acid receptor agonists in mice

Levetiracetam did not protect (ED_{50} value > 540 mg/kg, i.p.) against clonic convulsions induced by i.c.v. infusion of a maximal (CD_{97}) concentration of NMDA, kainic acid and AMPA whereas most of the other antiepileptic drugs were active in all three seizure tests (Table 2). The exceptions were vigabatrin in the NMDA and AMPA seizure models and ethosuximide in the NMDA seizure model.

3.3. Seizures induced by chemoconvulsants modulating the γ-aminobutyric acid (GABA) / benzodiazepine receptor and by pilocarpine, 4-aminopyridine and caffeine in mice

When tested against different chemoconvulsants, administered in maximal (CD_{97}) bolus doses (s.c. or i.p.) for the induction of clonic convulsions, levetiracetam showed a lack of anticonvulsant activity (ED_{50} value > 540 mg/kg, i.p.) in the 3-mercaptopropionic acid, bicuculline, picrotoxin, 4-aminopyridine and caffeine seizure tests (Table 3). However, minor protection appeared in the DMCM seizure test and potent protection in the pilocarpine seizure test. This profile differs from those of the other antiepileptic drugs which either showed general protection in these seizure tests (clonazepam, phenobarbital, valproate), a selective effect against chemoconvulsants modulating the GABA/benzodiazepine receptor complex (ethosuximide); pilocarpine seizures (gabapentin); 3-mercapto-propionic acid and 4-aminopyridine seizures (carbamazepine) or a general lack of effect (vigabatrin, phenytoin and lamotrigine).

3.4. Pilocarpine-induced seizures in rats

Levetiracetam (17 mg/kg, i.p.) significantly reduced the severity of pilocarpine-induced seizures in rats, from a mean score of 4 in the saline-treated group down to a mean score of 2.3. The minimum active dose of levetiracetam inducing significant protection against the secondarily generalised seizures (stage ≥ 3 seizures) was 17 mg/kg (Table 4).

Table 2
Effect of levetiracetam, established and new antiepileptic drugs against clonic convulsions induced by i.c.v. infusion of excitatory amino acid agonists in mice

Antiepileptic drugs	NMDA	Kainic acid	AMPA
	ED_{50} (mg/kg, i.p.)		
Levetiracetam	> 540	> 540[a]	> 540
Valproate	126 (79–165)	149 (116–187)	214 (165–317)
Phenobarbital	9 (6–12)	4 (1–7)	13 (8–19)
Clonazepam	0.09 (0.06–0.13)	0.05 (0.03–0.10)	0.04 (0.01–0.10)
Ethosuximide	> 452[a]	288 (224–387)	303 (219–449)
Carbamazepine	21 (14–38)	8 (5–12)	13 (8–37)
Phenytoin	29 (18–74)	7 (4–12)	10 (2–15)
Vigabatrin	> 2325	1588	> 2325[a]
Lamotrigine	5 (3–10)	4 (2–5)	12 (8–15)
Gabapentin	251 (149–475)	253 (61–596)	312 (159–693)

Intracerebroventricular microinfusion of subtype-specific ionotropic excitatory amino acid receptor agonists was performed into the lateral brain ventricle of unrestrained mice at a rate of 5 μl/min. NMDA (1 nmol), kainic acid (3 nmol) and AMPA (3 nmol) were infused at a concentration that induced clonic convulsions in all saline-treated animals within 1 min. The protective effect against clonic convulsions in all four extremities was assessed ($n = 8–11$). Values given are ED_{50} values, i.e., the doses protecting 50% of the animals, with associated 95% confidence intervals. All compounds were administered at their optimal pretreatment times (see Table 1).
[a]Protection against clonic convulsions in a significant proportion of the animals ($P < 0.05$).

All reference antiepileptics known to interact with the $GABA_A$/benzodiazepine receptor complex (clonazepam, phenobarbital, valproate and vigabatrin) reduced the severity of these seizures and produced significant protection against the expression of the secondarily generalised seizures (Table 4). In contrast, lamotrigine (up to 30 mg/kg, i.p.), carbamazepine (up to 50 mg/kg, i.p.) and phenytoin (up to 200 mg/kg, i.p.) were inactive and phenytoin even increased both the severity of the seizures and the lethality induced by pilocarpine at high doses (100 and 200 mg/kg, i.p.).

Only few antiepileptics (phenobarbital, valproate and vigabatrin) were active against stage 2 seizures and none protected against stage 1 seizures. However, the cholinergic receptor antagonist, scopolamine, dose dependently inhibited these seizures.

3.5. Kainic acid-induced seizures in rats

Levetiracetam dose dependently reduced the severity of kainic acid-induced seizures with significant protection appearing at a dose of 54 mg/kg (i.p.).

Among the reference antiepileptics, only clonazepam and phenobarbital reduced the severity of these seizures and protected a significant proportion of the rats against the expression of stage 4 and 5 seizures at 1.0 and 12.5 mg/kg (i.p.), respectively (Table 4). In contrast, valproate (up to 600 mg/kg, i.p.), vigabatrin (up to 1200 mg/kg i.p.), carbamazepine (up to 75 mg/kg, i.p.) and phenytoin (up to 400 mg/kg, i.p.) were inactive and phenytoin even increased the severity of the seizures at the highest dose tested (400 mg/kg, i.p.).

3.6. Effect of the benzodiazepine receptor antagonist, flumazenil, on the protective effects of diazepam and levetiracetam against audiogenic seizures in mice

The ability of diazepam (0.5 mg/kg, i.p.) to protect against clonic convulsions in audiogenic-susceptible mice was abolished by co-administration of flumazenil from a dose of 0.5 mg/kg (p.o.) (Fig. 1a). In contrast, even a dose of 1 mg/kg (p.o.) of flumazenil was without any effect on the protection afforded by levetiracetam (5.4–17 mg/kg, i.p.) (Fig. 1b).

3.7. Threshold for clonic convulsions induced by the inverse benzodiazepine receptor agonist, DMCM, in mice

The ability of diazepam (5 and 10 mg/kg, i.p.) to significantly elevate the threshold for clonic convulsions induced by i.v. infusion of DMCM disappeared after chronic administration of the same doses twice daily for 14 days (Fig. 2a). When compared to its effect in the control group, levetiracetam did not show a significant elevation in the threshold for clonic convulsions after its chronic administration twice daily of 17 mg/kg (i.p.) (Fig. 2b). In contrast, both 54 and 170 mg/kg remained equipotent after both acute and chronic administration (Fig. 2b).

3.8. Proconvulsant activity induced by the inverse benzodiazepine receptor agonist, FG 7142, in mice

Administration of FG 7142 to mice in a dose only inducing seizures during withdrawal from benzodiazepines (40 mg/kg, i.p.) 2 days after 14 days twice daily administration of saline did not induce any seizure activity whereas FG 7142 induced a significant incidence of clonic convulsions in mice when saline was replaced by diazepam (5–20 mg/kg, i.p.) (Fig. 3a). In contrast, FG 7142 did not induce seizure activity following chronic treatment with levetiracetam (17–170 mg/kg, i.p.) (Fig. 3b).

3.9. Effect of the metabolite, ucb L057, against audiogenic, pilocarpine and corneally kindled seizures in mice

The main acid metabolite of levetiracetam, ucb L057, did not protect in mice up to a dose of 548 mg/kg (i.p.)

Table 3
Effect of levetiracetam, established and new antiepileptic drugs against clonic convulsions induced by chemoconvulsants modulating the $GABA_A$/benzodiazepine receptor complex and by pilocarpine, 4-aminopyridine and caffeine in mice

Antiepileptic drugs	Bicuculline	Picrotoxin	3-MPA	DMCM	Pilocarpine	4-AP	Caffeine
	ED_{50} (mg/kg, i.p.)						
Levetiracetam	> 540	> 540	> 540	97 (32–609)	7 (3–12)	> 540	> 540
Valproate	255 (92–349)	175 (95–396)	88 (54–183)	145 (118–183)	247 (183–324)	180 (107–255)	223 (134–322)
Phenobarbital	10 (4–20)	14 (8–20)	14 (11–19)	11 (7–19)	14 (8–22)	20 (14–30)	58
Clonazepam	0.2 (0.1–0.4)	0.06 (0.04–0.10)	0.03 (0.02–0.05)	0.7 (0.3–1.2)	0.02 (0.01–0.08)	> 3[a]	0.7 (0.4–1.5)
Ethosuximide	324 (230–453)	299 (233–394)	> 452	223 (154–308)	> 452	> 452	> 452
Carbamazepine	> 42	> 42	17 (15–19)	> 42	> 42	27 (16–75)	> 42
Phenytoin	> 45	> 45	> 45[a]	> 45	> 45	> 45[a]	> 45
Vigabatrin	> 2325	> 2325[a]	Not tested	> 2325	> 2325	> 2325	> 2325
Lamotrigine	> 46	> 46	> 46	> 46	> 46	> 46	> 46
Gabapentin	> 665	> 665	> 665	> 665	228 (117–574)	> 665	> 665

All chemoconvulsants were administered at a dose inducing clonic convulsions in 97% of saline-treated animals (CD_{97}). The s.c. CD_{97} doses of bicuculline, picrotoxin and 4-aminopyridine (4-AP) were 3, 4.3 and 10.2 mg/kg, respectively and the i.p. CD_{97} doses of DMCM, 3-mercapto-propionic acid (3-MPA), pilocarpine and caffeine were 15, 31.5, 373 and 283 mg/kg, respectively. The protective effect against clonic convulsions in all four extremities was assessed ($n = 10$). Values given are ED_{50} values, i.e., the doses protecting 50% of the animals, with associated 95% confidence intervals. All compounds were administered at their optimal pretreatment times (see Table 1).
[a] Protection against clonic convulsions in a significant proportion of the animals ($P < 0.05$).

when tested after a pretreatment time similar to that for levetiracetam, or at T_{max} in the audiogenic, pilocarpine or corneally kindled seizure models. This was in contrast to the potent protection afforded by levetiracetam in the same models.

Table 4
Effect of levetiracetam, established and new antiepileptic drugs against pilocarpine- and kainic acid-induced seizures in rats

Antiepileptic drugs	Pilocarpine	Kainic acid
	Minimum active dose (mg/kg, i.p.)	
Levetiracetam	17	54
Valproate	300	> 600
Phenobarbital	5	12.5
Clonazepam	0.5	1.0
Carbamazepine	> 50	> 75
Phenytoin	> 200	> 400
Vigabatrin	800	> 1200
Lamotrigine	> 30	Not tested
Scopolamine	1	Not tested

Pilocarpine was administered i.p. at a dose of 375 mg/kg, i.e., a dose inducing clonic convulsions in the forelimbs (i.e., a stage 3 seizure) in 97% of saline-treated animals (CD_{97}). Kainic acid was administered s.c. at a dose of 13.2 mg/kg, i.e., a dose inducing severe forelimb clonus with rearing on hindlimbs, back fully extended (i.e., a stage 4 seizure) in 97% of saline-treated animals (CD_{97}). The protective effect against secondary generalised motor seizures was assessed ($n = 8$). Values given are minimum active doses, i.e., the doses inducing protection in a significant proportion of the animals ($P < 0.05$). All compounds were administered at their optimal pretreatment times, i.e., 15 min for valproate, 30 min for clonazepam, phenobarbital and scopolamine, 60 min for levetiracetam, carbamazepine, phenytoin and lamotrigine and 24 h for vigabatrin.

3.10. Adverse effects on behavioural parameters in normal and amygdala-kindled rats

Levetiracetam induced similar behavioural alterations in non-kindled and kindled animals. These alterations consisted in a minor reduction in spontaneous activity and abdominal tone only at the highest dose tested (1700 mg/kg, i.p.) (Table 5). A slight decrease in grip strength was also observed at high doses in some animals but ataxia was not observed at any dose level (54–1700 mg/kg). Head weaving and other stereotypic behaviours were not observed at any dose of levetiracetam but occasionally wet-dog shakes were observed at the highest dose (1700 mg/kg) in both kindled and non-kindled rats but the intensity was low.

With the classical antiepileptics, the adverse effects on behavioural parameters were in general somewhat more marked in both normal and kindled rats compared to levetiracetam (Table 5). Valproate (100 mg/kg, i.p.) and clonazepam (0.1–3 mg/kg, i.p.) induced a greater reduction in spontaneous activity in kindled than in non-kindled animals. Valproate (300 mg/kg, i.p.) also induced more marked ataxia and wet-dog shakes in the kindled rats. MK-801 (1.0 mg/kg, i.p.) reduced spontaneous activity more and induced marked ataxia (0.3 mg/kg, i.p.) and

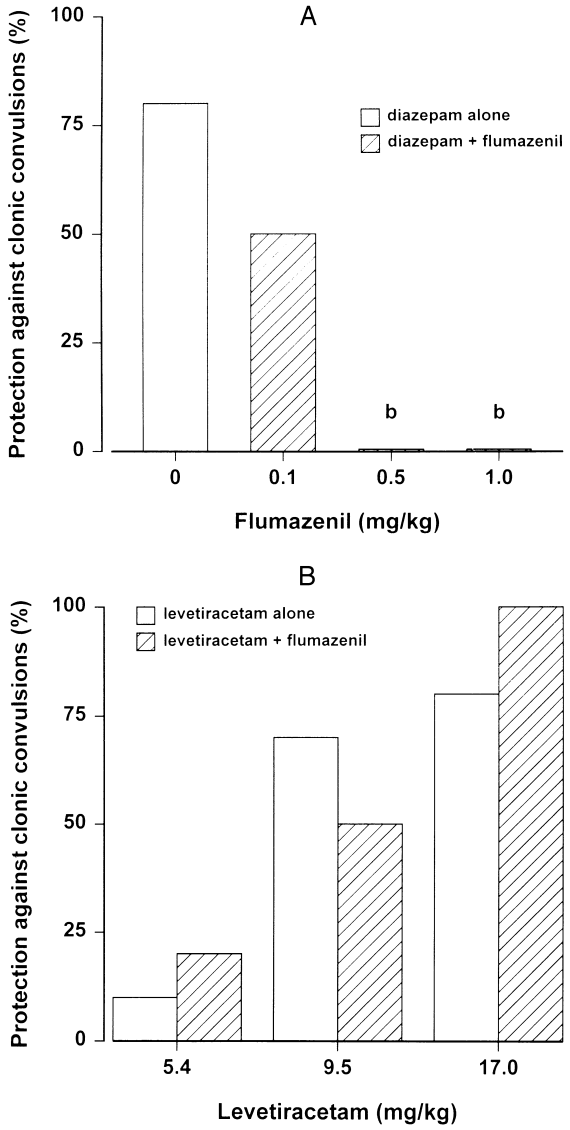

Fig. 1. Effect of flumazenil on the protection afforded by diazepam and levetiracetam against clonic convulsions in audiogenic-susceptible mice. Audiogenic seizures were induced by a 90 dB, 10–20 kHz, acoustic stimulus for 30 s and a protective effect against clonic convulsions was assessed ($n = 10$ animals per group). Diazepam was administered at a dose of 0.5 mg/kg (i.p. adm., −30 min) alone or in combination with different doses of flumazenil (p.o. adm., −15 min). (b) Indicates a statistically significant difference ($P < 0.01$) compared to diazepam alone (1a). Levetiracetam was administered at different doses (i.p. adm., −30 min) alone or in combination with flumazenil at a dose of 1 mg/kg (p.o. adm., −15 min) (1b).

head weaving (0.1 mg/kg, i.p.) at lower doses in kindled than in non-kindled animals. Generally levetiracetam and the other antiepileptic drugs had similar effects on abdominal tone in kindled and non-kindled animals.

3.11. Impairment of rotarod performance in normal and amygdala-kindled rats

Levetiracetam, the established antiepileptic drugs and MK-801 all had similar effects upon rotarod performance in normal and amygdala-kindled rats but levetiracetam, in contrast to the other antiepileptic drugs, impaired performance only at extremely high doses (Table 6).

3.12. Ratio between rotarod impairment and protection against corneally kindled seizures in mice

The safety margin between impairment of rotarod performance and protection against corneally kindled seizures was defined as the ratio between the TD_{50} value, determined in the rotarod test in fully corneally kindled mice and the protective ED_{50} value obtained against generalized seizures in the same animals. The TD_{50} values for leve-

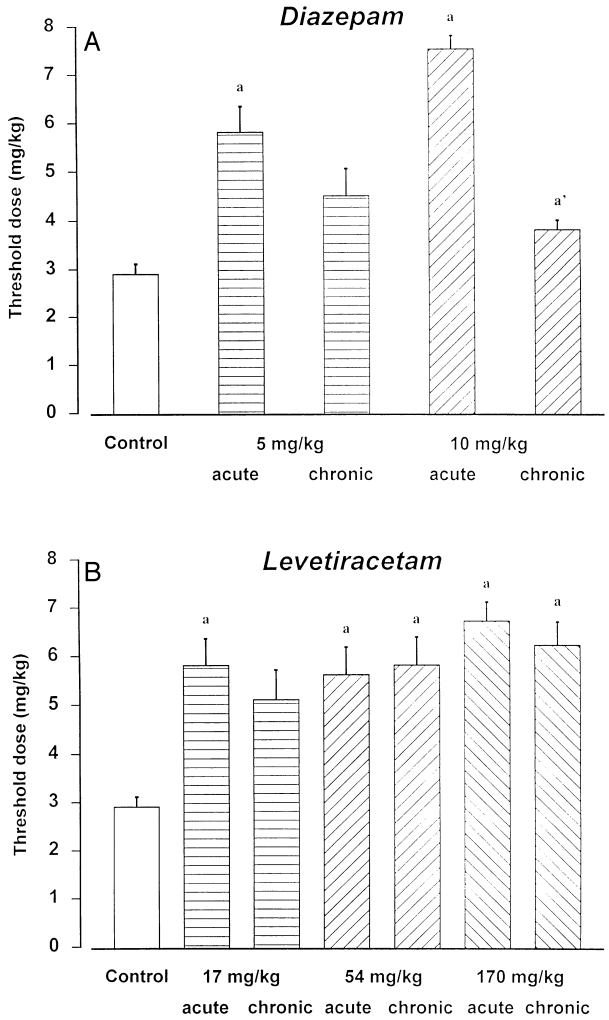

Fig. 2. Effect of chronic administration on the ability of diazepam and levetiracetam to elevate the threshold for DMCM-induced clonic convulsions in mice. Diazepam (2a) and levetiracetam (2b) were administered i.p. twice daily for 14 days and i.p. 60 min before testing on day 15 (chronic treatment). Vehicle was administered i.p. twice daily for 14 days and diazepam and levetiracetam were administered i.p. 60 min before testing on day 15 (acute treatment). DMCM (0.5 mg/ml) was infused into the tail vein of unrestrained freely moving mice at a rate of 0.25 ml/min. Values given are the mean DMCM threshold doses (±S.E.M.) inducing clonic convulsions in all four extremities ($n = 11–16$). (a and a') Indicate a statistically significant difference ($P < 0.05$) vs. control group and acutely treated group, respectively.

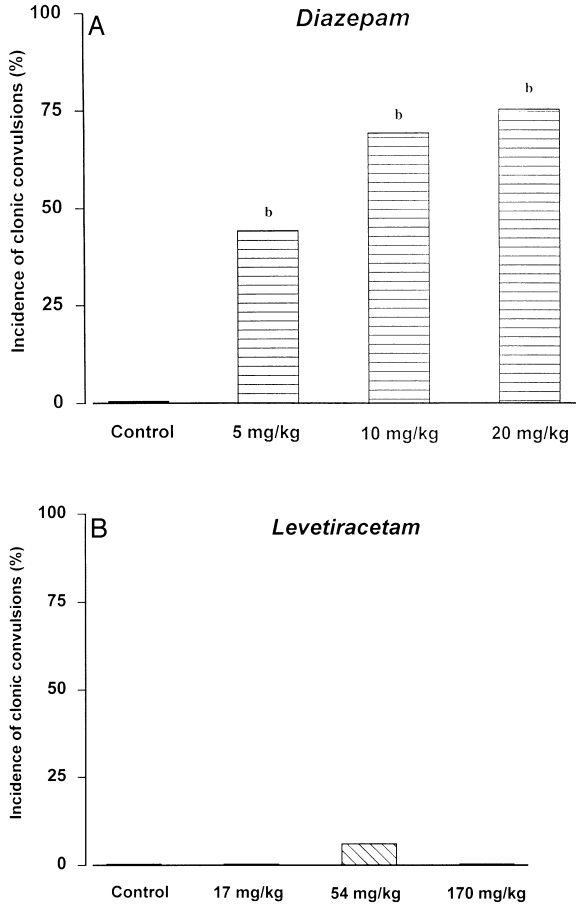

Fig. 3. Effect of chronic administration with diazepam and levetiracetam on the proconvulsant activity of FG 7142 in mice. Diazepam (3a) and levetiracetam (3b) were administered i.p. twice daily for 14 days. Two days later, FG 7142 was administered i.p. at a dose of 40 mg/kg and the incidence of clonic convulsions in all four extremities was assessed ($n = 16$). Values given are the percentage of mice showing a clonic convulsion. (b) Indicates a statistically significant difference ($P < 0.01$) vs. saline-treated group.

tiracetam (1036 mg/kg), valproate (184 mg/kg), phenobarbital (25 mg/kg), clonazepam (0.08 mg/kg), carbamazepine (33 mg/kg), phenytoin (99 mg/kg), vigabatrin (1464 mg/kg), lamotrigine (29 mg/kg) and gabapentin (887 mg/kg) and their corresponding ED_{50} values in the epilepsy model (see Table 1) reveal a very wide safety margin of 148 for levetiracetam when compared to ratios between 2 and 17 for the other antiepileptic drugs (Fig. 4). Due to the lack of effect of topiramate against corneally kindled seizures, a safety margin was calculated for this antiepileptic drug, using rotarod impairment in normal mice (249 mg/kg) and the protective ED_{50} value in the maximal electroshock seizure test (12 mg/kg). This gave a safety margin of 21 (Fig. 4).

3.13. Ratio between rotarod impairment and suppression of spike-and-wave discharges in GAERS rats

The safety margin in rats from the GAERS strain was determined as the ratio between impairment of the rotarod performance (TD_{50} value) and the minimum active dose (MAD) capable of inducing significant suppression of the mean duration of the spike-and-wave discharges. The TD_{50} values for levetiracetam (1272 mg/kg), valproate (221 mg/kg) and ethosuximide (152 mg/kg) and their corresponding MADs of 5.4, 100 and 30 mg/kg, respectively, again reveal a very wide safety margin of 235 for levetiracetam. In contrast, the two major anti-absence drugs, valproate and ethosuximide had similar, low ratios of 2 and 5, respectively (Fig. 5).

4. Discussion

Levetiracetam is the *S*-enantiomer of the ethyl analogue of piracetam, a drug widely used on account of its purported beneficial effects on cognition in the elderly. Previous studies with sound-sensitive mice have demonstrated that levetiracetam protects against seizure activity in this model after both oral (Gower et al., 1992) and intracerebroventricular administration (Gower and Matagne, 1994) whereas the *R*-enantiomer (ucb L060) was found to induce seizure protection only at very high doses (Gower et al., 1992). The present study demonstrated that the main metabolite of levetiracetam (ucb L057) was inactive as were all the minor metabolites when tested in this model (unpublished data). These results suggest that seizure suppression with levetiracetam is due to a central action of the unchanged compound.

Different results have been obtained with levetiracetam in the maximal electroshock seizure test. Gower et al. (1992) originally reported a protective effect in mice whereas levetiracetam was found to be inactive in both mice and rats in a later study (Löscher and Hönack, 1993). Our results confirm that levetiracetam is inactive against maximal electroshock seizures. Internal testing at UCB Pharma showed that differences between our results and those of Gower et al. (1992) were related to the stimulator being unable to deliver a maximal electroshock of 50 mA to mice in the latter study.

The lack of protection by levetiracetam against maximal electroshock seizures and pentylenetetrazol seizures in rodents contrasts markedly with the potent protection against seizures in mice kindled with corneal electroshocks or the chemoconvulsant, pentylenetetrazol. When an acute supramaximal electroshock and a maximal bolus dose of pentylenetetrazol is replaced by chronic, subconvulsive electroshocks or doses of pentylenetetrazol, the animals undergo an epileptogenic kindling process which gradually evolves into the expression of generalised motor seizures (Goddard et al., 1969). The results of the present study demonstrated that levetiracetam exerts a highly selective action in animals with 'epileptogenic brains'. This distinguishes levetiracetam from established antiepileptic drugs and most of the new antiepileptic drugs which have equipotent effects in normal and in kindled animals. The

Table 5
Behavioural alterations induced by levetiracetam, established antiepileptics and MK-801 in normal and amygdala-kindled rats

Antiepileptic drugs	Dose (mg/kg, i.p.)	Spontaneous activity (score)		Abdominal tone (score)		Ataxia (score)		Head weaving (score)	
		Non-kindled rats	Kindled rats	Non-kindled rats	Kindled rats	Non-kindled rats	Kindled rats	Non-kindled rats	Kindled rats
Levetiracetam	54	20 (20–20)	20 (19–20)	20 (20–20)	20 (20–20)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	170	20 (20–20)	20 (18–20)	20 (20–20)	20 (20–20)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	540	20 (20–20)	18 (18–20)	20 (20–20)	20 (17–20)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	1700	18 (15–19)	15 (14–16)	16 (14–19)	17 (16–18)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Valproate	100	20 (20–20)	18 (17–19)[b]	20 (20–20)	20 (20–20)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	200	20 (20–20)	20 (19–20)	20 (20–20)	19 (18–20)	1 (0–1)	1 (0–2)	0 (0–0)	0 (0–0)
	300	17 (16–18)	17 (16–18)	20 (19–20)	20 (20–20)	3 (2–4)	5 (4–6)[b]	0 (0–0)	0 (0–0)
Phenobarbital	20	19 (19–20)	19 (19–20)	20 (20–20)	20 (20–20)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	40	17 (16–17)	16 (15–16)	20 (20–20)	20 (20–20)	0 (0–2)	2 (2–2)	0 (0–0)	0 (0–0)
	60	14 (12–15)	13 (12–13)	17 (17–18)	20 (17–20)	9 (9–9)	9 (9–12)	0 (0–0)	0 (0–0)
Clonazepam	0.1	20 (20–20)	18 (18–19)[b]	20 (20–20)	20 (20–20)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	1.0	16 (15–18)	14 (12–14)	19 (18–20)	17 (15–20)	0 (0–0)	0 (0–2)	0 (0–0)	0 (0–0)
	3.0	15 (14–17)	12 (11–13)[b]	18 (16–20)	17 (14–19)	0 (0–1)	2 (0–4)	0 (0–0)	0 (0–0)
Carbamazepine	20	20 (20–20)	19 (18–20)	20 (20–20)	20 (20–20)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	30	18 (18–19)	17 (17–18)	20 (20–20)	19 (18–20)	0 (0–1)	1 (1–3)	0 (0–0)	0 (0–0)
	40	17 (15–18)	17 (15–17)	20 (20–20)	20 (20–20)	2 (1–3)	2 (1–2)	0 (0–0)	0 (0–0)
MK-801	0.03	20 (20–20)	20 (20–20)	20 (20–20)	20 (20–20)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	0.1	19 (18–19)	18 (17–18)	20 (20–20)	20 (20–20)	0 (0–0)	0 (0–0)	1 (0–1)	5 (4–7)[b]
	0.3	18 (17–19)	17 (16–17)	20 (20–20)	20 (20–20)	0 (0–2)	15 (3–16)[b]	9 (9–10)	13 (11–13)
	1.0	12 (12–13)	11 (10–11)[b]	18 (15–20)	17 (16–19)	18 (17–18)	18 (16–18)	10 (8–13)	10 (9–11)

Spontaneous activity, abdominal tone and head weaving were scored at 15, 30, 60, 120 and 180 min and ataxia at 30, 60 and 120 min after i.p. administration of each drug. Each score was summed over the five observation periods. A score of 20 (spontaneous activity and abdominal tone) or 0 (ataxia and head weaving) indicates that the parameter observed was normal. Values given are median and 1st and 3rd quartiles ($n = 8$).

[b] Indicates a statistically significant difference between kindled and non-kindled animals ($P < 0.01$).

Table 6
Effect of levetiracetam, established antiepileptics and MK-801 on the rotarod performance of normal and amygdala-kindled rats

Antiepileptic drugs	Impairment of rotarod performance	
	Non-kindled rats	Kindled rats
	TD_{50} (mg/kg, i.p.)	
Levetiracetam	1060 (335–2081)	1119 (766–1555)
Valproate	206 (132–261)	185 (122–268)
Phenobarbital	24 (17–34)	29 (22–40)
Clonazepam	0.8 (0.2–2.0)	0.8 (0.5–1.4)
Carbamazepine	32 (22–59)	36 (25–59)
MK-801	0.05 (0.03–0.08)	0.06 (0.03–0.11)

Values given are TD_{50} values, i.e., the doses impairing the rotarod performance in 50% of the animals, with associated 95% confidence intervals. All compounds were administered at their optimal pretreatment times (see Table 4).

exception is vigabatrin which also showed a relatively selective but less potent effect than that of levetiracetam against kindled seizures.

A general lack of anticonvulsant activity of levetiracetam was also observed in several other acute seizure tests where maximal CD_{97} doses of different chemoconvulsants were used. Indeed, levetiracetam only appears to have anticonvulsant activity on submaximal stimulation (Gower et al., 1992) and in threshold tests (Löscher and Hönack, 1993). Clonic convulsions induced by infusion with NMDA, kainic acid and AMPA into the lateral brain ventricle of mice were not affected by levetiracetam. In contrast, most established and new antiepileptic drugs tested offered general protection in these models. Levetiracetam was also inactive against CD_{97} doses of several chemoconvulsants modulating the $GABA_A$/BZ receptor complex with the exception of a minor effect against DMCM. Although DMCM is a selective inverse receptor agonist acting at the recognition site for benzodiazepine receptor ligands (Petersen, 1983) this finding does not indicate that levetiracetam has an action on this site since the protection obtained with levetiracetam in sound-sensitive mice was not modulated by the benzodiazepine receptor antagonist, flumazenil. Furthermore, levetiracetam neither had affinity for this binding site in vitro (Noyer et al., 1995), nor modulated muscimol-induced chloride fluxes (unpublished data) and did not show the characteristic tolerance development and withdrawal characteristics of benzodiazepine receptor ligands. Finally, several ubiquitous ligands like adenosine A_1 and A_2 receptor agonists (Klitgaard et al., 1993), NMDA receptor antagonists (Czuczwar and Meldrum, 1982) and AMPA receptor an-

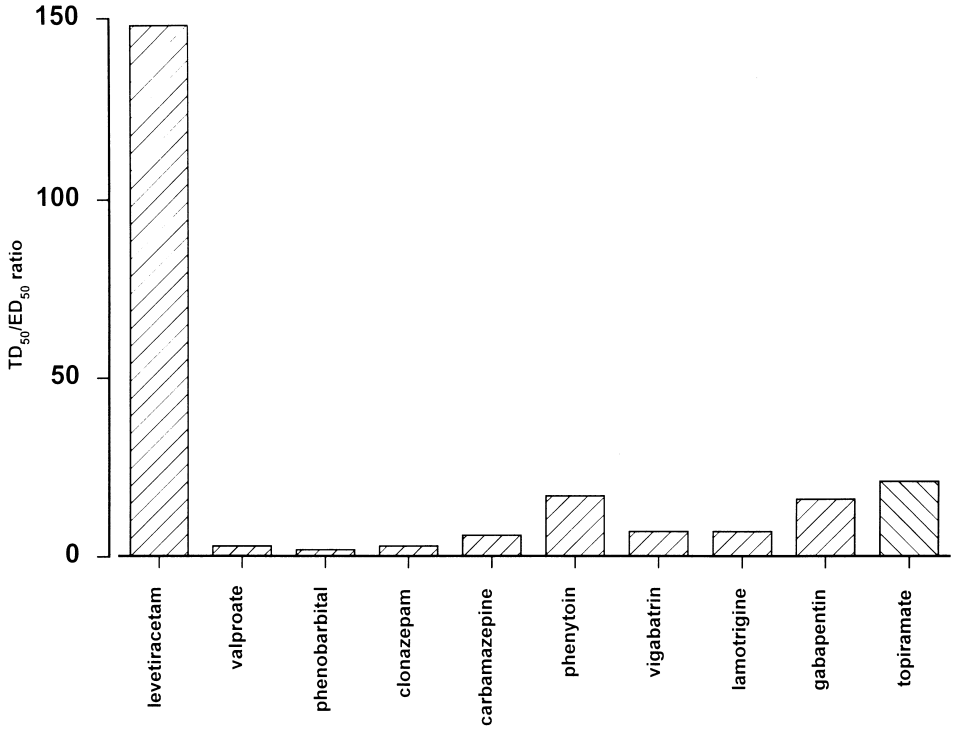

Fig. 4. Safety margin of levetiracetam, established and new antiepileptic drugs in corneally kindled mice. The safety margin was calculated by dividing the TD_{50} value for rotarod impairment in fully corneally kindled mice by the protective ED_{50} value determined against generalised motor seizures in the same animals. Due to the lack of effect of topiramate against corneally kindled seizures, a safety margin was calculated for this antiepileptic between the TD_{50} value for rotarod impairment in normal mice and the protective ED_{50} value in the MES test. All compounds were administered i.p. at their optimal pretreatment times (see Table 1).

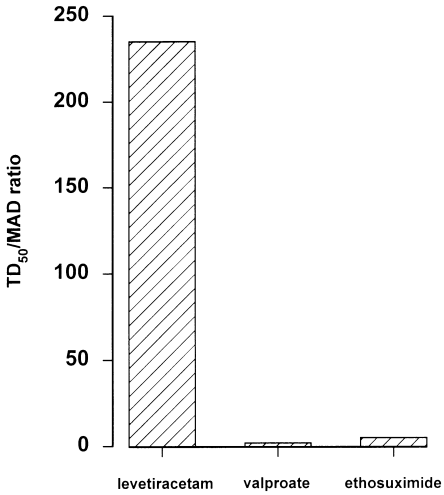

Fig. 5. Safety margin of levetiracetam, valproate and ethosuximide in rats from the GAERS strain. The safety margin was calculated by dividing the TD_{50} value determined in the rotarod test by the minimum active dose (MAD) inducing a significant reduction in the duration of spontaneous spike-and-wave discharges in GAERS rats. All compounds were administered i.p. at their optimal pretreatment times for inducing a reduction in the appearance of SWDs, i.e., 40 min for valproate and ethosuximide and 100 min for levetiracetam.

tagonists (Turski et al., 1992) have also shown anticonvulsant effects against DMCM-induced seizures, showing that protection in this model does not necessarily indicate a specific action on the benzodiazepine recognition site.

Several reports on the behavioural and electroencephalographic consequences of systemic administration of pilocarpine and kainic acid in the rat have shown that both drugs induce seizure activity with a focal limbic onset, most probably in the hippocampus (Nadler, 1981; Turski et al., 1983). With pilocarpine in particular this seizure activity becomes secondarily generalised, so such models have been suggested to mimic the complex partial seizures with secondary generalisation observed in humans. When tested against several different chemoconvulsants applied as the maximal CD_{97} doses, levetiracetam only produced potent protection against pilocarpine-induced seizures in mice. Further testing in rats confirmed the protection by levetiracetam against the relatively drug-resistant seizures induced by both pilocarpine and kainic acid in this species. This relatively selective action against pilocarpine-induced seizures in mice and rats appears unrelated to a scopolamine-like effect, since levetiracetam has been reported to oppose scopolamine-induced amnesia in mice (Verloes et al., 1988). Instead, levetiracetam exerts a relatively selective action against chemoconvulsants which induce seizures that resemble partial seizures in epileptic patients. This supports a previous observation (Löscher and Hönack, 1993) showing that levetiracetam induces marked suppression of several seizure parameters in fully amygdala-kindled rats, a model that has also been suggested to mimic complex partial seizures with secondary generalisation in man (Löscher et al., 1986).

The activity profile of the different antiepileptic drugs on pilocarpine-induced seizures in rats is similar following systemic and intranigral administration (Turski et al., 1990) and correlates with the ability of these antiepileptic drugs to alter firing in GABAergic neurons in the substantia nigra pars reticulata (Waszczak et al., 1986). This area is critically involved in modulating the generalisation of seizure activity, since a reduction in the neuronal activity of GABAergic projections from substantia nigra pars reticulata to areas such as superior colliculus, tegmentum and thalamus induces disinhibition of target cells in these areas, resulting in suppression of seizure activity (Gale, 1986). The potent ability of levetiracetam shown in the present study to protect against pilocarpine-induced seizures in both mice and rats is therefore consistent with a recent report showing that levetiracetam reduces spontaneous neuronal firing in the substantia nigra pars reticulata in anesthetized rats but that this does not appear to involve a direct enhancement of GABAergic inhibition in the substantia nigra pars reticulata (Löscher et al., 1996). The latter authors proposed that this action relates to alterations in GABA turnover in the striatum. This provokes a deficit of GABAergic-mediated inhibition in the striatum which disinhibits GABA pathways from this structure to the substantia nigra pars reticulata, thereby inhibiting neuronal firing in the substantia nigra pars reticulata. However, this effect was biphasic and was only observed at high doses of levetiracetam, which contrasts with the progressive decrease observed in substantia nigra pars reticulata neuronal firing. Focal microinjection of NMDA (Turski et al., 1987) and apomorphine (Turski et al., 1988) into the striatum has been shown to induce protection against pilocarpine-induced seizures, which suggests that modulation of neurotransmitter systems other than GABA may be involved in the effect of levetiracetam on neuronal firing in substantia nigra pars reticulata. The effect of levetiracetam against pilocarpine-induced seizures therefore suggests that at least part of its seizure suppression activity derives from an effect on neuronal firing in the substantia nigra pars reticulata. It, however, remains to be demonstrated through which anatomical network and molecular mechanism this action is mediated.

A concern when introducing a potential new drug for central nervous system diseases is whether it may lose efficacy after chronic use and give rise to problems of abuse and dependence. This limits the therapeutic use of currently available benzodiazepine receptor ligands (Haigh and Feely, 1988; Woods et al., 1995). The present study confirmed that chronic administration of diazepam is associated with marked tolerance development and also results in a lowered seizure threshold due to withdrawal hyperexcitability, which is one of the major characteristics of dependence on benzodiazepine receptor ligands (Little et al., 1988). In this respect it is important to note that levetiracetam remained equipotent after both acute and chronic treatment and did not lower the seizure threshold

after chronic treatment. Although further studies should be performed, this preliminary observation suggests that levetiracetam may not cause tolerance and withdrawal hyperexcitability in man as do benzodiazepines.

Epileptogenesis induced by amygdala kindling of rats revealed an unexpected neurotoxicity of NMDA receptor antagonists consisting of sedation, ataxia and psychomimetic behaviour (Löscher and Hönack, 1991). Subsequent clinical studies also showed such differences in susceptibility between healthy volunteers and patients with partial epilepsy (Sveinbjornsdottir et al., 1993). More recent experiments have shown that established antiepileptic drugs also produce exacerbated adverse effects in amygdala-kindled compared to normal rats (Hönack and Löscher, 1995). This finding could not be confirmed in the present study in the rotarod test, where a similar impairment was observed in normal and kindled rats. However, more qualitative evaluation showed that most of the classical antiepileptic drugs and, in particular the NMDA receptor antagonist, MK-801, produce more marked effects on different behavioural parameters in kindled than in normal rats. Levetiracetam only induced minor behavioural alterations in normal and kindled animals. These alterations were not associated with any psychomimetic effects and resulted in impairment of the rotarod performance in normal and kindled animals only at doses above 1000 mg/kg. These results clearly support previous observations (Gower et al., 1992; Löscher and Hönack, 1993) showing that levetiracetam has a low adverse effect potential in normal and kindled animals. Furthermore, the minimal adverse effects of levetiracetam combined with the potent protection results in an unusually high safety margin. Safety margins of over 100 were calculated in corneally kindled mice and in rats from the GAERS strain—two animal models reflecting partial and generalised epilepsy in humans, respectively. This suggest that levetiracetam may offer very safe, broad spectrum protection against epileptic seizures in man, which is not the case with existing antiepileptic drugs.

The selective protection by levetiracetam in animal models of chronic epilepsy appears to derive from a novel mode of action which involves inhibition of burst firing without interference with normal neuronal excitability. Thus, it appears that levetiracetam exerts a selective action against abnormal patterns of neuronal activity and thereby provides protection against the transition from interictal to ictal activity. This probably explains both the lack of anticonvulsant activity against acute seizures with immediate ictal activity induced by a maximal electroshock or maximal CD_{97} doses of different chemoconvulsants and also its unique tolerability. In vitro (Birnstiel et al., 1997) and in vivo (Margineanu and Wülfert, 1995) recordings from CA_3 neurons in hippocampus demonstrate that levetiracetam inhibits burst firing induced by synaptic activation in the presence of the chemoconvulsant, bicuculline. This effect is mediated via a non-$GABA_A$ receptor mechanism (Margineanu and Wülfert, 1997). Levetiracetam has been shown to have no direct interaction with a range of traditional drug targets implicated in the modulation of inhibitory and excitatory central neurotransmission. Potentiation of GABAergic inhibition has been proposed (Löscher et al., 1996) but extensive in vitro experiments have not revealed any significant displacement of ligands specific for 55 different binding sites including different receptor systems, reuptake sites, second messenger systems and channel proteins (see Noyer et al., 1995). Furthermore, levetiracetam was also inactive on glutamate receptor-mediated neurotransmission in the spinal cord and did not modulate chloride fluxes induced by muscimol (unpublished data). Finally, a lack of effect on GABA levels and the enzymatic activities of GABA transaminase and glutamic acid decarboxylase was recently reported from a neurochemical study on mouse brain (Sills et al., 1997). The apparent absence of any direct interaction with known mechanisms involved in inhibitory and excitatory neurotransmission parallels the recent discovery of a specific binding site for levetiracetam (Noyer et al., 1995). Ligand binding assays have revealed the existence of reversible, saturable and stereoselective binding specific for levetiracetam. This binding appears only in plasma membranes obtained from the central nervous system whereas no specific binding is obtained in peripheral tissues. Intensive investigations are in progress to isolate this binding site and help with further understanding of its possible functional role.

Kindling represents an animal model of epileptogenesis, and inhibition of the development of kindling can be used to identify antiepileptic drugs with potential antiepileptogenic effects. Among the major antiepileptic drugs in clinical use, only valproate and phenobarbital appear to exhibit antiepileptogenic effects in kindling models but only do so at doses producing adverse effects (Silver et al., 1991). Levetiracetam has been shown to prevent the development of pentylenetetrazol kindling in mice (Gower et al., 1992) and was also recently reported to have antiepileptogenic properties in the amygdala kindled rat (Löscher et al., 1998). A persistent effect of levetiracetam against the development of kindling after cessation of chronic administration and drug washout was found in the latter study, showing that levetiracetam did not simply mask the expression of kindled seizures (seizure suppression) but exerted a true, powerful antiepileptogenic effect. This, combined with a low incidence of adverse effects in both genetic and kindled rodents, suggests that levetiracetam is an efficacious and safe antiepileptic drug. Further studies are required to ascertain whether levetiracetam can influence underlying causes of this disease and provide the first pharmacotherapeutic approach to a cure.

In summary, the present study demonstrated selective seizure suppression by levetiracetam in genetic and kindled animals and against chemoconvulsants inducing partial epileptic seizures. This activity contrasts with the

general lack of activity against acute seizures induced by maximal electrical or chemical stimulation. This profile distinguishes levetiracetam from known antiepileptic drugs which have comparable activity in these models. The seizure protection obtained with levetiracetam derives from a central effect of the unchanged compound. No tolerance to levetiracetam was observed following chronic treatment and neither was rebound hyperexcitability produced upon withdrawal. Most importantly, levetiracetam showed a very low adverse effect potential in 'genetic' and kindled animals. Together with the potent protection in models reflecting partial and generalised epilepsy in humans this produces an exceptionally high safety margin for levetiracetam. In conclusion, these results have demonstrated a unique preclinical profile of levetiracetam, distinct from that of all known antiepileptic drugs. They also suggest that levetiracetam may constitute a very effective, broad spectrum approach to treatment of both partial and generalized epileptic seizures in patients, with a minimum of adverse effects. Finally, the powerful antiepileptogenic activity in kindling models indicates that this therapy may not be restricted to the symptomatic treatment of epilepsy but may also involve pharmacological inhibition of progress of the disease.

Acknowledgements

The authors are grateful to Miss Z. Povegliano, M. Petit, K. Leclercq, A. Gallisz, C. Chaussee, A.C. Michaux and M.-C. Tordeur for their skillful technical assistance and to Dr. E. Baltès for performing the plasma analysis for ucb L057. We are also grateful to Pr. C. Marescaux for supplying us with rats from the GAERS strain and to Miss A. Boehrer for her very helpful collaboration in this regard. Finally, we also thank Dr. P. Michel for the synthesis of gabapentin and Dr. R. Massingham for critical reading of an early version of this manuscript.

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