Antiepileptogenic Effects of the Novel Anticonvulsant Levetiracetam (ucb L059) in the Kindling Model of Temporal Lobe Epilepsy

WOLFGANG LÖSCHER, DAGMAR HÖNACK and CHRIS RUNDFELDT

Department of Pharmacology, Toxicology, and Pharmacy, School of Veterinary Medicine, Hannover, Germany

Received for publication April 15, 1997

ABSTRACT

We have previously shown that the novel anticonvulsant levetiracetam exerts potent anticonvulsant activity against both focal and secondarily generalized seizures in fully amygdala-kindled rats, i.e., a model of temporal lobe epilepsy. We examined whether levetiracetam also exhibits antiepileptogenic activity, i.e., prevents or retards acquisition or development of amygdala-kindling in rats. Before the experiments with chronic administration of levetiracetam at different doses, we determined the pharmacokinetics of the drug after i.p. injection. Levetiracetam had a relatively short half-life (about 2-3 hr) in rats, so that any lasting effects of the drug after chronic administration were certainly not due to drug accumulation. When rats were treated with levetiracetam during kindling acquisition at daily i.p. doses of 13, 27 or 54 mg/kg, the drug dose-dependently suppressed the increase in seizure severity and duration induced by repeated amygdala stimulation. After termination of daily treatment with 54 mg/kg, duration of behavioral seizures and of afterdischarges recorded from the amygdala remained to be significantly shorter compared to vehicle controls, although amygdala stimulations were continued in the absence of drug. These data thus indicate that levetiracetam not simply masked the expression of kindled seizures through an anticonvulsant action, but exerted a true antiepileptogenic effect. Adverse effects were not observed at any dose of levetiracetam tested in kindled rats. The powerful antiepileptogenic activity of levetiracetam in the kindling model indicates that levetiracetam is not only an interesting novel drug for symptomatic treatment of epilepsy but might be suited for pharmacological prevention of this disease in patients with a high prospective risk of the development of epilepsy.

Epilepsy, i.e., a group of chronic brain diseases characterized by recurrent spontaneous seizures, is a common and frequently devastating disorder, affecting at least 50 million people worldwide (Scheuer and Pedley, 1990). Pharmacotherapy is symptomatic in that available anticonvulsant drugs inhibit seizures, but no effective prophylaxis or pharmacotherapeutic cure of epilepsy is available. In view of the fact that seizures in 20 to 30% of all epileptic patients are resistant to current anticonvulsant drugs (Scheuer and Pedley, 1990), a drug that prevents or retards epileptogenesis, i.e., the process of development of epilepsy, would be an important advantage in therapy. Previous controlled randomized trials in patients with head injury associated with a high risk of the development of “posttraumatic” epilepsy have shown that early prophylactic administration of the major anticonvulsant drugs phenytoin and carbamazepine did not yield any favorable difference to placebo in the proportion of patients developing seizures (cf., Chadwick, 1995; Hernandez, 1997). Interestingly, this lack of antiepileptogenic efficacy of phenytoin and carbamazepine in patients is consistent with observations from the kindling model, i.e., the most widely used model of temporal lobe epilepsy (Wada, 1974; Albertson et al., 1984; Sato et al., 1990; Silver et al., 1991). Indeed, kindling, i.e., the phenomenon that repeated application of an initially subconvulsive electrical stimulus to limbic or cortical brain areas leads to the development of seizures increasing in severity and duration with ongoing stimulation, has repeatedly been proposed as a model to search for drugs with antiepileptogenic efficacy (Wada, 1974; Sato et al., 1990; Silver et al., 1991). Of the major anticonvulsant drugs in clinical use, only valproate and, to a lesser extent, phenobarbital, appear to exhibit antiepileptogenic effects in the kindling model, although only at high doses that are associated with sedative and ataxiogenic adverse effects (Silver et al., 1991).

More recently, several novel anticonvulsants have been developed and are currently evaluated in clinical trials (cf., Löscher and Schmidt, 1993; Bialer et al., 1996; Chadwick et al., 1996). One of these drugs is levetiracetam (ucb L059), i.e.,
a derivative of the nootropic piracetam with a wide spectrum of anticonvulsant effects in animal models of different types of epileptic seizures (Gower et al., 1992; Löscher and Hönack, 1993; Gower et al., 1995; Klitgaard et al., 1996). In clinical trials, levetiracetam appears to be an effective and well-tolerated agent in refractory partial epilepsy (Stables et al., 1995; Bialer et al., 1996). This prompted us to study whether levetiracetam exhibits antiepileptogenic properties in the kindling model.

Materials and Methods

Animals. Female Wistar rats (Harlan-Winkelmann, Borchen, Germany), weighing 210 to 230 g, were used. The animals were purchased from the breeder at a body weight of about 200 g. After arrival in the animal colony, the rats were kept under controlled environmental conditions (ambient temperature 24-25°C, humidity 50-60%, 12/12 hr light/dark cycle, light on at 7:00 A.M.) for at least 1 wk before being used in the experiments. Standard laboratory food (Altromin 1324 standard diet) and tap water were allowed ad libitum.

Pharmacokinetics of levetiracetam. As a basis for the dosing protocol in chronic experiments in kindled rats, the pharmacokinetics of levetiracetam were determined after acute drug administration in nonkindled female rats, which were age-matched with the rats used for the kindling experiments described below. Levetiracetam was injected i.p. at a dose of 54 mg/kg in a group of seven animals. Blood was sampled by orbital puncture after .25, .5, 1, 2, 4, 6 and 8 hr. Levetiracetam was determined in plasma by gas chromatography as described in below. In one experiment, plasma levels of levetiracetam were also determined in kindled rats.

For drug extraction from plasma, 0.1 ml plasma were mixed with 50 μl distilled water containing the internal standard (40 μg piracetam), and leviteracetam and piracetam were extracted with 8 ml chloroform. After centrifugation, the chloroform was evaporated, the residue was dissolved in 100 μl methanol, and 2 μl were used for analysis by gas chromatography. For analysis, a gas chromatograph equipped with a Megabore DB-1 column (30 m length with 0.545 mm inner diameter and a film thickness of 1.5 μm; Varian, Darmstadt, Germany) and a thermionic specific detector was used. The carrier gas was nitrogen at a flow rate of 25 ml/min, and the operating conditions were injector temperature of 280°C and detector temperature of 320°C. The temperature of the column oven was programmed with an initial temperature of 140°C for 1 min, increased by 40°C/min to 180°C, which was maintained for 2 min, then increased by 40°C/min to 220°C, which was maintained for 1 min. Retention times of piracetam and levetiracetam under these conditions were 2.2 and 2.7 min, respectively.

Electrode implantation. For the kindling experiments, the rats were anesthetized with chloral hydrate (360 mg/kg i.p.) and received stereotaxic implantation according to the surgery methods described in the atlas of Paxinos and Watson (1986) of one bipolar electrode in the right basolateral amygdala. Coordinates for electrode implantation were AP-2.2, L -4.8, V -8.5. All coordinates were measured from bregma. Skull screws served as the indifferent reference electrode. The electrode assembly was attached to the skull by dental acrylic cement.

Experiments on kindling development. Four groups of eight rats each were implanted with kindling electrodes as described above. Two weeks after implantation, the initial (prekindling) electrical susceptibility of the stimulated region (ADT) was determined in each rat using an ascending staircase procedure. The initial current intensity was 10 μA, and the current intensity was increased in steps of about 20% of the previous current at intervals of 1 min until an afterdischarge of at least 3 sec duration was elicited. On the next day, chronic treatment was started and the animals were stimulated with a suprathreshold current of 500 μA 1 hr after each i.p. injection in the morning. Group I received i.p. injections of vehicle (saline), group II i.p. injections of 13 mg/kg levetiracetam, group III i.p. injections of 27 mg/kg levetiracetam and group IV i.p. injections of 54 mg/kg levetiracetam. Vehicle or drug were injected once daily 5 days a week (i.e., except on weekends). After 21 injections, treatment and amygdala stimulations were terminated. After a wash-out period of 5 days, further amygdala stimulations with 500 μA were carried out, until all animals had exhibited stage 5 seizures. Seizure severity, seizure duration and afterdischarge duration were recorded after each stimulation as follows. Seizure severity was classified according to Racine (1972): 1, immobility, eye closure, twitching of vibrissae, sniffing, facial clonus; 2, head nodding associated with more severe facial clonus; 3, clonus of one forelimb; 4, rearing, often accompanied by bilateral forelimb clonus; 5, rearing with loss of balance and falling accompanied by generalized clonic seizures. Seizure duration was the duration of limbic (stage 1-2) and/or motor seizures (stages 3-5); limbic seizure activity (immobility associated with low amplitude afterdischarges and occasional facial clonus or head nodding) often occurring after termination of motor seizures was not included in seizure duration. Afterdischarge duration was the total duration of amygdala electroencephalogram spikes with an amplitude of at least twice the amplitude of the prestimulus recording and a frequency greater than 1/sec.

One week after the last stage 5 seizure, the post-kindling ADT was determined in each group as described above for the prekindling ADT.

Evaluation of behavioral effects. The extent of sedation, ataxia and muscle relaxation after administration of levetiracetam (or vehicle) was determined by a rating system shortly before amygdala stimulation on each experimental day (cf., Hönack and Löscher, 1995). In short, animals were taken out of the cage, placed in an open field, observed for about 1 min and sedation and ataxia/muscle relaxation were rated separately (abdominal muscle tone was evaluated by palpation at the end of the period of observation): Sedation: 0, normal forward locomotion; 1, slightly reduced forward locomotion; 2, reduced locomotion with rest periods in between (partly with closed eyes); 3, reduced locomotion with more frequent rest periods; 4, no forward locomotion, animal sits quietly with closed eyes. Ataxia/muscle relaxation: 0, no ataxia, no decrease in abdominal muscle tone; 1, slight ataxia in hind-legs (trotting of the hind quarters), no decrease in abdominal muscle tone; 2, more pronounced ataxia with dragging of hind legs and slight decrease of muscle tone; 3, further increase of ataxia and more pronounced dragging of hind legs and decrease in muscle tone; 4, marked ataxia, animals lose balance during forward locomotion, total loss of abdominal muscle tone; 5,
very marked ataxia with frequent loss of balance during forward locomotion, total loss of abdominal muscle tone. Behavioral alterations other than those described above were separately recorded.

In addition to rating of sedation and motor impairment in the open field, impaired motor function was quantitated by the rotarod test of Dunham and Miya (1957). The rotarod test was carried out with a foam rubber coated rod of 6 cm diameter which rotated at 8 r.p.m. Neurological deficit was indicated by inability of the animals to maintain their equilibrium for at least 1 min on the rotating rod. The kindled rats were trained before drug experiments to maintain on the rod. After drug treatment, rats not able to maintain their equilibrium on the rod for 1 min were put again on the rod twice. Only animals not able to remain on the rod for three subsequent 1-min attempts were considered to exhibit neurological deficit.

Statistics. All data are given as means ± S.E. Significance of differences between seizure readings in the same group of rats was calculated by the Wilcoxon signed-rank test for paired replicates. Significance of differences between seizure readings in different groups of rats was either calculated by the Mann-Whitney U test (seizure severity) or by Student’s t test (all other seizure parameters). In case of comparisons between more than two groups, analysis of variance preceded post hoc calculations by the tests described above.

Drugs. Levetiracetam and piracetam (as internal standard for analysis of levetiracetam) were obtained from UCB Pharma (Braine-l’Alleud, Belgium). Levetiracetam was freshly dissolved in distilled water prior to each injection. Injection volume was 2 ml/kg. In control experiments, rats received the same volume of saline.

Results

Pharmacokinetics of levetiracetam. The individual plasma concentration time curves of the seven female Wistar rats receiving injections with levetiracetam are shown in figure 1. The peak concentration ranged between 70.5 and 79.5 µg/ml (mean ± S.E.: 74.5 ± 3.1) and was determined after 15 to 60 min (average time of peak level was 26 min). The average half-life of levetiracetam in plasma was 2.6 hr (range 2.2-3.0 hr).

Kindling did not appear to alter pharmacokinetics of levetiracetam, because the average plasma level 1 hr after acute administration of 54 mg/kg in nine fully kindled rats was 54.8 ± 2.01 µg/ml (mean ± S.E.) compared to 60.3 ± 3.2 µg/ml in seven age-matched nonkindled rats, the difference being not significant.

Effects of levetiracetam on kindling development.

Based on previous results of acute experiments in fully kindled rats (Lösch and Höfack, 1993), three dosages were chosen for the chronic experiments: 13, 27 and 54 mg/kg. Pretreatment time was 1 hr before each amygdala stimulation. Results of the experiments on kindling development are shown in tables 1-4 and figures 2 and 3. In the control group, an average of 12.8 stimulations with 500 µA was necessary to induce stage 5 seizures (table 1). This figure was dose-dependently increased by levetiracetam, because the average plasma level 1 hr after acute administration of 54 mg/kg in nine fully kindled rats was 54.8 ± 2.01 µg/ml (mean ± S.E.) compared to 60.3 ± 3.2 µg/ml in seven age-matched nonkindled rats, the difference being not significant.

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than controls (table 2; fig. 2). Afterdischarge duration recorded at each stimulation was significantly reduced by chronic treatment with levetiracetam, particularly at the highest dose tested (fig. 3). However, calculation of cumulative afterdischarge duration demonstrated that the total focal seizure duration which was necessary for development of stage 5 seizures was not significantly altered by the drug, although there was a trend towards increased cumulative afterdischarge duration (table 1). After termination of treatment, all rats developed stage 5 seizures during subsequent stimulations (fig. 2). However, in the fully kindled rats, seizure and afterdischarge duration of rats that had been treated weeks before with levetiracetam, 54 mg/kg, were still significantly lower compared to controls (fig. 3; table 3). The focal seizure threshold in rats that had been kindled under medication with levetiracetam was not significantly different from rats treated with vehicle (table 4).

Adverse effects. Neither after administration of vehicle nor the different doses of levetiracetam, sedative or ataxogenic adverse effects were noted in the open field or rotarod test during the period of the chronic experiments (not illustrated). In some experiments, the rats appeared to be more active than vehicle-injected controls.

Discussion

Various previous studies have shown that the kindling model allows the distinction between antiepileptogenic and anticonvulsant effects of a compound (cf., Sato et al., 1990). The term “antiepileptogenic” refers to inhibition of processes underlying the development of the epileptic condition,
whereas “anticonvulsant” refers to inhibition of seizures in an epileptic condition. Drugs that inhibit the development of kindling may have antiepileptogenic properties in humans, whereas drugs that inhibit seizures evoked in kindled animals may have anticonvulsant properties in humans (Sato et al., 1990). Only some of the major anticonvulsant drugs in current clinical use, i.e., valproate and phenobarbital, inhibit both the development of kindling and the expression of kindled seizures (Wada, 1974; Sato et al., 1990; Silver et al., 1991). We have previously shown that levetiracetam exerts potent anticonvulsant effects in fully kindled rats (Lo¨scher and Honack, 1993). Our study demonstrates that levetiracetam also produces potent antiepileptogenic effects in the kindling model during kindling development. The antiepileptogenic effect is reached at doses that do not induce sedative or ataxic adverse effects. Indeed, previous studies with acute administration of levetiracetam in rats of the same sex, age and strain as used in the present experiments have demonstrated an outstanding tolerability of this drug with no sedation and no rotarod failures up to doses of 1700 mg/kg, and only slight ataxia (score 1-2) at doses exceeding 500 mg/kg (Lo¨scher and Honack, 1993).

Although all rats of our study could be kindled when electrical stimulations were continued after termination of treatment, electrographic and motor seizures in rats previously treated with daily doses of 54 mg/kg levetiracetam were significantly shorter compared to vehicle-treated rats. This indicates that levetiracetam not simply masked the expression of kindled seizures through an anticonvulsant action, but exerted a true antiepileptogenic effect. The shorter seizures in levetiracetam-treated rats, which were still observed weeks after treatment, were certainly not due to retarded elimination of levetiracetam, because the drug was rapidly eliminated in rats. Preliminary experiments in which levetiracetam was determined in plasma of rats after chronic treatment substantiated the absence of any significant drug accumulation during prolonged treatment (W Lo¨scher and C Rundfeldt, unpublished observations). Furthermore, the long-lasting antiepileptogenic effect of levetiracetam was certainly not due to retained active metabolite(s) because the major metabolite of levetiracetam is pharmacologically inactive, and other metabolites are produced at levels (1% and below) that are below those that are relevant for pharmacological activities (A Baltes, unpublished data).

To our knowledge, our data are the first demonstration of an antiepileptogenic drug effect that is present long after termination of treatment, indicating a permanent inhibitory effect on development of kindled seizures. In addition to the increase in seizure severity, enhanced seizure duration is the second hallmark of the kindling phenomenon (Sato et al., 1990). Drugs such as valproate or phenobarbital which have previously shown to exert antiepileptogenic efficacy in the kindling model retarded the increase in severity and duration of seizures during kindling acquisition, but both parameters rapidly reached control values after termination of treatment (Silver et al., 1991).

Before discontinuation of levetiracetam dosing in the experiments with the 27- and 54-mg/kg dose, the seizure score was increasing (see fig. 2), which might indicate that some pharmacodynamic tolerance to levetiracetam was developing during chronic treatment. Experiments with daily administration of levetiracetam in fully kindled rats for 3 wk indeed indicated a loss of anticonvulsant efficacy that was not related to an increased drug elimination, thus suggesting development of pharmacodynamic tolerance to the anticonvulsant effect of levetiracetam in this model (W Lo¨scher, D Honack and C Rundfeldt, unpublished data).

The mechanism by which levetiracetam exerts this antiepileptogenic action is uncertain. Interestingly, similar to valproate and phenobarbital, there is some evidence that levetiracetam may act, at least in part, through potentiation of GABAergic inhibition (Lo¨scher et al., 1986). Impairment of GABAergic inhibition is thought to play a crucial role in the processes underlying epileptogenesis in the kindling model (Sato et al., 1990). Therefore, the GABAmimetic effects of valproate, phenobarbital and levetiracetam could explain, at least in part, their antiepileptogenic activity in this model.

Apart from effects on the GABA system (cf., Lo¨scher et al., 1996), there are other cellular actions of levetiracetam that separate this drug from currently used anticonvulsant drugs (Margineanu and Wulffert, 1995; Noyer et al., 1995). Using in vivo recordings from rat hippocampus, it was shown that levetiracetam prevents increases in CA3 neuronal excitability by bicuculline through a non-GABAergic mechanism (Margineanu and Wulfert, 1995). Binding assays revealed the existence of specific stereoselective binding sites for levetiracetam on rat brain synaptic membranes prepared from several brain regions, including the hippocampus (Noyer et al., 1995). Interestingly, several levetiracetam homologues, including nootropics such as piracetam and aniracetam, exerted an affinity for the levetiracetam binding site that was linearly correlated with the anticonvulsant activity of these compounds, although commonly used antiepileptic drugs such as carbamazepine, phenytoin, valproate, phenobarbital or benzodiazepines did not displace [3H]levetiracetam bind-

### TABLE 3
Seizure parameters in fully kindled rats, which were treated with levetiracetam during kindling development (see legend to table 1 for details)

<table>
<thead>
<tr>
<th>Group</th>
<th>Seizure Severity (Score)</th>
<th>Seizure Duration (Sec)</th>
<th>Afterdischarge Duration (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>52.0 ± 6.5</td>
<td>61.8 ± 0.67</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>43.5 ± 4.3</td>
<td>58.4 ± 9.4</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>48.7 ± 7.2</td>
<td>52.7 ± 7.9</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>33.8 ± 4.0</td>
<td>40.8 ± 6.4**</td>
</tr>
</tbody>
</table>

All stimulations were carried out with 500 μA. Data are means and S.E. of eight rats per group recorded at the 10th stage 5 seizure, i.e., several weeks after the last administration of levetiracetam. Significance of differences to group I (control) was calculated by the Mann-Whitney U-test (for seizure severity) or by Student’s t-test (for all other seizure parameters) and is indicated by asterisks (* P < .05; ** P < .01).

### TABLE 4
Focal seizure threshold before and after chronic treatment with levetiracetam during kindling development (see legend to table 1 for details)

<table>
<thead>
<tr>
<th>Group</th>
<th>Prekindling ADT (μA)</th>
<th>Postkindling ADT (μA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>248 ± 41</td>
<td>120 ± 32</td>
</tr>
<tr>
<td>II</td>
<td>248 ± 31</td>
<td>76 ± 16</td>
</tr>
<tr>
<td>III</td>
<td>270 ± 47</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>IV</td>
<td>249 ± 43</td>
<td>88 ± 19</td>
</tr>
</tbody>
</table>

The table shows the initial (pre-kindling) afterdischarge threshold (ADT) determined 1 day before onset of treatment and the ADT determined in fully kindled rats 1 week after the 10th stage 5 seizure. In all groups, postkindling ADTs were significantly lower than pre-kindling ADTs (P at least <.05). There was no significant difference between groups in either prekindling or postkindling ADTs. All data are shown as means ± S.E.
T-type Ca


References

Displacement of [3H]levetiracetam binding by amiloride, a T-type calcium channel antagonist, whereas the N-type Ca2+ antagonist o-conotoxin and the Na+ channel antagonist saxitoxin were without effect. However, extensive experiments on the receptor binding assay profile of levetiracetam indicated that levetiracetam was inactive at all of the more than 30 neurotransmitter receptors and ion channel sites tested, including T-type calcium channels, which indicates that the levetiracetam binding site in the brain is not directly related to any site on which standard antiepileptic drugs act (Noyer et al., 1995). This might explain the unique features of this novel agent, i.e., anticonvulsant activity against various seizure types, including partial and secondarily generalized kindled seizures, in animal models at doses well below those inducing adverse effects (Gower et al., 1995; Löschner and Hönnack, 1993). The antiepileptogenic activity shown in our study extends this favorable profile of levetiracetam, indicating that levetiracetam might not only be a novel drug for treatment but also for pharmacological prevention of epilepsy, particularly in patients with head injury that have a high prospective risk of the development of epilepsy (Chadwick, 1995; Hernandez, 1997). Furthermore, in view of the fact that many epileptic patients have memory disturbances, the antiamnesic effect of levetiracetam observed in animal experiments (Verloes et al., 1988) might be an added advantage of this compound.

Acknowledgment

The authors thank Mrs. D. Pieper for skillful technical assistance.

References


Send reprint requests to: Prof. W. Löschner, Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Bunteweg 17, 30559 Hannover, Germany.