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Profile of the new pyrrolidone derivative seletracetam (ucb 44212) in animal models of epilepsy

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ABSTRACT

Seletracetam is a pyrrolidone derivative with a one-log-unit higher affinity for the synaptic vesicle protein 2A (SV2A) than levetiracetam (Keppra®). This study explored its anticonvulsant properties in animal models of epilepsy. Seletracetam reduced both the amplitude and repetitive firing of population spikes induced by a high K^+/low Ca^{2+} concentration fluid (HKLCF) in rat hippocampal slices. The reduction of HKLCF-induced increases in population spike amplitude was particularly pronounced, and occurred at ~10 times lower seletracetam concentrations than previously observed for levetiracetam. These *in vitro* data suggest that desynchronisation of epileptiform activity may contribute significantly to the antiepileptic properties of seletracetam. Seletracetam also showed a potent anti-seizure activity in animal models mimicking partial-onset (kindled animals) and generalized epilepsy (audiogenic seizure susceptible mice and genetic absence epilepsy rats from Strasbourg (GAERS)). In amygdala-kindled rats, seletracetam increased the generalized seizure threshold current and decreased the duration of the after-discharge and the seizure severity observed at the after-discharge threshold current, and generally had a much more potent effect than previously observed for levetiracetam. Seletracetam showed no psychomimetic effects and a very high central nervous system (CNS) tolerability in both kindled and GAERS rats, markedly superior to that of levetiracetam and other antiepileptic drugs. These results suggest that seletracetam may represent an effective and very well tolerated broad-spectrum agent for the symptomatic treatment of epilepsy.

1. Introduction

Seletracetam (ucb 44212; (2S)-2-[(4S)-4-(2,2-difluorovinyl)-2-oxopyrrolidin-1-yl]butanamide; Fig. 1), is a recently discovered pyrrolidone derivative, structurally related to levetiracetam, which displays one-log-unit higher affinity than levetiracetam to the synaptic vesicle protein 2A (SV2A) (Lynch et al., 2004). Levetiracetam ((S)-α-ethyl-2-oxopyrrolidine acetamide) is an antiepileptic drug with a unique profile in animal models of seizures and epilepsy and a novel mechanism of action (Klitgaard et al., 1998; Margineanu and Klitgaard, 2002). The latter involves an interaction with the SV2A. The importance of this interaction is highlighted by a strong correlation between the affinity of levetiracetam analogues for SV2A and their anticonvulsant potencies (Lynch et al., 2004; Kaminski et al., 2008). This suggests that levetiracetam's primary antiepileptic mechanism of action relates to its interaction with SV2A and triggered a drug discovery programme which led to the identification of both brivaracetam (Kenda et al., 2004; Matagne et al., 2008), currently in phase III clinical trials for epilepsy, and seletracetam.

Like levetiracetam, seletracetam is devoid of affinity for a number of binding sites associated with different receptors, second messenger systems and ion channels (Noyer et al., 1995). This suggests that seletracetam resembles levetiracetam by selective binding to SV2A, albeit with a markedly higher affinity.

A significant number of *in vitro* studies have been conducted with whole-cell patch clamp techniques to ascertain if seletracetam modulates voltage- and/or ligand-operated ion channels. Seletracetam, in contrast to another new pyrrolidone derivative, brivaracetam, did not modify tetrodotoxin-sensitive fast Na^+ currents in rat cortical neurons and did not alter persistent Na^+ currents, like phenytoin, in neurons from the CA1 region of rat hippocampal slices (Zona et al., 2005). Likewise, another study on rat hippocampal neurons reported that seletracetam is devoid of any significant effect on GABA-, glycine-, NMDA-, kainic acid- and AMPA-gated currents, with the exception of a minor inhibition of the plateau phase of the NMDA current (Rigo et al., 2005). Therefore, seletracetam does not appear to directly modulate Na^+ channels as well as other key ligand-gated ion channels involved in inhibitory and excitatory neurotransmission.

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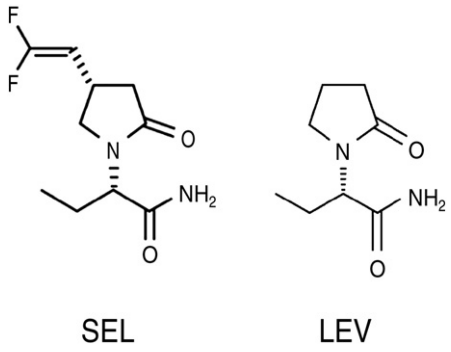

Fig. 1. Chemical structure of seletracetam (SEL) and levetiracetam (LEV).

Other studies have revealed effects that may contribute to an antiepileptic potential of seletracetam beside its high affinity for SV2A. Seletracetam resembles levetiracetam by its more potent ability to reverse the inhibitory effect of zinc and ß-carbolines on glycine-gated currents in rat hippocampal neurons but also differs from levetiracetam by an absence of effect on these negative allosteric modulators on GABA-gated currents (Rigo et al., 2005). Likewise, seletracetam resembles levetiracetam by its more potent inhibition of high-voltage-operated Ca^{2+} currents and epileptiform elevations on intracellular Ca^{2+} concentrations but this effect also differs for levetiracetam by involving more than N-type Ca^{2+} channels (Martella et al., 2009).

The purpose of the present study was to characterize the ability of seletracetam to induce seizure suppression in a variety of *in vitro* and *in vivo* models of epilepsy. Adverse CNS/motor effects of seletracetam were also evaluated in fully kindled rodents and in genetic absence epilepsy rats from Strasbourg, using a qualitative behavioural evaluation and a rotarod test.

2. Materials and methods

All experiments were approved by the local ethics committee for animal experimentation according to Belgian law.

2.1. Chemicals

Seletracetam (ucb 44212) was synthesized in the chemical laboratories of UCB Pharma SA, Belgium. Pentylenetetrazol was obtained from Acros Organics, Geel, Belgium and pentobarbital from Siegfried AG, Zofingen, Switzerland. NaCl, KCl and CaCl2 were obtained from Vel, Leuven, Belgium.

2.2. Rat hippocampal slices

Transverse hippocampal slices were prepared from Sprague–Dawley rats according to standard procedures, as previously reported (Margineanu and Klitgaard, 2000, 2001). Field potentials were recorded extracellularly in the CA3 area of the slices, with 2 M NaCl-filled glass microelectrodes. Field potentials were evoked upon fimbrial stimulation with constant-current pulses producing ~50% of the maximal population spike.

Epileptiform responses were induced by changing from a normal perfusion fluid (aCSF, artificial CSF) to an aCSF with high K^+ and low Ca^{2+} concentration (HKLCF), containing 7.5 mM K^+ and 0.5 mM Ca^{2+}. Field potentials were recorded at 10 min intervals, upon evoking with constant pulses eliciting a single population spike when in aCSF. Spontaneous bursts were also recorded at 10 min intervals. Seletracetam was added to the perfusion fluid 20 min before shifting from aCSF to the HKLCF, and was kept in the perfusion fluid throughout the experiment.

The acquisition software averaged on-line three successive samples of field potentials, calculated the population spike amplitudes, and counted the number of population spikes with amplitude ≥10% of the first one.

Drug effects were quantified through Δpopulation spikes 1 — the increase in amplitude vs. aCSF perfusion of the first and largest population spike the number of repetitive population spikes per evoked burst and, also by the spontaneous burst number and amplitude per 2-min recordings.

2.3. Mice in vivo models of seizures and epilepsy

2.3.1. Corneal kindling

Kindling was induced in male NMRI mice (20–25 g) by BID corneal stimulations (3 mA, 3 s). Animals were considered kindled after the appearance of 4 consecutive generalised seizures; forelimb clonic convulsions with or without rearing, falling and loss of balance (Matagne and Klitgaard, 1998). Fully kindled mice ($n=10$ per group), pre-treated with saline in the morning, were stimulated and observed for convulsive behaviour, similarly in the afternoon after pre-treatment with saline or seletracetam administered i.p. 60 min before the stimulation. The proportion of mice protected against generalised seizures was the end point for evaluation of anticonvulsant activity.

2.3.2. Audiogenic seizures

Male genetically sound-sensitive mice (16–28 g; $n=10$ per group) responding with wild running, clonic and tonic convulsions to an acoustic stimulation were used. Audiogenic seizures were induced by an acoustic stimulus (90 dB, 10–20 kHz) applied for 30 s. Mice were pre-treated with either saline or seletracetam, administered i.p. 60 min before the stimulation, and the proportion of mice protected against clonic convulsions used as the end point to assess anticonvulsant activity.

2.3.3. Maximal electroshock seizures

Maximal electroshock seizures were induced in male NMRI mice (25–30 g; $n=10$ per group) by a stimulator (WITT IndustrieElektronic, Berlin, Germany) using a 50 mA current delivered with a 50 Hz pulse frequency for 0.2 s through corneal electrodes. Before stimulation, a drop of saline containing Unicaïne (0.9%) was placed on the eyes of the animals to promote conductivity and induce slight anaesthesia. Initial testing with these stimulation parameters showed tonic hindlimb extensions in 100% of vehicle-treated animals from a 14 mA current. Animals were observed for 10 s and the proportion of mice protected against tonic hind limb extension used as the anticonvulsant activity evaluation endpoint.

2.3.4. Pentylenetetrazol-induced seizures

Generalised seizures were induced in male NMRI mice (20–25 g; $n=10$ per group) by s.c. administration of pentylenetetrazol at a dose of 89 mg/kg, a dose inducing clonic convulsions of all four extremities in 97% of saline-treated animals. Animals were placed individually in Perpex cages and observed for clonic convulsions in all four extremities for 60 min. The proportion of mice protected against clonic convulsions was used as the anticonvulsant activity evaluation endpoint.

2.4. Rat in vivo models of seizures and epilepsy

2.4.1. Hippocampal kindled rats

Male Sprague–Dawley rats (250–350 g) were implanted with a bipolar stimulation/recording electrode of stainless steel into the right dorsal hippocampus (coordinates = AP −3.5, L −2.5 and V −2.5) (Paxinos and Watson, 1986) under pentobarbital anaesthesia. Kindling was induced by twice daily stimulations, 5 days per week, with 600 µA

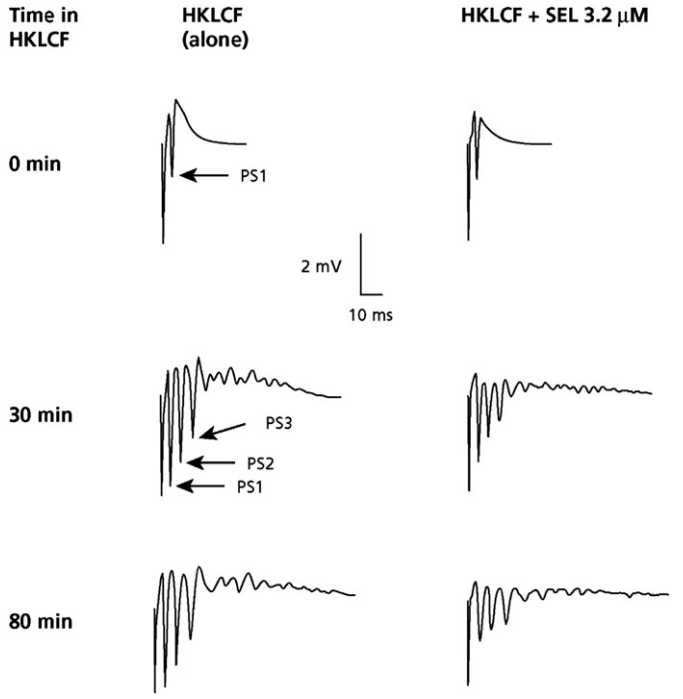

Fig. 2. Field potentials, evoked in CA3 area of rat hippocampal slices by constant fimbrial stimulation, in normal perfusion fluid (uppermost traces), or in HKLCF, alone or after addition of seletracetam (SEL), 3.2 µM.

monophasic square-wave pulses, 50 Hz for 1 s. Rats were defined as kindled, and used for drug experiments, after the appearance of at least 3 consecutive stage 4 or 5 seizures according to the scale of Racine (Racine, 1972). Fully kindled rats ($n=8$ per group) were stimulated once with the same stimulation parameters as above 60 min after oral water administration. Two days later, this protocol was repeated with the same animals, 60 min after oral administration of either water or seletracetam. The behavioural effect of stimulation was graded according to the Racine score.

2.4.2. Amygdala-kindled rats

Female Wistar rats (200–220 g) were implanted with a bipolar stimulation/recording electrode of teflon-coated stainless steel into the right hemisphere basolateral amygdala (stereotaxic coordinates = 2.2 mm caudal, 4.8 mm lateral, 8.5 mm ventral, all respective from bregma) (Paxinos and Watson, 1986) under chloral hydrate anaesthesia. Following a post-operative recovery period of two weeks, constant-current stimulations (500 µA, monophasic square-wave pulses, 50-Hz for 1 s) were delivered to the amygdala once daily until at least 10 sequential fully kindled stage 5 seizures were elicited. In these fully kindled rats, the threshold for inducing afterdischarges in the amygdala (after-discharge threshold) was repeatedly determined as described previously (Löscher et al., 1998). Seizure severity at the after-discharge threshold current was rated by the Racine scale (Racine, 1972). Furthermore, the after-discharge and seizure duration were recorded. After all rats exhibited reproducible after-discharge thresholds, the effect of seletracetam on after-discharge threshold current was determined 60 min after i.p. administration. If only focal seizure activity was induced at the after-discharge threshold current, stimulations were continued to determine the generalized seizure threshold as described previously (Löscher et al., 1998).

2.4.3. Genetic absence epilepsy rats from Strasbourg (GAERS)

Male GAERS were implanted with 4 platinum electrodes into the left and right frontal cortex and left and right occipital cortex, under Imalgene® 50 mg/kg/Domitor® 0.5 mg/kg i.m. anaesthesia. Cortical, spontaneous spike-and-wave discharges (SWDs) were recorded bilaterally from the rats placed into Plexiglass boxes, and prevented from falling asleep by gentle sensory stimulation. After a 20-min habituation period, the rats were injected i.p. with either saline or seletracetam, EEGs recorded continuously over consecutive 20 min intervals up to 120 min, and the cumulative SWD duration was calculated.

2.5. Rotarod performance in kindled mice and rats and GAERS

Behavioural alterations were evaluated qualitatively with particular attention to potential psychomimetic effects (head weaving and stereotyped behaviors). Potential adverse effects on motor function were assessed quantitatively in a rotarod test (Treadmill for mice or rats, Ugo Basile) using 3 cm diameter rod for mice, 6 cm rod diameter for rats, rotating at a constant speed of 6 rpm. Fully corneally-kindled mice ($n=10$ per group), amygdala-kindled rats or GAERS ($n=8$ per group) were pre-trained; only animals able to remain on the rod for at least 60 s in 3 consecutive trials were retained. The following day, either saline or seletracetam was administered i.p. 60 min before testing, and the number of animals unable to remain on the rod for at least 60 s was recorded.

2.6. Statistical analysis

ED_{50} values (dose protecting 50% of the animals) and TD_{50} values (dose inducing impairment of rotarod performance in 50% of the animals) were calculated using a log-probit analysis (SAS/STATR Software). The significance of differences between individual control recordings and recordings after drug treatment in both amygdala-kindled rats and hippocampal-kindled rats was calculated by the Wilcoxon sign rank test for paired replicates (level of significance: $P<0.05$). The significance of differences between individual control recordings and recordings after drug treatment in GAERS rats was calculated using a Friedman analysis of variance test for paired samples, followed by a post hoc Dunn's multiple comparison test (level of significance: $P<0.05$). Significance of differences vs. the respective control groups for Δpopulation spike 1 and the number of repetitive population spikes were assessed with two-tailed *t*-tests, corrected (Bonferroni) for multiple comparisons.

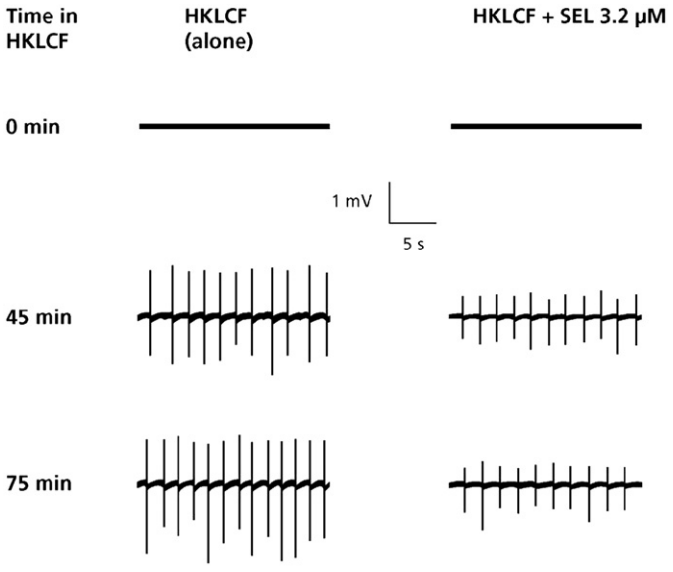

Fig. 3. HKLCF-induced spontaneous bursting in CA3 area of rat hippocampal slices and the effect of seletracetam (SEL) 3.2 µM (traces at right).

3. Results

3.1. Epileptiform activity in rat hippocampal slices

Elevating K^+ concentrations in the perfusion fluid from 3 mM to 7.5 mM, and lowering Ca^{2+} concentrations from 2.4 mM to 0.5 mM, produced epileptiform field potentials in the CA3 area of the slices, evoked upon constant-current fimbrial stimulation. The population spike amplitude gradually raised and bursts of repetitive population spikes (PS2, PS3 etc.) were evoked by single stimuli (Fig. 2).

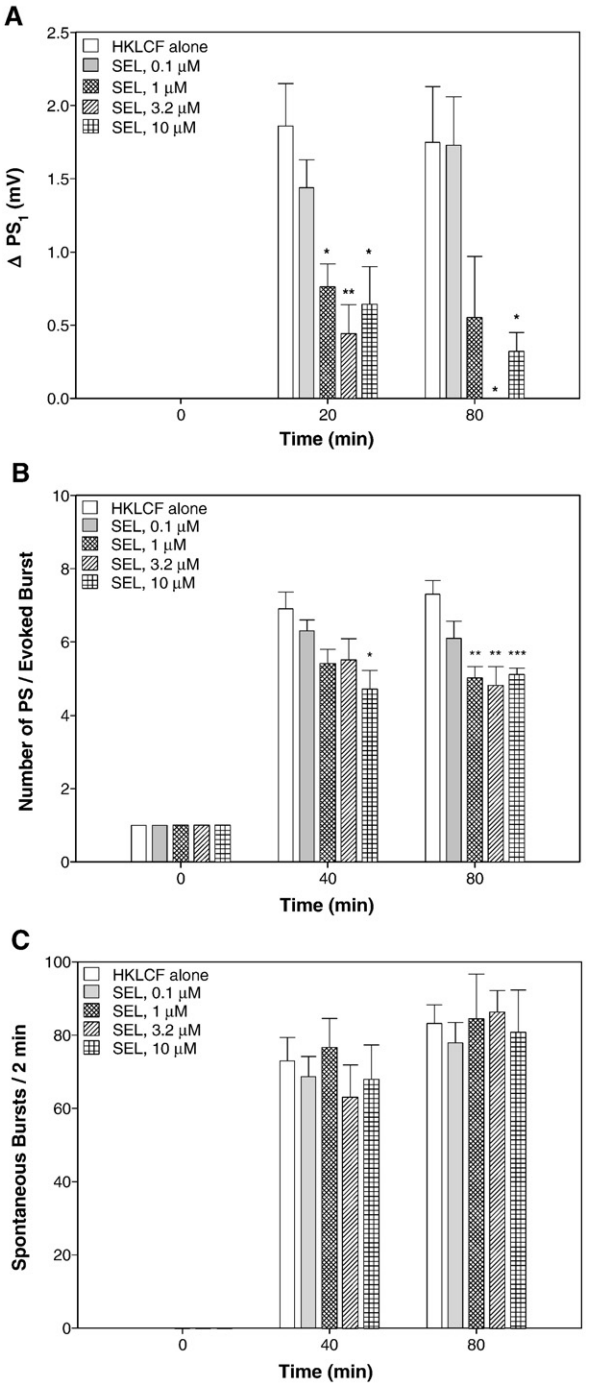

Fig. 4. Effect of seletracetam (SEL), 0.1–10 µM, on (A) the increase in amplitude of the first population spike (ΔPS1), (B) the number of repetitive population spikes per evoked burst induced by HKLCF and (C) the rate (number of bursts per 2 min intervals). Mean values ± SEM in groups of 10 slices/group. Significant differences with respect to the control (HKLCF alone) are indicated with *P<0.05, **P<0.005 or ***P<0.0005.

Table 1
Effect of seletracetam in various models of seizures and epilepsy.

Model	Predicting	ED_{50} (mg/kg i.p.)
Corneal kindling (mice)	Generalised motor seizures	0.31 (0.07–1.21)
Audiogenic seizures (mice)	Clonic convulsions	0.17 (0.09–0.29)
Maximal electroshock seizures (mice)	Tonic hindlimb extension	>232
Pentylenetetrazol (mice)	Clonic convulsions	>232 MAD (mg/kg p.o.)
Hippocampal kindling (rats)	Generalised motor seizures	0.23

Values in parentheses: 95% confidence interval. MAD: minimal active dose, the first dose inducing a significant reduction in seizure severity score *vs.* pre-drug value (P<0.05).

Seletracetam reduced the HKLCF-induced increase of population spike 1 (Fig. 2, traces at right). The reduction of the HKLCF-induced increase of population spike 1 induced by seletracetam appeared at around 10 times lower concentrations than previously observed for levetiracetam (Margineanu and Klitgaard, 2000). After more than 10 min in HKLCF, spontaneous bursts occurred regularly (Fig. 3).

Seletracetam, 1–10 µM, markedly decreased the HKLCF-induced increase in amplitude of the first population spike (Fig. 4A). The reduction of Δpopulation spike 1 induced by seletracetam, 10 µM, was however less than for 3.2 µM. The effect of seletracetam on reducing Δpopulation spike was superior to the reported effect of levetiracetam (Margineanu and Klitgaard, 2000). Seletracetam, 1–10 µM also depressed the number of HKLCF-induced repetitive population spikes (Fig. 4B). Seletracetam 0.1 µM concentration had only minor inhibitory tendencies on these epileptiform markers (Fig. 4). Seletracetam (0.1–10 µM) had no significant effect on the occurrence rate of spontaneous bursts (Fig. 4C), though it tended to reduce the spontaneous burst amplitude (see traces in Fig. 2).

3.2. Mice in vivo models of seizures and epilepsy

Seletracetam displayed potent protection against secondary generalised motor seizures in fully corneally-kindled mice (ED_{50} 0.31 mg/kg; i.p. adm) (Table 1). In audiogenic seizure susceptible mice, seletracetam protected against clonic convulsion expression with an ED_{50} value of 0.17 mg/kg i.p. (Table 1).

The seizure suppression by seletracetam in these chronic epilepsy models was markedly more potent than previously reported for levetiracetam (Gower et al., 1992; Klitgaard et al., 1998) and contrasts to its absence of anticonvulsant activity in the acute tests, in maximal electroshock seizures and in pentylenetetrazol-induced seizures (Table 1), as also previously observed with levetiracetam (Klitgaard et al., 1998).

3.3. Rat in vivo models of seizures and epilepsy

3.3.1. Hippocampal kindled rats

Seletracetam displayed a protection against secondary generalised motor seizures in hippocampal-kindled rats (minimal active dose 0.23 mg/kg; p.o.) (Table 1), and was markedly more potent than previously reported for levetiracetam (54 mg/kg; p.o.) (Matagne and Klitgaard, 1999).

3.3.2. Amygdala-kindled rats

The effects of seletracetam on after-discharge threshold and generalized seizure threshold are shown in Fig. 5, together with the after-discharge duration and seizure severity score recorded at the after-discharge threshold current. Seletracetam, administered i.p. 60 min before seizure threshold determination, significantly increased the after-discharge threshold current at doses of 0.0074 mg/kg (+86%) and 74 mg/kg (+104%) compared to respective control

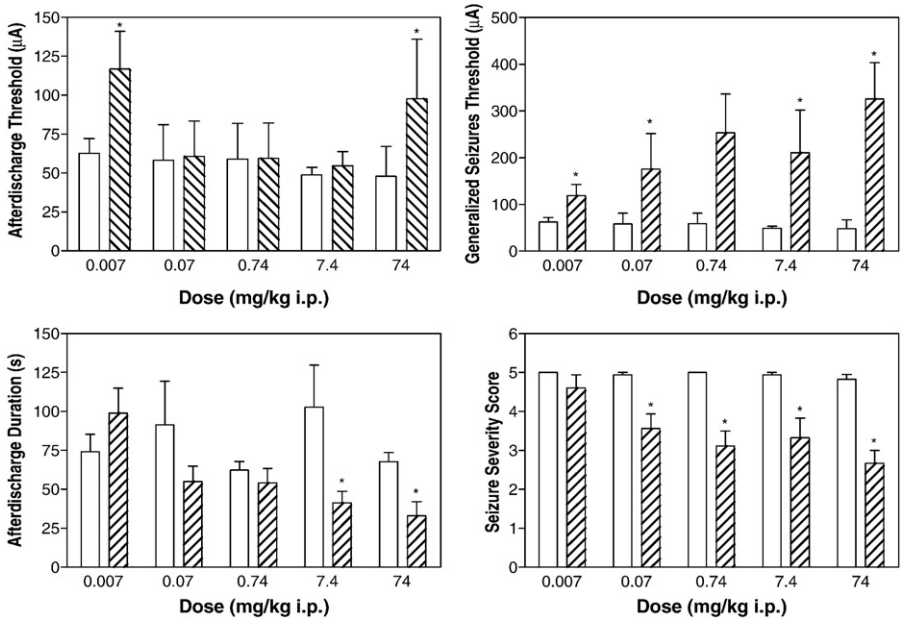

Fig. 5. Effect of seletracetam on after-discharge threshold and generalized seizure threshold in amygdala-kindled rats, and on after-discharge duration and seizure severity score recorded at after-discharge threshold. *Significant difference between vehicle and test substance ($P<0.05$).

values. However, no effect on the after-discharge threshold current was seen at doses of 0.074, 0.74 and 7.4 mg/kg. In another series of experiments with the same model, levetiracetam displayed a similar profile with the lowest (1.25 mg/kg) and highest (50 mg/kg) doses tested significantly increasing the after-discharge threshold current but the doses in-between (12.5 and 25 mg/kg) being inactive (data not shown). The duration of the after-discharge observed at the after-discharge threshold was significantly decreased by a seletracetam dose of 7.4 mg/kg, and the seizure severity was significantly reduced at doses ranging from 0.074 to 74 mg/kg (Fig. 5). Seletracetam had a markedly more potent effect than did levetiracetam on all parameters recorded in amygdala-kindled rats. In comparison, levetiracetam had similar effects but was less potent, and significantly increased the after-discharge threshold current at a dose of 12.5 mg/kg, decreased the after-discharge duration at a dose of 50 mg/kg, and reduced the seizure severity score at 12.5 mg/kg (data not shown).

As also shown in Fig. 5, seletracetam markedly increased the generalized seizure threshold at all doses tested, with increases of 190% (0.0074 mg/kg), 302% (0.074 mg/kg), 429% (0.74 mg/kg), 433% (7.4 mg/kg) and 679% (74 mg/kg) compared to control values. At a dose of 74 mg/kg, it was not possible to induce a generalized seizure in two animals even when increasing the stimulation strength to 840 μA, which was the technical limit of the stimulator used. In comparison, levetiracetam increased the generalized seizure threshold current at doses starting at 1.25 mg/kg and above (data not shown).

At the generalized seizure threshold current, the seizure and after-discharge durations were significantly reduced following administration of a dose of 7.4 mg/kg, while the after-discharge duration was increased at the lowest dose (0.0074 mg/kg) and seizure duration was increased at the highest applied dose of 74 mg/kg (not illustrated).

3.3.3. Genetic absence epilepsy rats from Strasbourg (GAERS)

Seletracetam, administered i.p. in doses from 0.02 mg/kg to 23.22 mg/kg, produced a significant and dose-related suppression of spontaneous spike-and-wave discharges in GAERS, with an ED_{50} value of 0.146 mg/kg i.p. This reveals a markedly more potent effect than previously reported for levetiracetam (Gower et al., 1995). As shown in Fig. 6, the effect appeared already during the first 20 min test interval, and persisted throughout the observation period up to 120 min. A statistically significant suppression of the duration of SWDs was observed from a dose of 0.23 mg/kg and a nearly complete suppression

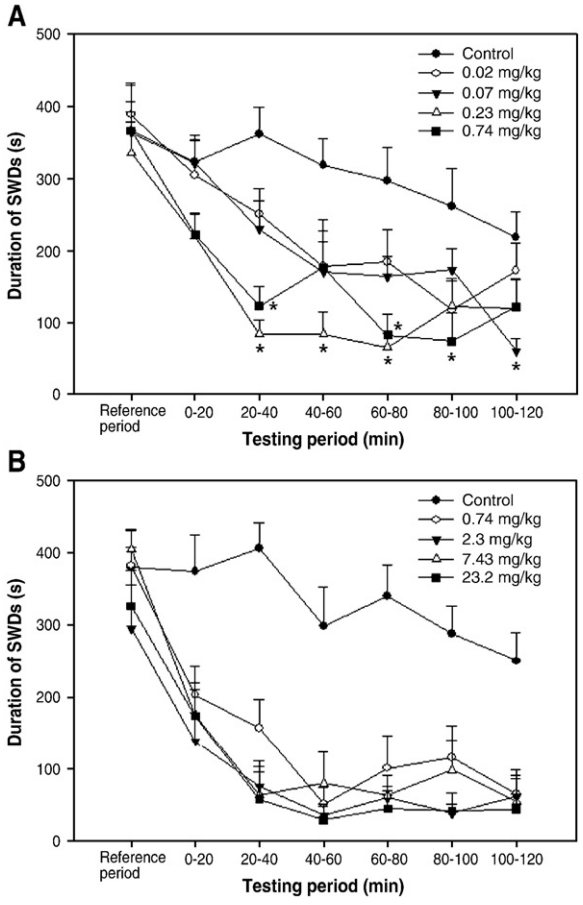

Fig. 6. Effect of seletracetam on spike-and-wave discharge duration in GAERS, for each 20-min post-injection period. Mean values ± SEM ($n=8$ per group). A: *significantly ($P<0.05$) different from the control group. B: all drug-treated groups were significantly ($P<0.05$) different from the control group.

Table 2
Effect of seletracetam, administered i.p. 60 min before the experiment, on rotarod performance.

Animals	TD_{50} (mg/kg)
Fully corneally-kindled mice	325 (no limits calculated)
Fully amygdala-kindled rats	520 (388–702)
Genetic absence epilepsy rats from Strasbourg	449 (295–842)

Values in parentheses: 95% confidence interval.

of spontaneous SWDs was observed at a dose of 23.2 mg/kg (Fig. 6). However, the statistical analysis was hampered by a relatively high variability of the recorded parameter and the low number of animals tested ($n=8$).

3.4. Rotarod performance in kindled mice and rats and GAERS

Seletracetam, administered i.p. 60 min before the experiment, dose-dependently impaired the performance of fully corneally-kindled mice in the rotarod test, with a TD50 value of 325 mg/kg (Table 2). Seletracetam also dose-dependently impaired the rotarod performance of fully amygdala-kindled and GAERS rats, with TD50 values of 520 mg/kg and 449 mg/kg, respectively (Table 2). Compared to the protective ED_{50} values obtained in the latter animals, this resulted in an unusually high CNS tolerability index of 1048 and 3075, superior to that for levetiracetam as well as other marketed antiepileptic drugs (Fig. 7). None of the animals assessed showed head weaving or stereotyped behaviours indicative of a potential for psychomimetic effects of seletracetam.

4. Discussion

In the CA3 area of rat hippocampal slices, the HKLCF milieu produced time-increasing rises of: 1) population spike amplitude, which indicates that a higher number of cells are firing synchronously during an action potential (Andersen et al., 1971), and of 2) the number of repeated population spikes, indicating that the neurons are firing bursts of several action potentials in response to single stimuli. The interictal-type spontaneous bursts induced by HKLCF account for spontaneous neuronal hyper-synchronization in the highly seizure-prone CA3 area of rat hippocampal slices. This study demonstrates a clear antiepileptic effect of seletracetam in this *in vitro* model of epilepsy, at concentrations approximately 10 times lower than those required for levetiracetam (Margineanu and Klitgaard, 2000). In this model, the antiepileptic activity of seletracetam was maximal at a concentration of 3.2 μM, which completely eliminated the HKLCF-induced increases in population spike amplitudes (Δpopulation spike 1). Seletracetam hereby differs from levetiracetam by a more pronounced ability to reduce population spike amplitudes, and even more from the classical antiepileptic drugs valproate, clonazepam and carbamazepine (Margineanu and Klitgaard, 2000). All this suggests a stronger effect of seletracetam than of levetiracetam on neuronal synchronization, and that desynchronization of epileptic activity may contribute a significant feature to seletracetam's antiepileptic properties.

Seletracetam induced a potent protection against seizure activity in sound-sensitive mice, which is a genetic animal model of generalized epilepsy. In fully corneally-kindled mice, where partial seizures with secondary generalization gradually are induced by chronic subconvulsive electroshocks, seletracetam also showed a potent ability to suppress generalized motor seizures. In both these two animal models of epilepsy, seletracetam demonstrated a markedly increased anticonvulsant potency compared to that of levetiracetam (Klitgaard et al., 1998). However, up to the high dose of 232.2 mg/kg, seletracetam did not reveal any significant anticonvulsant activity in the two classical screening tests for antiepileptic drugs, the maximal electroshock and pentylenetetrazol seizures tests. These results demonstrate that seletracetam has a profile similar to that of levetiracetam (Klitgaard et al., 1998; Löscher et al., 1998) in these acute mice models of seizures and epilepsy, but differs by a markedly increased potency. Amygdala kindling in rats is a reputed animal model of temporal lobe epilepsy, in which seizures with a focal/partial onset are induced by direct electrical stimulation of the amygdala. These seizures are characterized by a sequential development of behavioural patterns and electrical activity which originates from a partial phase, occurring within amygdala, and then develops into a generalized phase, expressing secondary generalized seizures. Seizure protection in this model is therefore considered to predict efficacy against partial complex seizures with secondary generalization in humans (Löscher and Schmidt, 1988). Seletracetam, in the doses from 0.0074 mg/kg to 74 mg/kg, induced a pronounced increase in the generalized seizure threshold current in amygdala-kindled rats, with an effect of 679% versus control values at the highest dose tested. The severity of the motor seizures elicited at after-discharge threshold current was reduced by a dose of 0.074 mg/kg, and the duration of the after-discharge was significantly decreased by a dose of 7.4 mg/kg. For comparison, in this model levetiracetam reduced the severity of the motor seizures recorded at the after-discharge threshold current at a dose of 12.5 mg/kg. These findings demonstrate significant anti-convulsant properties of seletracetam in fully amygdala-kindled rats, with an increased potency compared to that of levetiracetam. Taking into account the good predictability of the amygdala kindling model for drug efficacy against complex partial seizures in humans, these

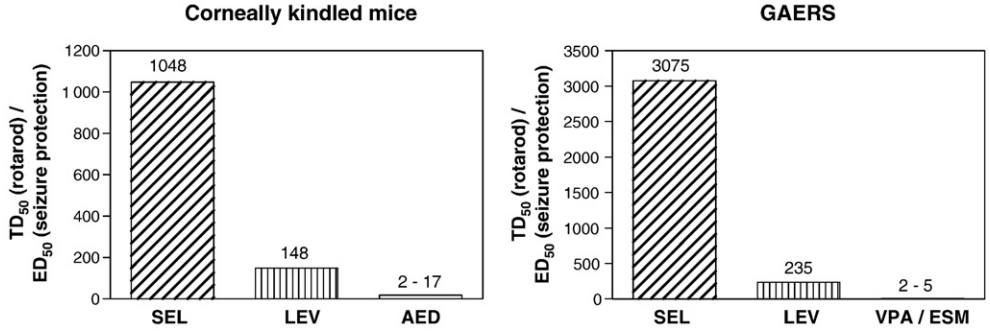

Fig. 7. CNS tolerability margin of seletracetam (SEL) in corneally-kindled mice and GAERS, calculated as the ratio of the TD_{50} value for rotarod impairment in these animals and the protective ED_{50} value against generalised seizure activity in the same animals. VPA valproate; ESM ethosuximide; AED antiepileptic drugs. Values for levetiracetam (LEV) and reference AEDs are from (Klitgaard et al., 1998).

results suggest that seletracetam should possess clinical activity against temporal lobe epilepsy in man.

Genetic absence epilepsy rats from Strasbourg represent a genetic animal model characterized by the appearance of spontaneous, cortical spike-and-wave discharges. Both the seizure phenomenology and pharmacology of this model mimic absence epilepsy in humans (Löscher and Schmidt, 1988). Seletracetam suppressed spontaneous spike-and-wave discharges recorded in genetic absence epilepsy rats from Strasbourg from the lowest dose tested (0.02 mg/kg; 32% reduction), and induced a marked inhibition at the highest applied dose (23.2 mg/kg; 80% reduction). In comparison, levetiracetam significantly suppressed SWDs from a dose of 5.4 mg/kg i.p. but did not completely inhibit these SWDs up to the highest dose tested (170 mg/kg i.p.) (Gower et al., 1995). These results demonstrate a more potent and complete seizure protection of seletracetam in this genetic animal model of absence epilepsy than observed for levetiracetam.

Previous studies demonstrated a high separation between doses of levetiracetam inducing significant rotarod impairment and seizure protection in corneally-kindled mice and GAERS, compared to classical and newer antiepileptic drugs as well as an absence of psychomimetic effects (Klitgaard et al., 1998). A behavioral assessment did not reveal any evidence for psychomimetic effects of seletracetam and the results of the rotarod testing suggest that seletracetam possesses a markedly improved CNS tolerability, compared to levetiracetam. Seletracetam impaired the rotarod performance of fully corneally-kindled mice with a TD50 of 325 mg/kg (Table 2); the corresponding TD50 value for levetiracetam was 1036 mg/kg (Klitgaard et al., 1998). This resulted in a tolerability index between doses inducing seizure protection and those inducing motor adverse effects in corneally-kindled mice of 1048 for seletracetam and 148 for levetiracetam. The corresponding ratios for several classical and other newly developed antiepileptic drugs were between 2 and 17 (Klitgaard et al., 1998). In fully amygdala-kindled rats, seletracetam impaired the rotarod performance with a TD_{50} value of 520 mg/kg (Table 1), compared to 1119 mg/kg for levetiracetam (Klitgaard et al., 1998). The very potent ability of seletracetam to elevate the threshold current for induction of generalized motor seizures in these animals resulted in a tolerability index of 70270, substantially higher than the ratio of 895 observed for levetiracetam.

The tolerability index of seletracetam in rats from the GAERS strain was determined as the ratio between impairment of the rotarod performance (TD50 449 mg/kg, Table 2) and the ED_{50} value (0.146 mg/kg) inducing a 50% reduction of the spike-and-wave discharge duration, which resulted in a high tolerability index of 3075. This ratio is higher than the corresponding of 235 for levetiracetam, and the corresponding ratios of 2 and 5 observed for valproate and ethosuximide, respectively (Klitgaard et al., 1998). These results demonstrate that seletracetam possesses a wide safety margin between doses inducing seizure protection and doses producing motor adverse effects in animal models mimicking partial or generalized epilepsy in man.

Seletracetam is similar to levetiracetam by its absence of protection against acute seizures but reveals superior anticonvulsant properties and a higher tolerability index in kindled and genetic animals. This appears to correlate a potent and complete suppression of neuronal hyper-synchronization suggesting that a more marked desynchronization of epileptiform activity may distinguish seletracetam from levetiracetam. The molecular correlate of this effect may relate to the one-log-unit higher affinity of seletracetam to SV2A, which probably represent its primary mechanism of action. However, it may also partly reflect that seletracetam differs from levetiracetam by a very potent and selective effect against Zn^{2+} inhibition of glycine-gated currents as well as a more potent inhibition of high-voltage-operated Ca^{2+} currents and epileptiform elevation of intracellular Ca^{2+} concentrations involving multiple high-voltage-operated Ca^{2+} channels (Klitgaard and Matagne, 2009).

The findings of the present study demonstrate that seletracetam induces a more potent and complete suppression of neuronal synchronisation than does levetiracetam in the *in vitro* HKLCF model of epilepsy. This appears to be related to an anti-seizure activity *in vivo*, superior to that of levetiracetam, against the expression of secondary and primary generalized seizure activity in animal models that mimic partial-onset (kindled animals) and generalized epilepsy (audiogenic seizure susceptible mice and GAERS). Furthermore, seletracetam showed no psychomimetic effects and a very high tolerability index in both kindled and GAERS rats, markedly superior to that of levetiracetam and other antiepileptic drugs. These results suggest that seletracetam may represent an effective and very well tolerated broad-spectrum agent for the symptomatic treatment of epilepsy.

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