Discovery of Levetiracetam (Keppra®): The first SV2A ligand for the treatment of epilepsy

Prof. Herik Klitgaard

Levetiracetam – Chemical Structure

(S)-α-Ethyl-2-oxo-1-pyrrolidine acetamide

CAS 102767-28-2

C₈H₁₄N₂O₂

Mol. weight: 170.21

Levetiracetam - Originally Discovered in Sound-sensitive Mice

Protective ED₅₀ values against clonic convulsions

- S-enantiomer: 17 mg/kg, i.p.
  31 µg/mouse, i.c.v.
- R-enantiomer: > 1400 mg/kg, i.p.
- Main metabolite: > 540 mg/kg, i.p.

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Antiepileptic Drug Discovery - A Strong Heritage of Animal Models

The MES and the CD₉₇ PTZ tests in rodents

<table>
<thead>
<tr>
<th>AEDs</th>
<th>MES 50 mA 0.2 s</th>
<th>CD₉₇ PTZ 89 mg/kg s.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valproate</td>
<td>168 106</td>
<td>106 106</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>12 11</td>
<td>12 5</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>&gt; 10 0.02</td>
<td>&gt; 10 0.02</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>6 &gt; 45</td>
<td>6 38</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>6 17</td>
<td>6 17</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>&gt; 452 &gt; 540</td>
<td>&gt; 254 117</td>
</tr>
<tr>
<td>Levetiracetam (2000)</td>
<td>&gt; 540 &gt; 540</td>
<td>7 36</td>
</tr>
</tbody>
</table>


Levetiracetam - Inactive in both the MES and S.C. PTZ Seizure Tests

<table>
<thead>
<tr>
<th>AEDs (first entry into market)</th>
<th>MES 50 mA 0.2 s</th>
<th>CD₉₇ PTZ 89 mg/kg s.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valproate (1967)</td>
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<td>&gt; 10 0.02</td>
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<td>Phenytoin (1938)</td>
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<td>Carbamazepine (1963)</td>
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<tr>
<td>Ethosuximide (1958)</td>
<td>&gt; 452 &gt; 540</td>
<td>&gt; 254 117</td>
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<td>Levetiracetam (2000)</td>
<td>&gt; 540 &gt; 540</td>
<td>7 36</td>
</tr>
</tbody>
</table>


Levetiracetam - Selective Action Against Kindled Electroshock and Kindled PTZ Seizures in Mice

<table>
<thead>
<tr>
<th>AEDs (first entry into market)</th>
<th>MES 50 mA 0.2 s</th>
<th>CD₉₇ PTZ 89 mg/kg s.c.</th>
<th>Electroshock</th>
<th>PTZ</th>
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<tbody>
<tr>
<td>Valproate (1967)</td>
<td>168 106</td>
<td>106 106</td>
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<td>Carbamazepine (1963)</td>
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<td>Ethosuximide (1958)</td>
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Levetiracetam - Protects Against Seizures in the Fully Amygdala-kindled Rat Model of Partial Epilepsy

Seizure Severity

After-Discharge Duration

All values are means ± S.D.

* and ** indicate significant statistical difference to individual control responses of P<0.05 and P<0.01, respectively.


Levetiracetam - Suppresses Spontaneous Spike-and-wave Discharges in the GAERS Model of Absence Epilepsy

Mean duration of SWDs (s)

Gower et al., Epilepsy Res. 22: 207-213, 1995

Levetiracetam – a High Therapeutic Index

Corneally-kindled mice

Antiepileptic drugs

Safety margin = Dose at which 50% of the animals fail to perform the rotarod test

Dose at which 50% of the animals are protected against seizures


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Levetiracetam - Absence of Negative Impact on Cognitive Function

Morris water maze test

- Mean total latencies to find the hidden platform, statistical significance: * P<0.05 vs. control group

Lamberty et al., Epilepsy & Behavior 1: 333-342, 2000

Levetiracetam - Absence of Negative Impact on Cognitive Function (2)

Morris water maze test

- Mean total latencies to find the hidden platform, statistical significance: * P<0.05 vs. control group

Lamberty et al., Epilepsy & Behavior 1: 333-342, 2000

Levetiracetam - Inhibits both Neuronal Hyper-synchronization and Hyper-excitability in Vitro

- Electrophysiological studies were performed both in vitro and in vivo


- Number of population spikes – measures hyper-excitability
- Population spikes amplitude – measures hyper-synchronization

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Levetiracetam - Distinct from Other AEDs by its Ability to Inhibit Hyper-synchronization in Vitro

- Hyper-excitability
  Levetiracetam, carbamazepine, valproate, and clonazepam decreased repetitive neuronal firing
- Hyper-synchronization
  Only levetiracetam reduced the number of cells firing synchronously (or amplitude) of the evoked PS

Levetiracetam – Significant and Persistent Effect Against the Development of Amygdala Kindling in Rats

Values given are the mean seizure severity score and after-discharge duration observed in each group during kindling development.

Levetiracetam - Effect Against the Appearance of Seizures in Genetic Models of Epilepsy

- Tonic convulsions in Spontaneously Epileptic Rat
  (Prof. Sasa, Hiroshima, Japan)
  Once daily administration of LEV (80 mg/kg i.p.)
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Levetiracetam - Chemical Structure and Pharmacological Profile
Distinct from Other AEDs

- Novel chemical structure
- Lack of anticonvulsant activity in traditional seizure models
- Potent broad spectrum activity with a wide safety margin in animal models of partial and generalized epilepsy
- Inhibition of neuronal hyper-synchronization
- Antiepileptogenic properties revealed in kindling and genetic models

Molecular Mechanisms Targeted in Antiepileptic Drug Discovery

- GABA$_A$ agonists
- Enhanced GABA levels
- K' channels modulators

Current Antiepileptic Mechanism of Action

Inactivation of Na$^+$ channels

Topiramate, Felbamate, Phenytoin, Lamotrigine, Zonisamide, Carbamazepine, and Valproate

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Current Antiepileptic Mechanism of Action (2)

Reduction of current through T-type Ca²⁺ channels

Levetiracetam - No Conventional GABAergic Effects

No effect on ... 
• The activities of the GABA-synthesizing enzyme GAD and GABA-degrading enzyme GABA-T in mouse brains
• GABA transport and metabolism in rat astrocyte culture
• GABA levels in rat hippocampus and frontal cortex
• Amplitude of GABA-induced current recorded by whole-cell patch-clamp in cultured rat hippocampal and cerebellar granule neurons

Molecular Mechanisms Targeted in Antiepileptic Drug Discovery

Epilepsy – a critical balance

Excitation

• Na⁺ channel antagonists
• Ca²⁺ channel antagonists
• Glutamate receptor antagonists

Inhibition

• GABA₄ agonists
• Enhanced GABA levels
• K⁺ channels modulators
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Levetiracetam Binding Site - Characteristics

- LBS is an integral membrane protein
- Brain and spinal cord specific
- Autoradiography shows ubiquity of LBS in brain gray matter
- High expression level
- Present in neuronal cell types and PC12 cells
- Experiments suggest no N-linked glycosylation
- Localization to synaptic vesicles
- Photoaffinity labeling identifies a 90 kDa protein

SV2 Proteins - Candidates for the Levetiracetam Binding Site

- Membrane glycoproteins of 80-100 kDa present in synaptic vesicles
- Three isoforms of SV2 protein exist:
  - SV2A (Bajjalieh et al., 1992; Feany et al., 1992)
  - SV2B (Bajjalieh et al., 1993)
  - SV2C (Janz and Südhof, 1999)
- SV2A is the most common isoform, expressed ubiquitously throughout the brain, present in secretory granules of endocrine cells
- SV2B expression is not as prevalent, but is co-expressed with SV2A in many neurons
- SV2C expression is less prevalent than 2A or 2B
- SV2A knockout mice experience seizures (Crowder et al., 1999; Janz et al., 1999)

SV2 Proteins as Levetiracetam Binding Site Candidates - Knockout Studies

[Image showing experimental results]

[3H]ucb 30889 binds only to wild type and knockout mouse brains containing the SV2A isoform

Lynch BA et al., Proc Natl Acad Sci USA 98 (26):15811-6, 2001
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SV2A Cloning and Binding Studies

- None of clinically-used AEDs, including valproate, carbamazepine, phenytoin, ethosuximide, felbamate, gabapentin, tiagabine, vigabatrin and zonisamide, at concentrations of up to 100 µM, competed with [3H]ucb 30889 for binding to SV2.

Correlation Between Affinities for hSV2A and Anti-seizure Potencies

Lynch BA et al., Proc Natl Acad Sci USA, 101(26): 9861-6, 2004

SV2A - a Novel Intracellular and Presynaptic Target

- At the cellular level, deletion of SV2A impairs neurotransmitter release
- Function of SV2 remains unknown
- Still remain to elucidate the mechanism by which levetiracetam and SV2A interact to prevent epileptiform activity

Lynch et al., Proc Natl Acad Sci USA 101: 9861-9866, 2004
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SV2A - Broad-spectrum Anticonvulsant Target

Audiogenic seizures pED50
SV2A pIC50

Corneal kindling pED50
SV2A pIC50

GAERS pED50
SV2A pIC50


Accelerated Kindling Acquisition in SV2A (+/−) Mice

Corneal Kindling

Pro-epileptic Phenotype of SV2A+/- Mice Matches with the Protective Activity of Levetiracetam

<table>
<thead>
<tr>
<th>Epilepsy model</th>
<th>SV2A phenotype difference</th>
<th>Protective activity of levetiracetam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala kindling</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- afterdischarge threshold</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- kindling development</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cornelia kindling</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Kainic acid</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>PTZ (iv)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>6 Hz</td>
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<td>Yes</td>
</tr>
<tr>
<td>MES</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Kaminski et al., Epilepsia, 50: 1729-1740, 2009

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Reduced Anticonvulsant Activity of Levetiracetam in SV2A(+/-) Mice – but Not of Valproate

SV2A - Target Validation
- SV2A affinity – anticonvulsant potency correlations in animal models of epilepsy indicate SV2A is a broad spectrum anticonvulsant target
- SV2A(-/-) mice have severe, spontaneous seizures
- Accelerated kindling acquisition in SV2A (+/-) mice
- Lower seizure threshold of SV2A(+/-) mice exclusively in epilepsy models where levetiracetam is active
- Reduced anticonvulsant activity of levetiracetam in SV2A(+/-) mice

Levetiracetam - the Preclinical Profile of a New Class of Antiepileptic Drugs
- Lack of anticonvulsant activity in traditional seizure models
- A potent broad spectrum activity with a wide safety margin in animal models reflecting both partial and generalized epilepsy
- Antiepileptogenic properties revealed in kindling and genetic models
- Inhibition of neuronal hyper-synchronization
- Novel, primarily SV2A, mechanism of action
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