The Science of Epilepsy: Mechanisms of Action of Drugs and Biologics

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Molecular Targets of Antiepileptic Drugs

- **Na⁺ Channels**
  - Phenytoin 1938
  - Carbamazepine 1974
  - Lamotrigine 1994
  - Fosphenytoin 1996
  - Oxcarbazepine 2000
  - Eslicarbazepine acetate

- **Ca²⁺ Channels**
  - Ethosuximide 1960

- **GABA_A Receptors**
  - Phenobarbital 1912
  - Primidone 1954
  - Clonazepam 1975

- **GABA Transporter**
  - Tiagabine

- **GABA Transaminase**
  - Vigabatrin 2009

- **Mixed**
  - Valproate 1978
  - Felbamate 1993
  - Topiramate 1996
  - Zonisamide 2000
  - Rufinamide 2009

- **α₂δ**
  - Gabapentin 1993
  - Pregabalin 2004

- **SV2A**
  - Levetiracetam 1999
  - Brivaracetam

- **KCNQ K⁺ Channel**
  - Retigabine

- **Na⁺ Channel Slow Inactivation**
  - Lacosamide 2008
Conventional wisdom —

• Epilepsy market is saturated

• Generic competition provides little opportunity for new entrants

• New drugs provide limited incremental benefit; a magic bullet is what is needed but there are no good ideas on how to find one

• Epilepsy drug development is a poor investment
Lyrica, an AED, is the Second Biggest Selling Product for the World’s Largest Pharmaceutical Company

- Lyrica global sales 2009 $2.8B, 10% increase from 2008
- Q4 $820MM
- Second most profitable product, after lipitor

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Outline for this Talk

• **Small Molecule AEDs** — two examples, pregabalin and levetiracetam: How are such drugs discovered?
  ᵇ Conclusion: to discover new and important agents, *position to take advantage of the unexpected.*

• **Non-conventional treatment approaches** – future of epilepsy therapy?
Drug Treatments for Epilepsy
History of Bromides

Sir Charles Locock, during discussion of paper by the neurologist Edward Sieveking at the Royal Medical and Chiurgical Society of London (May 12, 1857), made following points:

- Epilepsy may be caused by crowded teeth;
- The practice of masturbation may account for the great increase in epilepsy in recent years;
- There is a form of hysterical epilepsy connected with the menstrual period;
- Locock had read about a German who had become impotent taking 2 g of potassium bromide daily;
- Tried it in 14 or 15 women with menstrual epilepsy, only 1 remained uncured.

This occasion calls for exercise of imagination. Imagine that you are one of the nearly 730 fellows of the Royal Medical and Chiurgical Society of London and that you are attending the biennial session held on May 12, 1857. Lacking five months, this is just a century ago, this Society having been founded fifty-two years before. In 1857 it occupied the ground floor of the house at 55 Bernera Street; the average attendance this year is 46 members and 952 visiters, and yearly dues are 3 guineas. The twenty-sixth president of the Society is Sir Charles Locock, who is serving the second year of his two-year term (Fig. 1). Sir Charles had been elected to fellowship at the age of twenty-four in 1826. He is now fifty-eight years old, having waited thirty-two years before receiving the honor of the presidency. Over and above his present position is the more intimate and responsible one of being first physician in attendance to her Majesty, Queen Victoria. More mundane jobs include those of consulting physician to the Westminster Lying-in Hospital and member of the faculty of the University Hospital. He lives at 28 Euston Street, Mayfair (a house to be vandalized in 1927).

Froth on this occasion is being read by Dr. Edward Henry Sieveking, the neurologist, later to become Sir Edward, but now fellow of the Royal College of Physicians, physician to the late Duke of Cambridge, lecturer on materia medica and assistant physician at St. Mary's Hospital (Fig. 2). This is two years before the establishment of the National Hospital for the Paralysed and Epileptic, and seven years before Sieveking will become a member of its staff. His present paper includes an analysis of the remedies employed in the 15 most successful of 52 cases of epilepsy: zinc salts, purges, turions of antimony elixir to the neck, ceylonan umbilical, diuretics, tinctures, ferr and quinse cinnas, calomel, digitals, tr. ferris nitrici, morphina, OJ.儿茶, vincam ferris, astringa nitrazis and nitrazis argent.
Bromide used to treat epilepsy for 55 years until 1912, when phenobarbital was introduced.

We assume that the current process by which successful AEDs are discovered occurs on a more rational basis than in Locock’s time . . .
History of the Discovery of Gabapentin and Pregabalin

1977, **Gabapentin** synthesized as a BBB-permeable GABA analog by Satzinger a chemist at Gödecke AG

1989, **Rick Silverman**, Northwestern chemist, searching for a better GABA-T inhibitor synthesized **pregabalin**
Brain GABA (mM)

Control  Vigabatrin  Gabapentin  Pregabalin

[Errante & Petroff, Seizure 12, 300, 2003]
$\alpha_2\delta$ is an Auxiliary Subunit of Voltage-Activated Calcium Channels
Gabapentin, Pregabalin

Presynaptic

Postsynaptic

NMDA

AMPA

Kainate

α₂δ

Ca²⁺
Cacn2d2 (α₂δ) Knockout Mice Have Enhanced Seizure Susceptibility and Spontaneous Seizures

Pregabalin Activity in MES Test Is Less Effective in R217A Mutant Mouse

Mutation also eliminates analgesic and anxiolytic activity.

[Acknowledgement: C. Taylor, Pfizer]
History of the Discovery of Levetiracetam

UCB launched **Nootropil®** in 1972 for the treatment of memory and balance disorders (H1 2009 sales €37)

Keppra®
2008 sales €1.26B
20 mice placed in airtight cage; exposed to N₂ until only 3 of 20 survive in control group; (S)-Enantiomer (levetiracetam) 10-times more potent than racemate in increasing survival

(S)-Enantiomer 4-times more potent than racemate in protecting against lethality by ligature of carotids

Levoratatory (S)-enantiomer “more suitable for the treatment and prevention of hypoxic and ischemic type aggressions of the central nervous system.”
ucb L059, a novel anti-convulsant drug: pharmacological profile in animals

Alma J. Gower, Michel Noyer, René Verloes, Jean Gobert and Ernst Wülffert

UCB Pharmaceutical Sector, Chemin du Foriest, 1420 Braine l’Alleud, Belgium

Received 14 May 1992, revised MS received 2 July 1992, accepted 11 August 1992

The anticonvulsant activity of ucb L059 ((S)-α-ethyl-2-oxo-pyrrolidine acetamide) was evaluated in a range of animal models. ucb L059 was active after oral and intraperitoneal administration in both rats and mice, with a unique profile of action incorporating features in common with several different types of antiepileptic drugs. The compound was active, with ED50 values generally within the range of 5.0–30.0 mg/kg, in inhibiting audiogenic seizures, electrically induced convulsions and convulsions induced chemically by pentylentetrazole (PTZ). bicuculline, picrotxin and N-methyl-D-aspartate (NMDA). ucb L059 retarded the development of PTZ-induced kindling in mice and reduced PTZ-induced EEG spike wave discharge in rats. The R enantiomer, ucb L060, had low intrinsic anticonvulsant activity, showing the stereospecificity of action of the molecule although the actual mechanism of action remains unknown. Neurotoxicity, evaluated with an Irwin-type observation test, the rotarod test and open-field exploration, was minimal, with only mild sedation being observed, even at doses 50–100 times higher than the anticonvulsant doses; at pharmacologically active doses, the animals appeared calm but slightly more active. ucb L059 thus presents as an orally active, safe, broad-spectrum anticonvulsant agent, with potential antiepileptogenic and anti-absence actions.

1. Introduction

The term epilepsy refers to a wide range of neurological disorders characterised by an abnormal discharge of cerebral neurones. The prevalence of epilepsy is estimated at between 3 and 6 per 1000 (Griffin and Wyles, 1991; Sander and Shorvon, 1987). There is a general consensus for the need for new, improved drugs to treat epilepsies (Löschner and Schmidt, 1988; Porter, 1986). Although epilepsy is adequately con-

Launched in April 2000
Photoaffinity Labeling with [³H]3-Azidophenyl-levetiractam

SV2A Binding
Screened 12,000 analogs and other compounds

Levetiracetam

Seletracetam
Brivaracetam
Older Na\(^+\) Channel Blocking AEDs
(PHT, CBZ, LTG, zonisamide)

New AEDs
(levetiracetam, brivaracetam, gabapentin, PGB)

Presynaptic

Postsynaptic

NON-GABA-RELATED AEDs

Ca\(^{2+}\)

EPSP
47% of cases seizure free with first drug; treatable in 64% of cases. 36% are medically intractable.

[Brodie MJ. Do we need any more new antiepileptic drugs? Epilepsy Res 2001;45:3–6.]
Nontraditional Epilepsy Treatment Approaches

• **Physical Approaches**
  - Magnetic stimulation
  - Electrical stimulation (VNS, DBS, direct stimulation of focus)
  - Cooling
  - Optical (caged compounds, light sensitive channels)

• **Novel Delivery Approaches**
  
  *Invasive*
  - Intracerebroventricular
  - Transmeningeal delivery
  - Convection-enhanced delivery
  - Errodable microparticles
  - Encapsulated cells
  
  *Noninvasive*
  - Intranasal
  - Intrapulmonary
• **Cell Therapy**
  - Engineered adenosine-releasing cells (human mesenchymal stem cells and human ES cells)
  - Engineered GABA-releasing cells
  - GABAergic neural progenitors (embryonic medial ganglionic eminence)
  - Embryonic stem cell grafts

• **Gene Therapy**
  - Neuropeptide (galanin, NPY) overexpression using recombinant AAV vector
  - Knockdown of epileptogenesis genes
  - Restarting neuronal migration by reactivating developmental programs (subcortical band heterotopia)
Cell Transplantation
Adenosine Releasing Human Mesenchymal Stem Cells and ES Cell Grafts Prevent Spontaneous Seizures After Intraamygdaloid KA (Detlev Boison)

Intrahippocampal Transplant of Adk−/− ES Cell-Derived Neural Precursors

Lentiviral vector (micro-RNA) knockdown of Adk

Sham

Wild-type Neural Precursor Cells

Adk −/− Neural Precursor

[Not antiepileptogenic as reversed by adenosine antagonists]

[Li et al., J Clinical Invest 118:571–582, 2008]
NsGene EC Biodelivery Platform: Long-term Galanin Delivery

- Human genetically-modified cell line.
- Membrane allow influx of nutrients and outflow of galanin.
- No direct contact between therapeutic cells and host tissue
- Immune cells cannot access modified cells.
- >12 months; replaceable.
Conclusions

• To find the next useful (and profitable drug), position for the unexpected.

• Non-conventional epilepsy treatment approaches are entering the mainstream of epilepsy research.

• Non-conventional approaches may be amenable to “engineering.”

• “Magic bullets” of the future may not look like conventional small molecule AEDs.
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COLLABORATORS
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The End
Gene Therapy
rAAV Vector with NPY Gene Causes NPY Overexpression that Is Releasable by Depolarization

Enhanced NPY expression in surviving neurons and ectopic expression
Neuropeptide Y Gene Therapy in a Rat Model of Temporal Lobe Epilepsy

Increased Expression of NPY in the Hippocampus Is Associated Reduced Progression To Greater Seizure Frequency

AAV Transduces Post-Mitotic Cells with High Efficiency, Is Non-pathogenic, and Does Not Stimulate Immune Response
R217A Mutation Reduces Binding of Gabapentin and Pregabalin

\( \alpha_2\delta-1 \) Transiently Expressed in COS7 Cells

\[ ^{\text{3}}\text{H}\text{Pregabalin} \] binding

\[ K_D = 36 \text{ nM} \]

\[ K_D \approx 654 \text{ nM} \]

[Acknowledgement: C. Taylor & Z. Li, F. Bian, L. Walker, Pfizer]
Binding of $[^3\text{H}]$Gabapentin to Recombinant $\alpha_2\delta$ Subunits Transiently Transfected Into COS-7 Cells

$K_d$, 59 nM

Lyrica, an AED is the Second Biggest Selling Product for the World’s Largest Pharmaceutical Company

- Lyrica global sales 2009 $2.8B, 10% increase from 2008
- Q4 $820MM
- Second most profitable product, after lipitor
Evoked EPSC in EC Neurons

Gabapentin
S-(+)-3-isobutyl-GABA

Pregabalin
S-(−)-3-isobutyl-GABA

R-IBG
S-(−)-3-isobutyl-GABA

Evoked EPSC in EC Neurons

GBP (20 μM)

PGB (20 μM)

R-IBG (20 μM)

mean eEPSC amplitude (pA)

Control
GBP
Control
PGB
Control
R-IBG

[Cunningham et al., Eur J Neurosci 20:1566–1576, 2004.]
The Molecular Target of Levetiracetam Is SV2A, a Synaptic Vesicle Protein

SV2 is an abundant component of all synaptic vesicles, not essential for synaptic transmission.

Normal Synapse Structure in Hippocampal Neurons from SV2A Knockout Mice

SV2B Knockout (Control)  SV2A/SV2B Knockout

Type 2 synapse

[Janz et al., Neuron 24:1003-1016, 1999]
SV2A Knockout Eliminates Specific (Levetiracetam Displaceable) [3H]ucb 30889 Binding

[3H]ucb 30889 bound

Wild-type

SV2A knockout

+ 1 mM Levetiracetam

Anticonvulsant Efficacy of Levetiracetam is Reduced in SV2A-Deficient Mice

Change in threshold in 6 Hz model.

Correlation Between Binding Affinity and Protective Potency of SV2A Ligands

- Audiogenic Seizures
  - \( r^2 = 0.77 \)

- Corneal Kindling
  - \( r^2 = 0.8 \)

- Absence Seizures
  - \( r^2 = 0.72 \)