Anticonvulsant and Improved Side-Effect Potential of LY3130481: The First γ-8-Selective Transmembrane AMPA Receptor Regulatory Protein (TARP) Receptor Antagonist

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Abstract

AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors are a primary protein involved in epileptogenic phenomenon and the AMPA receptor antagonist, perampanel (®Fycompa), was recently approved in Europe and the United States for the treatment of partial seizures. Transmembrane receptor regulatory protein (TARP) g-8- is an auxiliary protein associated with some AMPA receptors and has high brain localization in the hippocampus. We hypothesized that we could maintain the antiepileptic functionality while eliminating the motoric side effects inherent in globally-acting (non-TARP-dependent) AMPA receptor antagonists (i.e., perampanel) with a small molecule selectively targeting forebrain AMPA receptors. Specifically, AMPA receptors associated with TARP g-8- (high localization in hippocampus associated with seizures) while simultaneously avoiding pharmacological interaction with TARP g-2- (high localization in cerebellum associated with dizziness and motor coordination). We discovered LY3130481 (6-[(1S)-1-[1-[5-(2-hydroxyethoxy)-2-pyridyl]pyrazol-3-yl]ethyl]-3H-1,3-benzothiazol-2-one)), an orally-bioavailable compound and the first molecule rationally designed to target selective regions of the central nervous system while providing a relative sparing of interactions with non-desired brain regions. LY3130481 produced broad-ranging anticonvulsant efficacy in rodents including in models utilizing both acute and subchronic aconvulsant stimulation and either chemical or electrical stimuli. As AMPA receptors are involved in sensitization within the central nervous system, the blockade of seizure sensitization (kindling) was also demonstrated, thereby revealing a potential for disease modification of epilepsy. Moreover, the relative sparing of cerebellar Perkinje neurons from blockade of AMPA currents translated to a marked reduction in deleterious effects on motor function. Given these findings, the door has opened to the utilization of the TARP scaffolding proteins as a means of designing brain region-specific interactions along with the large therapeutic potential it offers.

Introduction

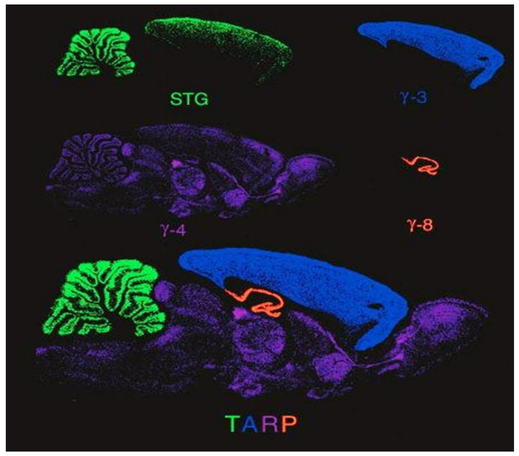
A general principle of medicinal therapy posits that the selectivity of a drug for its protein receptor is required for the specificity of target-directed biological activity (Paul Erlich, 1908 Nobel Prize). In neurology and psychiatry, drugs that impact specific neural pathways are predicted engender enhanced efficacy and reduced side-effects than those with actions across the nervous system. The diversity of proteins and their localization in specific neural circuits creates an opportunity for the discovery of selective medicines. Unfortunately, the rational discovery of molecules that selectively engage disease-impacted neural pathways is difficult due to the high amino acid sequence homology of differentially-localized receptor subtypes. We present here, for the first time, proof of principle that proteins associated with drug receptors can be used to create highly specific modulation of disease-relevant biology for therapeutic gain.

α-amino-3-hydroxy-5-methyl- 4-isoxazolepropionic acid (AMPA)-type glutamate receptors are cation channels controlling fast excitatory synaptic transmission throughout the brain. AMPA receptors are associated with a number of auxiliary proteins, including transmembrane AMPA receptor regulatory proteins (TARPs) that regulate receptor pharmacology and trafficking. TARPs are differentially expressed in specific brain regions. TARP γ-8 is expressed in forebrain regions with enrichment in hippocampus but not in cerebellum where TARP γ-2 predominates.

Perampanel (Fycompa), used to control refractory partial seizures, blocks cerebellar AMPA receptors with similar potency and efficacy to hippocampal receptors and induces sedation and ataxia in rodents and dizziness and falling in patients. We hypothesized that a molecule that selectively attenuates AMPA receptor-mediated synaptic transmission in the forebrain with minimal blockade in the cerebellum would be antiepileptic without impacting the control of motor coordinating functions in the cerebellum.

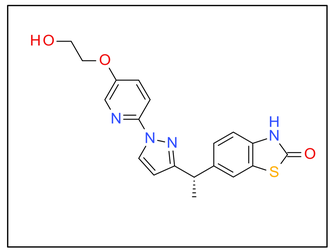
We first developed a method for screening compound libraries for compounds blocking iGluA1+TARP g-8 while having minimal ability to block iGluA1+TARP γ-2 (see POSTER 207.16/A91). From this screening tool, we found molecules with which to apply iterative medicinal chemistry methods to ultimately discover LY3130481 (see POSTER 207.16/A92). LY was fully characterized as a selective antagonist of AMPA receptors associated with TARP γ-8 without potent impact upon γ-2 (see POSTER 207.16/A93). We here provide proof of concept that LY3130481 is anticonvulsant without motor impairing effects. LY3130481 was also shown to be efficacious in multiple models predicting efficacy as a pain therapeutic (see POSTER 207.16/A94).

Localized expression of TARP-γ8 to hippocampus potentially avoids motor control impairment – a significant differentiation from non-selective AMPA receptor antagonists.



LY3130481 is a potent and selective antagonist of AMPA receptors associated with TARP γ-8

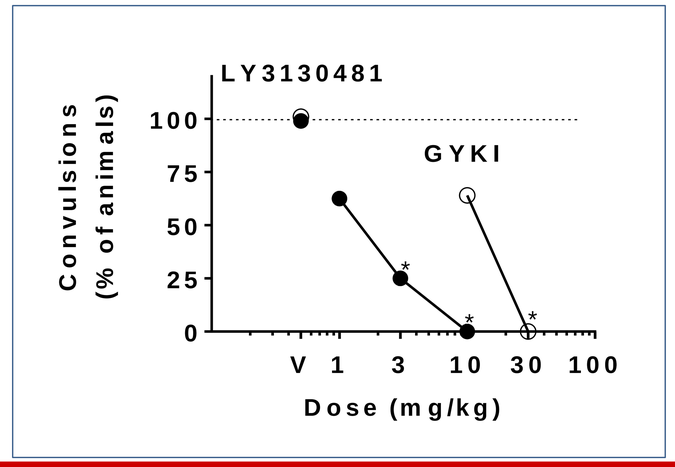
LY3130481



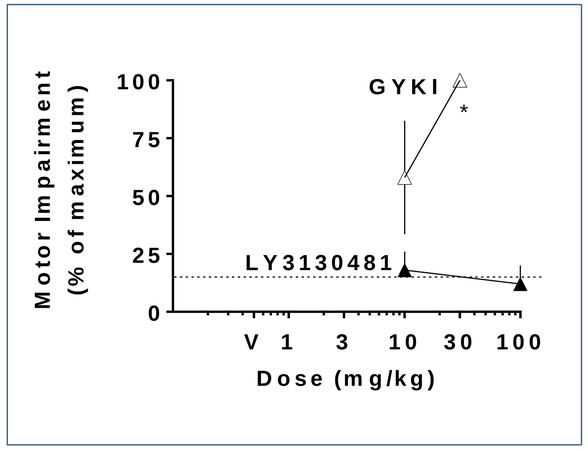
Potency and Selectivity

Target	Activity uM (SD, n)
hIGlu1-γ8 Antagonist	0.065 (0.04,30)
hIGlu1-γ8 Potentiator	68.5 (5.66, 24)
hIGlu1-γ2 Antagonist	>100 (5.7, 28)
hIGlu1 Antagonist	>83uM (0, 26)
hIGlu3 Antagonist	>100 (0,2)
hIGlu4 Antagonist	>100 (0,2)
hIGlu6 Antagonist	>100 (0,2)

LY3130481 (po) and the non-TARP-dependent AMPA receptor antagonist GYKI52466 (ip) prevent penylenetetrazole-induced clonic convulsions in rats



The non-TARP-dependent AMPA receptor antagonist GYKI52466 (ip) is motor impairing at anticonvulsant doses in rats; LY3130481 (po) is not

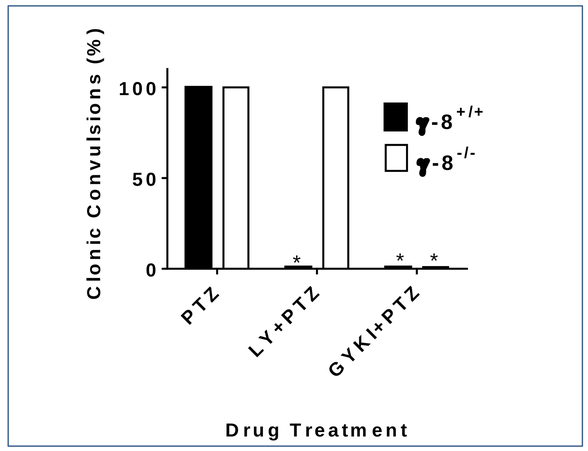


LY3130481 Demonstrates Efficacy in Multiple Acute Anticonvulsant Models

Assay	Potency
PTZ - Rat	1.7 (0/3-11.5)
Inverted Screen- rat	>100
PTZ - Rat	3.6 (2.0-6.2)
PTZ Rat	4.0 (1.6-7.3), i.p.
Motor Tox. Rat	>500, i.p.
PTZ Mouse	<3, i.p.
PTZ Mouse	8.0 (4.5-12), i.p.
Tonic Seizures and Lethality from PTZ - rat	10 mg/kg
6Hz Stimulation Mouse, 32mA	18 (9.8-30), i.p.
6Hz Stimulation Mouse, 44mA	23 (13-34), i.p.
Motor Tox. Mouse	>500, i.p.
MES Mouse	>300, at 0.5h
Fringes Mouse	4.4 (2.78- 6.5), i.p.
Bicuculine Mouse	>150 ip, 30 min
Pictrotoxin Mouse	Efficacious – quantitation pending

Doses are oral unless otherwise noted. Data are ED_{50} (95% confidence limits) or minimal effective dose in mg/kg. If 95% confidence limits are not shown, they could not be calculated based upon the dose-effect curve.

Deletion of TARP g-8 in mice eliminates the anticonvulsant efficacy of LY3130481 but not that of the non-TARP-dependent AMPA receptor antagonist GYKI52466



LY3130481 Demonstrates Efficacy in Multiple Chronic Kindling Anticonvulsant Models

Assay	Potency
Corneal Kindling - Mouse	4.6 (1.8-7.0), i.p.
Hippocampal Kindling — Rat	16 mg/kg, i.p. active
Lamotrigine-Insensitive Kindled Rat	5 mg/kg, i.p. inactive; data at higher doses pending
PTZ Kindling Mouse – Acquisition	<30
PTZ Kindling Mouse – Development	<30
PTZ Kindling Mouse — Fully Kindled	<30
Amygdala Kindling rat — After Discharge Threshold	MED =10
Amygdala Kindling rat — After Discharge Duration	6.4 (4.9 -8.4)
Amygdala Kindling rat — Seizure Severity	7.9 (4.6-14)
Amygdala Kindling rat — 400 µA Stimulation	MED =10

Doses are oral unless otherwise noted. Data are ED_{50} (95% confidence limits) or minimal effective dose in mg/kg. If 95% confidence limits are not shown, they could not be calculated based upon the dose-effect curve.

Conclusions

- LY3130481 is the first known antagonist that selectively binds AMPA receptors in the hippocampus excluding cerebellar AMPA receptors.
  - LY3130481 shows a lack of motor impairment at efficacious exposures in animal models.
- LY3130481 might demonstrate antiepileptic efficacy in patients without the motor-impairing effects of non-selective AMPA receptor antagonists
- **Therefore, LY3130481 presents a unique opportunity to**
  - **provide greater efficacy than non-selective AMPA receptor antagonists**
  - **require reduced or no dose ramp-up to achieve efficacious blood levels**

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