



Galanin Receptors Modulate Seizures

Galanin Receptor 1 Deletion Exacerbates Hippocampal Neuronal Loss After Systemic Kainate Administration in Mice.

Schauwecker PE. *PLoS One* 2010;5(12):e15657.

BACKGROUND: Galanin is a neuropeptide with a wide distribution in the central and peripheral nervous systems and whose physiological effects are mediated through three G protein-coupled receptor subtypes, GalR1, GalR2, and GalR3. Several lines of evidence indicate that galanin, as well as activation of the GalR1 receptor, is a potent and effective modulator of neuronal excitability in the hippocampus. **METHODOLOGY/PRINCIPAL FINDINGS:** In order to test more formally the potential influence of GalR1 on seizure-induced excitotoxic cell death, we conducted functional complementation tests in which transgenic mice that exhibit decreased expression of the GalR1 candidate mRNA underwent kainate-induced status epilepticus to determine if the quantitative trait of susceptibility to seizure-induced cell death is determined by the activity of GalR1. In the present study, we report that reduction of GalR1 mRNA via null mutation or injection of the GalR1 antagonist, galantide, prior to kainate-induced status epilepticus induces hippocampal damage in a mouse strain known to be highly resistant to kainate-induced neuronal injury. Wild-type and GalR1 knockout mice were subjected to systemic kainate administration. Seven days later, Nissl and NeuN immune-staining demonstrated that hippocampal cell death was significantly increased in GalR1 knockout strains and in animals injected with the GalR1 antagonist. Compared to GalR1-expressing mice, GalR1-deficient mice had significantly larger hippocampal lesions after status epilepticus. **CONCLUSIONS/SIGNIFICANCE:** Our results suggest that a reduction of GalR1 expression in the C57BL/6J mouse strain renders them susceptible to excitotoxic injury following systemic kainate administration. From these results, GalR1 protein emerges as a new molecular target that may have a potential therapeutic value in modulating seizure-induced cell death.

GalR2-Positive Allosteric Modulator Exhibits Anticonvulsant Effects in Animal Models.

Lu X, Roberts E, Xia F, Sanchez-Alavez M, Liu T, Baldwin R, Wu S, Chang J, Wasterlain CG, Bartfai T. *Proc Natl Acad Sci U S A* 2010;107(34):15229–15234; Comment 14943–14944.

Galanin receptors type 1 (GalR1) and/or type 2 (GalR2) represent unique pharmacological targets for treatment of seizures and epilepsy. Previous studies have shown that the endogenous peptide ligand galanin exerts powerful anticonvulsant effect through activation of these two G protein-coupled receptors, which are highly expressed in the temporal lobe of rodent brain. Here we report the characterization of a putative GalR2-positive allosteric modulator CYM2503. CYM2503 potentiated the galanin-stimulated IP1 accumulation in HEK293 cells stably expressing GalR2 receptor, whereas it exhibited no detectable affinity for the (125)I galanin-binding site of GalR2 receptor, an effect consistent with that of a positive allosteric modulator. In the rat Li-pilocarpine status epilepticus model, CYM2503, injected intraperitoneally, increased the latency to first electrographic seizure and the latency to first stage 3 behavioral seizure, decreased the latency to the establishment of status epilepticus, and dramatically decreased the mortality. In a Li-pilocarpine seizure model in mice, CYM2503 increased the latency to first electrographic seizure and decreased the total time in seizure. CYM2503 also attenuated electroshock-induced seizures in mice. Thus, CYM2503 provides a starting point for the development of anticonvulsant therapy using the galanin R2 receptor as target.

Commentary

Galanin is a 29 amino acid peptide (30 in humans), which is widely distributed in the brain. Galanin has been implicated in multiple physiological actions such as appetite, mood,

anxiety, and learning. Galanin is also believed to play a role in diseases including epilepsy (1). Much of the knowledge about galanin's role in epilepsy comes from studies of status epilepticus involving the hippocampus. The hippocampus and, particularly, the dentate gyrus receive a rich innervation of galanin-containing fibers. Cholinergic fibers from septum contain galanin in addition to acetylcholine. Noradrenergic fibers from locus caeruleus innervating the hippocampus also contain the neuropeptide. Galanin appears to be secreted

Epilepsy Currents, Vol. 11, No. 4 (July/August) 2011 pp. 125–127
© American Epilepsy Society

OPEN ACCESS Freely available online



during high frequency stimulation and modulates neurotransmitter release from the presynaptic terminals.

Initial evidence of a role for galanin in hippocampal seizures came from studies by Mazarati and colleagues (2). When galanin or its analogs were infused into the hippocampus, it prevented or terminated status epilepticus. Furthermore, status epilepticus itself depleted galanin in the hippocampus. Thus, there were effects of galanin on seizures and effects of seizures on galanin itself. Of interest, repeated seizures can induce galanin expression in neurons and interneurons over a period of time (2).

Further evidence for the role of galanin in seizures and epilepsy has come from mice overexpressing galanin and receptor knockouts, and the development galanin receptor-specific drugs. Galanin binds to three kinds of receptors: GalR-1, GalR-2, and GalR-3. Both GalR-1 and GalR-2 receptors are expressed in the hippocampus. GalR-1 receptors are linked to a pertussis toxin-sensitive G-protein, which inhibits the synthesis of cyclic AMP. The GalR-2 receptor is linked to G-proteins, which are pertussis toxin insensitive and appear to modulate inositol phosphate (IP) synthesis and turnover. These receptors also have other actions (3).

The seizure threshold is raised in mice that oversynthesize the neuropeptide. In contrast, when the GalR-1 receptor is knocked out, approximately 25% of the animals have seizures spontaneously. The remaining mice with this gene knocked out had increased sensitivity to seizures. In galanin-overexpressing mice, there is a reduced release of glutamate following high-frequency stimulation, suggesting that galanin may be reducing neurotransmitter release. Other studies report reduced excitability of postsynaptic neurons in galanin-overexpressing mice. Reduction of GalR-2 receptors by RNA knock down also results in increased susceptibility of seizures. Other studies have used the galanin gene to modulate seizures. Galanin gene delivered by adeno associated virus (AAV) into the hippocampus reduces the severity and the intensity of kainate-induced seizures (4). Thus, multiple converging lines of evidence suggest an important role for galanin in modulating seizures. However, several questions remain unresolved with regards to the role of galanin in seizures and the potential use of galanin as an anticonvulsant.

Small molecule drugs that cross the blood brain barrier to modulate galanin receptors have not been available. Most analogs used in the experimental studies were small peptide fragments of the large galanin molecule, which do not cross the blood brain barrier. These peptides have limited therapeutic value for treating epilepsy. Furthermore, the goal of gene therapy with galanin remains in the distant future until the challenges of safe and effective gene delivery to central neurons are overcome. A practical solution to these problems would be to find nonpeptide molecules that would bind to specific galanin receptors and terminate seizures. However, the identity of the receptor that modulates seizures is unclear, and previous studies would suggest that GalR-1 receptors would be the targets for drug development.

The recent article by Lu et al. makes progress in this regard in a surprising direction. CYM 2503 was identified by screening compounds for activity on GalR-2 receptor-ex-

pressing Human Embryonic Kidney (HEK) cells, as a nonpeptide allosteric modulator of GalR-2 receptors. The compound increased IP accumulation in the presence of galanin but had limited intrinsic effect. It shifted the galanin concentration response curve to the left but did not displace it from binding sites.

Peripheral (intraperitoneal) administration of CYM 2503 suppressed seizures. The authors confirmed the efficacy of this new nonpeptide modulator of GalR-2 receptors in the maximal electroshock model. It increased latency to hind limb clonus, suppressed seizures, and decreased mortality. In a model of lithium pilocarpine-induced status epilepticus, it CYM 2503 was as effective as levetiracetam in terminating seizures. The treatment of status epilepticus was carried out without giving benzodiazepines. The treatment of status epilepticus almost always involves initial treatment with benzodiazepines, and a second line drug given in combination with these drugs. It is possible that the analog would have terminated status epilepticus in all animals if it had been used in combination with benzodiazepines.

Schauwecker's study describes another emerging role for galanin receptors (5). An important consequence of prolonged seizures or status epilepticus is the loss of principal neurons of the hippocampus and interneurons in the hilus. This seizure-induced neuronal loss is believed to be mediated by excitotoxins. Schauwecker and colleagues in a seminal study demonstrated that susceptibility to neuronal loss caused by excitotoxins is genetically determined (5). Certain genetic background mice are resistant to excitotoxic cell death, whereas others are susceptible. In a systematic set of studies over a decade, the group has searched for the genes that confer susceptibility to seizure-induced cell death. The region of interest was localized to an expanse on mouse chromosome 18 that contains the GalR-1 receptor gene.

Schauwecker demonstrates in this study that animals resistant to excitotoxic cell death become sensitive to it when the GalR-1 receptor is knocked-out. These GalR-1 knockouts were characterized in detail, and they did demonstrate compensatory overexpression of GalR-2 receptors or galanin. Latency, severity, and duration of severe seizures were similar in wild-type and knock-out mice. Pharmacologic block of GalR-1 receptors also rendered wild-type mice susceptible to neuronal damage.

These studies suggest that the susceptibility to excitotoxic cell death is mediated at least in part by the GalR-1 receptor. It is possible that there are polymorphisms or mutations of the GalR-1 receptor in susceptible mice, which render the receptor less effective. However, it is also possible that the second messenger systems activated by GalR-1 receptors are less sensitive to galanin activation. Further studies are needed to figure out the molecular mechanisms of susceptibility. However, these findings are very exciting because they begin to unravel the mystery of differential sensitivity to excitotoxic cell death.

Finally, the two studies together raise further questions about the role of GalR-1 and GalR-2 receptors in seizures and epilepsy. The study by Lu et al. suggests that GalR-2 receptors are important for seizure termination and should be targeted for further drug development. Presumably, prolonged



seizures were responsible for excitotoxic cell death observed in mice. However, the study by Schauwecker suggests that GalR-1 receptors should be targeted for preventing cell death. It is possible that generation of seizures and generation of cell death are mediated by two distinct pathways even though both may involve glutamenergic transmission. A further careful analysis of electrographic seizures in these knock-out mice and perhaps generation of GalR-2 receptor knock-out mice will address these questions. Overall, these studies point to galanin receptors as an important therapeutic target for treatment of seizures and status epilepticus and perhaps a pathway towards preventing seizure-induced cell death in people.

by Jaideep Kapur, MD, PhD

References

1. Lundstrom L, Elmquist A, Bartfai T, Langel U. Galanin and its receptors in neurological disorders. *Neuromolecular Med* 2005;7:157–180.
2. Mazarati AM, Liu HT, Soomets U, Sankar R, Shin D, Katsumori H, Langel U, Wasterlain CG. Galanin modulation of seizures and seizure modulation of hippocampal galanin in animal models of status epilepticus. *J Neurosci* 1998;18:10070–10077.
3. Branchek TA, Smith KE, Gerald C, Walker MW. Galanin receptor subtypes. *Trends Pharmacol Sci* 2000;21:109–117.
4. Haberman RP, Samulski RJ, McCown TJ. Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. *Nat Med* 2003;9:1076–1080.
5. Schauwecker PE, Steward O. Genetic determinants of susceptibility to excitotoxic cell death: implications for gene targeting approaches. *Proc Natl Acad Sci U S A* 1997;94:4103–4108.

2011

AMERICAN EPILEPSY SOCIETY

65TH ANNUAL MEETING

IMPORTANT DEADLINES

October 28.....Early Bird Discount

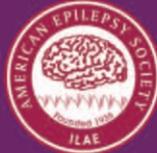
October 30.....Hotel Reservations

November 17.....Pre-Registration

www.AESNET.org

BUILDING
FOR THE
FUTURE

65TH ANNUAL
MEETING



December 2-6, 2011
Baltimore, MD

75
years
1936-2011

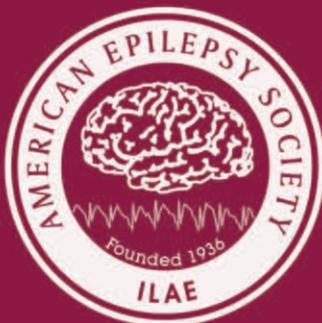
Committed to
Research,
Education &
Training

BALTIMORE, MD

BALTIMORE CONVENTION CENTER

December 2 - 6, 2011

Future Annual Meeting Dates



2012

San Diego, CA

San Diego Convention Center
November 30 – December 4

2013

Washington, D.C.

Washington Convention Center
December 6 – 10

2014

Seattle, WA

Washington State Convention and
Trade Center
December 5 – 9

2015

Philadelphia, PA

Pennsylvania Convention Center
December 4 – 8

2016

Houston, TX

George R. Brown Convention Center
December 2 – 6



American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Section #1 Identifying Information

1. Today's Date: _____ April 5 th 2011 _____
2. First Name _____ Jaideep _____ Last Name _____ Kapur _____ Degree ____ MD, PhD _____
3. Are you the Main Assigned Author? Yes No
If no, enter your name as co-author _____
4. Manuscript/Article Title: _____ Galanin receptors modulate seizures _____
5. Journal Issue you are submitting for: _____ 11.4 _____

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking "No" or providing the requested information. If you have more than one relationship just add rows to this table.

| Type | No | Money Paid to You | Money to Your Institution* | Name of Entity | Comments** |
|---|----|-------------------|----------------------------|----------------|------------|
| 1. Grant | X | | | | |
| 2. Consulting fee or honorarium | X | | | | |
| 3. Support for travel to meetings for the study or other purposes | X | | | | |
| 4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | X | | | | |
| 5. Payment for writing or reviewing the manuscript | X | | | | |
| 6. Provision of writing assistance, medicines, equipment, or administrative support. | X | | | | |
| 7. Other | | | | | |

* This means money that your institution received for your efforts on this study.

** Use this section to provide any needed explanation.

Section #3 Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the “Add” box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

| Type of relationship (in alphabetical order) | No | Money Paid to You | Money to Your Institution* | Name of Entity | Comments** |
|--|----|-------------------|----------------------------|----------------|---|
| 1. Board membership | X | | | | |
| 2. Consultancy | X | | | | |
| 3. Employment | X | | | | |
| 4. Expert testimony | X | | | | |
| 5. Grants/grants pending | X | | | | |
| 6. Payment for lectures including service on speakers bureaus | | | X | Pfizer | Spoke at ILAE Merrit Putnam in 2009 June. |
| 7. Payment for manuscript preparation. | X | | | | |
| 8. Patents (planned, pending or issued) | | | | UVA | GABA receptor antibody anti δ and γ2 subunits |
| 9. Royalties | | | X | UVA | GABA receptor antibody anti δ and γ2 subunits |
| 10. Payment for development of educational presentations | X | | | | |
| 11. Stock/stock options | X | | | | |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed.** | X | | | | |
| 13. Other (err on the side of full disclosure) | X | | | | |

* This means money that your institution received for your efforts.

** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

Section #4 Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

No other relationships/conditions/circumstances that present a potential conflict of interest.

Yes, the following relationships/conditions/circumstances are present:

Thank you for your assistance.
Epilepsy Currents Editorial Board