

A PROMISING GENETIC APPROACH FOR THE TREATMENT OF EPILEPSY

Recombinant AAV-mediated Expression of Galanin in Rat Hippocampus Suppresses Seizure Development

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Galanin, a 29- or 30-amino acid neuropeptide, has been implicated in the modulation of seizures. In this study, we constructed a recombinant adeno-associated viral (AAV) vector to constitutively overexpress galanin (AAV-GAL). The vector mediated efficient transduction of HEK 293 cells *in vitro* and robust galanin expression *in vivo* when injected into the rat dorsal hippocampus. Rats were administered kainic acid intrahippocampally 2.5 months after AAV-GAL or empty vector (AAV-Empty) injection to study the effect of vector-mediated galanin overexpression on seizures. AAV-GAL-injected rats showed a decreased number of seizure episodes and total time spent in seizures compared with AAV-Empty rats, despite similar latencies to development of the first EEG seizure and similar levels of neuronal damage in the CA3 region for both groups. These data show that recombinant AAV mediates strong and stable overexpression of galanin when injected into the rat hippocampus, resulting in a significant anticonvulsive effect. The seizure-suppression effect of galanin expression in the hippocampus by viral vectors may lead to novel therapeutic strategies for the treatment and management of intractable seizures with focal onset, such as temporal lobe epilepsy.

Attenuation of Seizures and Neuronal Death by Adeno-associated Virus Vector Galanin Expression and Secretion

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Seizure disorders present an attractive gene-therapy target, particularly because viral vectors such as adeno-

associated virus (AAV) and lentivirus can stably transduce neurons (1–3). When we targeted the *N*-methyl-D-aspartic acid (NMDA) excitatory amino acid receptor with an AAV-delivered antisense oligonucleotide, however, the promoter determined whether focal seizure sensitivity was significantly attenuated or facilitated (4). One potential means to circumvent this liability would be to express an inhibitory neuroactive peptide and constitutively secrete the peptide from the transduced cell. The neuropeptide galanin can modulate seizure activity *in vivo* (5,6), and the laminar protein fibronectin is usually secreted through a constitutive pathway (7,8). Initially, inclusion of the fibronectin secretory signal sequence (FIB) (9) in an AAV vector caused significant gene product secretion *in vitro*. More important, the combination of this secretory signal with the coding sequence for the active galanin peptide significantly attenuated *in vivo* focal seizure sensitivity, even with different promoters, and prevented kainic acid-induced hilar cell death. Thus neuroactive peptide expression and local secretion provide a new gene-therapy platform for the treatment of neurologic disorders.

COMMENTARY

Focal epilepsies, such as temporal lobe epilepsy, are often difficult to control with medication, and seizures persist in approximately 35% of patients (1). In some cases, it is possible to identify and successfully remove the seizure focus by using surgery. However, in $\geq 20\%$ of cases, surgery fails to control the seizures and many patients cannot be treated surgically because of the risk of loss of brain function. Therefore novel treatment approaches are needed, and recent studies show that gene therapy may be a realistic option for the treatment of refractory focal epilepsies.

Gene therapy, as a treatment for focal epilepsy, requires a safe, efficient method for delivering genes to specific regions in the brain. Adeno-associated virus (AAV) is a nonpathogenic DNA virus that has a unique set of characteristics that make it useful for human gene therapy. AAV vectors have the ability to deliver long-term gene expression without toxicity or immune response. In addition, the ability to infect nondividing

cells make AAV vectors ideal for delivering genes to the central nervous system. But what genes do we deliver? What genes will protect neurons against the hyperexcitability that leads to seizures? Two recent reports have shown promising results in rat models of epilepsy. In both studies, an AAV vector was used to deliver the neuropeptide galanin.

Galanin, a 29-amino-acid neuropeptide widely distributed in the central nervous system, has previously been shown to be involved in seizure activity. Galanin expression levels are altered during seizure activity; blocking galanin receptors causes seizures; and in rats, injection of galanin during status epilepticus has powerful anticonvulsant effects (2). In addition, mice overexpressing galanin show increased resistance to seizures (3). The importance of galanin for seizure control may arise from its ability to inhibit glutamate release by means of activation of presynaptic, adenosine triphosphate (ATP)-dependent K^+ channels, thus dampening seizure activity (4).

Lin et al. injected an AAV vector carrying the galanin gene into the dorsal dentate hilus of the hippocampus of rats. Two and a half months after unilateral injection, high levels of galanin (an increase of >200%) were observed around the injection site. Galanin was detected in hilar interneurons and dentate granule cells of the hippocampus, demonstrating successful delivery and expression of the galanin gene. Galanin also was observed in the inner third of the molecular layer of the dentate gyrus in the contralateral hippocampus, suggesting that galanin can be efficiently transported and may exert its action beyond the focal injection site. By using a kainic acid (KA) model of epilepsy, Lin et al. demonstrated that rats that were pretreated with AAV-galanin had fewer seizures, with less ictal spiking activity. The time to seizure onset was unaltered, suggesting that galanin has a role in the maintenance rather than in the initiation of seizures. Analysis of cell numbers within the injected hippocampus showed that galanin did not protect the rats against KA-induced neuronal damage.

Haberman et al. also used an AAV vector carrying the galanin gene to study seizure control in a rat model; however, their approach was slightly different from that of Lin et al. They added a fibronectin secretory signal sequence to the galanin gene, so that galanin was not only expressed but also secreted by neurons. Electrical stimulation threshold for seizure genesis increased significantly in rats that received prior cortical injections of AAV-galanin, and this resistance to seizures was evident only when the secretory signal sequence was included.

Similar to the study by Lin et al., Haberman et al. studied the effect of galanin gene therapy on KA-induced seizures. Injection of AAV-galanin into the hilar region of the hippocampus before KA treatment did not alter the time to onset or severity of seizures. Lin et al. also found no alteration in the time to

seizure onset, but they did find a reduced number of seizures. Haberman et al. did not report a decrease in the number of seizures; however, closer analysis of seizure duration and severity may reveal subtle changes associated with AAV-galanin injection. In contrast to the findings of Lin et al., hilar neurons around the injection site showed no cell death. This effect was seen only when the fibronectin signal sequence was used to induce secretion of galanin, suggesting that increased extracellular concentrations of galanin are required to protect neurons. The reduction in cell death is not associated with reduced seizure activity, which was unaltered. It was not determined whether normal function was retained in the surviving cells.

To summarize, both these reports show that the neuropeptide galanin can be efficiently delivered to the mammalian brain by using a viral vector and that the resulting increase in galanin expression suppresses seizure activity. Galanin may be suppressing seizures by presynaptic inhibition of glutamate release from principal hippocampal neurons or by inhibition of synaptic potentiation in hippocampal circuits. Haberman et al. also demonstrated that by using a signal sequence that induces secretion of galanin, neurons are protected against KA seizure-induced cell death. Further studies in animal models are required to determine the safety and efficacy of long-term galanin gene expression, and many questions must be resolved before human trials can begin.

One important issue is whether galanin gene therapy can suppress seizures in rats that have had seizures for many months before the treatment. In both reports, gene therapy was carried out before seizures were induced. Patients with intractable epilepsy have an established seizure focus; therefore to parallel more closely the situation in humans, galanin gene therapy should be tested in rats with established spontaneous seizures, such as kindling models. Certain safety issues also must be addressed before human trials. Haberman et al. showed that attachment of the fibronectin signaling sequence to galanin improves resistance to seizures and reduces neuronal damage; however, this procedure results in production of a novel protein that could produce an inflammatory response in humans.

Long-term gene expression is crucial for the successful use of gene therapy to treat focal seizures in humans, as repeated brain injections could be detrimental. Haberman et al. demonstrated that galanin was expressed 4 months after vector injection, and this finding must be followed up to determine exactly how long galanin gene expression persists. This information also would help address the question of whether any side effects are associated with the overexpression of galanin. Galanin coexists with classic transmitters in several systems, and evidence exists for involvement of galanin in multiple neuronal functions. It has been suggested that elevated expression of galanin in the forebrain contributes to Alzheimer's disease (5); therefore it is

critical that overexpression of galanin be limited to the site of seizure focus. Whereas galanin did suppress seizures in the rat epilepsy models, it did not stop seizures completely, raising the question of whether galanin is an appropriate gene to use for human therapy. Human galanin is not identical to galanin of other species and may have a variety of biologic properties that differ from those of the rat peptide.

In conclusion, gene therapy may be an effective treatment for intractable focal seizures, provided a suitable gene can be safely and effectively delivered. Rather than undergoing resective surgery, patients could opt for injection of a vector carrying a therapeutic gene such as galanin. Ideally, this would be a one-time procedure, resulting in localized, long-term gene expression and successful control of seizures.

by Robyn Wallace, Ph.D.

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