

# ON DEMAND UP-REGULATION OF THERAPEUTIC GENES IN THE BRAIN: FICTION OR REALITY?

**Enhancing GABA<sub>A</sub> Receptor  $\alpha$  1 Subunit Levels in Hippocampal Dentate Gyrus Inhibits Epilepsy Development in an Animal Model of Temporal Lobe Epilepsy** Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR. *J Neurosci* 2006;26(44):11342–11346. Differential expression of GABA<sub>A</sub> receptor (GABR) subunits has been demonstrated in hippocampus from patients and animals with temporal lobe epilepsy (TLE), but whether these changes are important for epileptogenesis remains unknown. Previous studies in the adult rat pilocarpine model of TLE found reduced expression of GABR  $\alpha$ 1 subunits and increased expression of  $\alpha$ 4 subunits in dentate gyrus (DG) of epileptic rats compared with controls. To investigate whether this altered subunit expression is a critical determinant of spontaneous seizure development, we used adeno-associated virus type 2 containing the  $\alpha$ 4 subunit gene (GABRA4) promoter to drive transgene expression in DG after status epilepticus (SE). This novel use of a condition-dependent promoter upregulated after SE successfully increased expression of GABR  $\alpha$ 1 subunit mRNA and protein in DG at 1–2 weeks after SE. Enhanced  $\alpha$ 1 expression in DG resulted in a threefold increase in mean seizure-free time after SE and a 60% decrease in the number of rats developing epilepsy (recurrent spontaneous seizures) in the first 4 weeks after SE. These findings provide the first direct evidence that altering GABR subunit expression can affect the development of epilepsy and suggest that  $\alpha$ 1 subunit levels are important determinants of inhibitory function in hippocampus.

## COMMENTARY

The development of gene transfer techniques *in vivo* has allowed for modification of cell phenotype either by ectopic expression of foreign peptides/proteins or by increased expression or suppression of endogenous molecules. These approaches can be exploited to study the function of specific genes in a variety of tissues and organs or to treat or eliminate the causes of some diseases. The delivery of genes into the CNS provides a special challenge because of the blood–brain barrier (which precludes entry of various xenobiotics, thus impairing noninvasive routes of gene delivery); the inaccessibility of various deep brain regions; and the nonmitotic nature of most cells, which prevents use of gene transduction methods that require DNA integration. Neurotropic adeno-associated viral (AAV) vectors represent one of the best tools for gene delivery to the CNS, because they provide long-term neuronal expression in a controllable manner and are nonpathogenic. Thus far, clinical applications for gene transfer to the CNS have been developed for Parkinson's and Alzheimer's disease ([\[nlm.nih.gov/medlineplus/genesandgenetherapy.html\]\(http://www.nlm.nih.gov/medlineplus/genesandgenetherapy.html\)\), while a protocol for neuropeptide Y gene transfer in epilepsy is under evaluation at the U.S. Food and Drug Administration.](http://www.</a></p></div><div data-bbox=)

Two distinct goals are relevant to the design of a gene therapy approach for epilepsy: first, to achieve an anticonvulsant effect (to suppress spontaneous recurrent seizures), and second to provide an antiepileptogenic effect (to prevent the development of epilepsy). Most antiepileptic drugs control neuronal hyperexcitability by decreasing excitatory neurotransmission or by enhancing inhibitory neurotransmission (1). A decrease in GABA-mediated inhibition resulting from molecular changes in the GABA<sub>A</sub>-receptor complex is one of the maladaptive alterations in brain injury that can contribute to epileptogenesis. Accordingly, reduced expression of GABA<sub>A</sub>-receptor  $\alpha$ 1 subunits concomitant with increased  $\alpha$ 4-subunit was found in the dentate gyrus of surgical specimens of temporal lobe epilepsy patients (2,3). These receptor subunit modifications also occur in adult rats experiencing status epilepticus (SE) (2), while opposite changes were observed in neonatal rats undergoing SE but not developing epilepsy (4).

Using this information, Raol and collaborators tested the hypothesis of a causal link between changes in GABA<sub>A</sub>-receptor  $\alpha$ 1 subunit and the development of epilepsy. They introduced the GABA<sub>A</sub> receptor  $\alpha$ 1 subunit gene (GABR  $\alpha$ 1) into the

rat hippocampus, where it remained quiescent until an injury-dependent stimulus (i.e., SE) was provided. The human minimal GABA<sub>A</sub>-receptor  $\alpha 4$ -subunit gene promoter was applied to drive the transcription of the *GABRA1* gene (encoding GABR  $\alpha 1$ ) in an AAV vector cassette, since it previously was identified that this promoter activity is upregulated in dentate gyrus after SE (5). The promoter chosen in the work of Raol et al., however, did not provide long-term expression of the transgene, as upregulation of the GABR  $\alpha 1$  subunit was observed in the hippocampus at 14 days after SE, rapidly declining thereafter. The reason for this transient effect still is unresolved but several potential mechanisms exist, including promoter silencing or transduced cell loss. Transcriptional down-regulation of the transgene from depletion of transcriptional factors (e.g., brain-derived neurotrophic factor or early growth response factor 3) appears unlikely since depletion should have, but did not, affected the levels of the GABR  $\alpha 4$  subunit. Influences of the immune system also are unlikely because the inhibitory effect of anti-AAV antibodies on transduction in the brain is very limited (6).

Other promoters, such as the cytomegalovirus/chicken  $\beta$ -actin promoter, can provide AAV-mediated transgene expression for up to 9 months in rodents, particularly when regulatory elements are included to increase the steady-state levels of the mRNA (7). However, this promoter cannot be induced by SE, but permits stable enhanced expression of the transgene independent of the injurious event. Another approach is regulation of the promoter activity by including a tetracycline-sensitive cassette, which has been successfully used to modulate the AAV-mediated galanin (*GAL*) gene expression in the rat brain (8). In this study, the elevated expression of galanin, an anticonvulsant peptide, in the inferior colliculus increases the threshold of wild-running seizures triggered by local electrical stimulation. However, when doxycycline, which binds to the tetracycline cassette, was added to drinking water, *GAL* gene transcription and consequent protein synthesis were blocked and the threshold to seizures decreased to baseline levels within 1 week. This effect was reversed upon doxycycline removal, highlighting the possibility of switching on or off a specific gene.

The choice of the vector serotype for transgene delivery is important since the viral capsid protein composition can influence vector spread in the injected tissue and the type of cells transduced (9,10); the type and extent of the cells transfected will determine the functional outcome. Eleven distinct AAV serotypes have been isolated to date but the transduction properties of the vast majority have not yet been characterized in the brain. AAV vectors generally have preferential uptake by neurons (15), indicating that the lack, or minimal expression in glia, is not due to absence of promoter activity. Raol et al. chose the AAV5 serotype capsid because by binding to the platelet-derived growth factor- $\alpha$  receptor it preferentially targets neurons.

Raol and colleagues demonstrate that the *GABRA1* gene was transcribed following AAV vector injection by measuring the corresponding mRNA levels; additionally, by western blot analysis they showed that the corresponding protein was synthesized in tissue. Also important is to determine whether the protein is expressed in the proper cell compartment (e.g., at the cell membrane for a receptor protein) and to identify, by immunohistochemistry, the cell populations in the hippocampus where GABR  $\alpha 1$  subunit is increased, as the location will determine the functional outcome. An elegant study by Haberman et al. showed that the NMDA-receptor gene antisense sequence leads to down-regulation of the receptor protein in the rat collicular cortex—either in GABA interneurons or in excitatory output neurons (depending on the AAV vector cassette promoter used), resulting in a decrease or increase in focal seizure sensitivity, respectively (11). Therefore, although neurotransmitter receptors and ion channels represent an obvious target for inhibition of seizures, a detailed knowledge of the expression patterns in the injected area is required so that the therapeutic strategy can be properly designed to avoid an undesired increase in seizure sensitivity.

Raol et al. induced SE in rats 2 weeks after the vector injection to evaluate whether epileptogenesis was impaired and found no difference in the amount of pilocarpine needed to provoke seizures or SE onset between controls and rats injected with the vector. This is a crucial finding since SE represents the injurious event leading to epilepsy and, theoretically, onset should not be altered by the experimental conditions. An analysis of the EEG characteristics during epileptic activity and quantification of relevant parameters could demonstrate unequivocally that SE was unaltered by experimental manipulations. The authors found a significantly lower percentage of AAV-*GABRA1* injected rats developing spontaneous behavioral seizures within 4 weeks of SE induction as compared to control rats. EEG analysis was performed on a subgroup of rats, revealing that behavioral seizures were invariably associated with EEG seizures. The authors acknowledge that the conservative interpretation of their results is that overexpression of the GABR  $\alpha 1$  subunit of the receptor in the hippocampus retards the occurrence of spontaneous seizures in a substantial population of rats. However, whether transgene overexpression produces an anticonvulsant effect or a true antiepileptogenic effect remains unclear and requires a longer follow-up study of spontaneous seizures, possibly including a viral vector construct that produces a more persistent elevation of the transgene. In addition to the effects on seizures, these authors analyzed the presence of side effects in rats overexpressing the transgene and found that 30% of the rats showed sedation, anorexia, and weight loss, raising the concern that brain functions may be significantly affected when gene expression is modified.

Numerous studies now have demonstrated that gene therapy interventions in acute and chronic models of seizures result in anticonvulsant effects, may be antiepileptogenic, and may afford neuroprotection (8,10–14). Although moving from pre-clinical research to clinical applications requires that several concerns be addressed, these experimental findings open the possibility of developing novel therapeutic strategies for the treatment of intractable seizures with focal onset, such as temporal lobe epilepsy, and possibly for preventing symptomatic epilepsies.

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