Parkinson's disease gene therapy

GAD Zooks! Excitement to inhibition in one easy step?

HJ Federoff

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In a recent issue of *Science*, Matt During's group presents intriguing results that highlight an imaginative new gene therapy approach for treating Parkinson's disease (PD).¹

The rationale behind the researchers' approach is that by delivering glutamic acid decarboxylase (GAD) genes to an excitatory neuron they can convert it into an inhibitory neuron. To fully appreciate this rationale and to assess how successful this approach could be for treating PD, it is vital to review several key tenets of neuroscience.

The mammalian central nervous system comprises excitatory and inhibitory neurons organized into networks. The neurotransmitters glutamate and GABA are, respectively, the principal vehicles for excitation and inhibition. In neurons, these neurotransmitters are loaded into synaptic vesicles that when they depolarize are released into synaptic clefts. After release, these neurotransmitters diffuse, interact with specific receptors, trigger alterations in cellular

ion channels and are then rapidly cleared by reuptake mechanisms.

In inhibitory neurons, GAD converts glutamate into GABA. Specific machinery in these neurons regulates the release and synaptic uptake of GABA. Synapses involving inhibitory neurons are morphologically distinct from excitatory synapses. Presumably, the morphological differences between the two groups also reflect biochemical differences.

In PD, the dopamine projections from the substantia nigra pars compacta (SNc) to the striatum are lost (Figure 1). In a widely accepted model for the disease, the reduced dopamine in the striatum results in 'disinhibition' (reduced GABA neurotransmission) in the subthalamic nucleus (STN). This, coupled with direct disinhibition from the striatum itself, results in increased glutamate neurotransmission and thus excessive excitation of the major basal ganglia output nuclei, the substantia nigra pars reticulata (SNr) and the globus pallidum internal segment (GPi).

In this model of PD, the STN overdriving of the GPi/SNr decreases activity in the thalamus leading to reduced excitation of the cortex that, in turn, causes characteristic symptoms such as tremors and rigidity (Figure 1). PD therapies have therefore targeted the silencing of the hyperexcited STN or GPi/SNr.

Neurosurgical approaches that silence either the GPi or STN can control PD symptoms. These approaches are not uniformly successful, but do suggest other therapeutic avenues. One alternative is to increase GABA-mediated inhibition of the STN. Studies on both monkeys and humans show that drugs such as lidocaine and muscimol can effectively silence the excessively active nucleus.^{2,3} However, while these experimental pharmacological studies demonstrate that PD symptoms can be controlled they also show that these drugs can cause other motor side effects.

During and his colleagues are, in effect, also taking a pharmacologic approach in their new study, but are delivering the therapeutic agent via genetic means. In a rat model of PD, lesioned with 6-hydroxydopamine (6OHDA), they examined the effects of delivering the genes for both isoforms of GAD into the STN with a recombinant adeno-associated virus vector (rAAV). Several of the findings of this study are noteworthy.

First, the investigators showed that gene therapy with GAD65 but not GAD67 led to an increase in GABA release in SNr. Second, GAD65 gene therapy shifted SNr single-unit recording responses from predomi-

Figure 1 Model of basal ganglia function in unaffected, parkinsonian subjects and after GAD65 gene therapy. The schemata show the functional organization of the basal ganglia circuitry: (a) Normal, (b) Parkinsonian and (c) Parkinsonian after GAD65 gene transfer. The loss of dopamine in the striatum results in (b) diminished inhibitory tone at the STN. Lacking adequate inhibitory tone the STN is hyperactive and produces excessive stimulation of GPi/SNr and its consequent inhibition of thalamus causing the reduction in cortical outflow. GAD65 gene transfer to STN (c) is believed to phenocopy excitatory (glutamate releasing) to inhibitory (GABA releasing) neurons. The provision of GABA in the GPi/SNr quiets these nuclei and restores normal thalamic tone. (Figure adapted from Parent et al.⁷).

nantly excitatory to inhibitory. Third, both histological and behavioral tests showed delivering GAD65 genes 3 weeks before administration of 6OHDA protected rats from its neurotoxic effects.

It seems, then, that GAD65 gene therapy can, effectively, convert excitatory neurons to inhibitory neurons and that this is sufficient to alter the neurotransmitter milieu of at least the SNr. Most of the transduced neurons would be those with cell bodies within the STN nucleus, where the genes were delivered. However, results from other studies using rAAVs suggest that it is possible that other neurons projecting to STN may also have been transduced via retrograde transfer.⁴⁻⁶ It is still unclear to what extent this has occurred and what impact it could have in the long term on the intended beneficial actions within the STN.

The microdialysis and electrophysiology data from the SNr certainly suggest that transduction of STN excitatory neurons resulted in *de novo* synthesis of GABA, its transport to presynaptic terminals and release in the SNr. However, further experiments are required to confirm this and many questions remain to be answered.

For example, do excitatory neurons possess the machinery to load GABA into synaptic vesicles? Does the GABA compete with glutamate for vesicle loading on a

stoichiometric basis? Do excitatory neurons manifest compensatory mechanisms to up-regulate glutamate vesicular loading, and if so, does this exceed the transduced GAD's biosynthetic capacity to generate GABA? If GABA is found in excitatory neuron vesicles, do they dock and recycle as would those delivering glutamate? In the absence of a GABA uptake mechanism in normally excitatory STN neurons, which cells in the SNr would internalize and metabolize GABA? Additionally, which receptor types and on which SNr neurons is the GABAergic neurotransmitter response mediated?

The neuroprotective effect of GAD65 expression in limiting toxicant-induced damage is interesting. The authors propose that the effective conversion of excitatory to inhibitory neurons in the SNr is the critical factor behind this effect. However, one possible alternative explanation is that forced GAD65 expression reduces 6OHDA uptake, attenuating its toxicant action. Thus in the future, we really need to investigate the mechanism(s) behind this strikingly robust neuroprotective effect.

The novel approach to treating PD that During and his colleagues have employed is clearly promising and points the way to numerous new avenues of research. In particular, we need to find out what are the long-term consequences of effectively

converting excitatory neurons to inhibitory neurons.

There is no doubt that this approach is an attractive strategy for silencing hyperactive excitatory neurons. However, given the inherent plasticity of the central nervous system, the likelihood of lifelong rAAV expression, and the need to treat human PD for many years it is vital to comprehensively evaluate this innovative strategy for longer time periods in animal models prior to considering its use as a clinical treatment. The prudent path to the clinic mandates completion of systematic and well-designed experiments to fully illuminate the consequences of long-term neuronal phenocconversion. ■

Howard J. Federoff is in the University of Rochester School of Medicine, Rochester, New York, USA.

Email: howard_federoff@urmc.rochester.edu

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