Priming the PD pump

By Lev Osherovich, Senior Writer

The precise cause of neurodegeneration in Parkinson's disease remains unknown, and as a result no one has been able to develop a truly disease-modifying therapy. Now, independent teams in Europe and the U.S. have found that the dopamine transporter VMAT2 has a potentially causative role in the disease^{1,2}—figuring out how to enhance its activity is the next challenge.

PD involves degeneration of nigrostriatal dopaminergic neurons, leading to insufficient dopamine levels in brain circuits that influence cognition and motor function. Symptomatic therapies for PD work by raising dopamine levels at synapses of affected neurons but do not arrest the degeneration of dopaminergic neurons. Eventually, the drugs become ineffective.

Genetic studies suggest 5%–10% of PD cases are caused by mutations in genes involved in metabolism and intracellular transport, but the origin of most cases is unclear or idiopathic.

One leading hypothesis about the origins of idiopathic PD relates to dopamine itself. Because dopamine is highly reactive and can damage intracellular proteins, the neurotransmitter must be stored inside intracellular vesicles until it is ready to be released at synapses. Researchers have long suspected that one potential cause of PD is a breakdown among cellular systems that protect neurons from intracellular dopamine. The result is acceleration of neurodegeneration.

"There are probably multiple mechanisms that work to protect against dopamine, which undergoes spontaneous oxidation and free-radical formation in the cytoplasm," said Robert Edwards, a professor of neurology at the **University of California, San Francisco**.

Chief among these dopamine-detoxifying mechanisms is VMAT2 (vesicular monoamine transporter 2; SLC18A2), which pumps dopamine from the cytoplasm into synapse-bound vesicles. In the 1990s, Edwards cloned VMAT2 while searching for ways to counteract the toxic effect of

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a dopamine-like toxin used in rodent models of PD.³

Since then, PD researchers have debated whether diminished VMAT2 activity plays a central role in disease

or whether the transporter is merely a secondary participant that gets overwhelmed when cytoplasmic dopamine levels rise due to other factors.

Now, a team led by Christian Pifl, an associate professor of pharmacology

at the **Medical University of Vienna**, has found the best evidence to date of compromised VMAT2 function in the brains of patients with idiopathic PD.

"This is as close as possible with current techniques to see if there are changes in VMAT2 activity in people with disease," said David Sulzer, a professor of psychiatry, neurology and pharmacology at **Columbia University**. "They are looking at brains from people preserved just hours after they've died."

Meanwhile, a team led by Gary Miller, a professor of environmental health and associate dean for research at the **Emory University Rollins School of Public Health**, has shown that VMAT2 overexpression enhances dopamine sequestration and protects mice from MPTP toxicity.

"When you impair the storage of dopamine, it's bad," said Miller. He added that it was not clear until now that extra VMAT2 could increase the ability of neurons to sequester dopamine and ameliorate disease.

Smoking gun

Pifl's team measured dopamine levels and VMAT2 activity in brain lysates from six patients with idiopathic PD and four healthy controls. The lysates were harvested shortly after death, before dopamine-laden intracellular vesicles could dissipate.

"The question was whether we could study the pumping efficiency of VMAT2," said Pifl. Working with lysates from fresh brain tissue, "we were surprised to find that vesicular transport was still active" in these patients. The team found that overall levels of dopamine and VMAT2 in patients with PD were markedly lower than those in healthy controls because dopaminergic cells degenerated.

The researchers then used pharmacological methods to show that the remaining VMAT2 was unable to function normally in patients with PD, unlike VMAT2 from healthy controls that showed normal functional activity. "Parallel studies of dopamine uptake and binding showed that in PD the VMAT2 transporter is not working as efficiently as in healthy controls," said Pifl.

Results were reported in *The Journal of Neuroscience* and are not patented.

Sulzer said that the results support the hypothesis that PD is caused by mislocalization of dopamine from intracellular vesicles to the cytoplasm resulting from insufficient VMAT2 activity. "It does now look like PD patients have reduced levels of dopamine transport," said Sulzer.

The next step is to understand how VMAT2 activity becomes compromised in PD. Pifl said that his team has ruled out some simple possibilities such as defective vesicular proton gradients. The group now is looking for cellular factors that differentially regulate VMAT2's activity in patients with PD versus controls.

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Medical University of Vienna

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Edwards cautioned that Pifl’s findings do not prove that compromised VMAT2 activity is the sole cause of idiopathic PD because there could be other ways to increase cytoplasmic dopamine besides inactivating VMAT2.

Going to the VMAT

Whereas Pifl’s findings imply that increasing VMAT2 activity could have a therapeutic effect, Miller’s mouse findings show this is indeed the case.

Miller’s team overexpressed murine Vmat2 in the dopaminergic regions of mouse brains and saw increased dopamine transport into synaptic vesicles in *ex vivo* brain lysates compared with what was seen with endogenous Vmat2 expression. The engineered mice stored and secreted more dopamine than controls, mimicking the pro-dopaminergic effects of PD therapies such as L-dopa.

Vmat2 overexpression increased locomotion and decreased anxiety in mouse behavioral assays, as would be expected from raising dopamine levels. Miller’s Vmat2-enriched mice also proved more resistant to MPTP toxicity than wild-type animals, likely because the now-abundant transporter could safely dispose of the toxin into vesicles.

The findings were reported in the *Proceedings of the National Academy of Sciences* and are not patented.

Altogether, Miller’s results show that elevating VMAT2 activity could stimulate intracellular vesicular transport as a way to detoxify dopamine. “People said that the vesicles were maximally full and increasing dopamine transport wouldn’t do anything, but our data clearly show that this is not the case,” said Miller.

“Miller shows that artificial overexpression of VMAT2 is protective against MPTP toxicity, which is the best available model for PD,” said Pifl. “Increased dopamine sequestration by extra VMAT2 might thus be protective.”

Sulzer said that Miller’s results are in line with previous findings by Edwards’ and Sulzer’s teams indicating that overexpression of VMAT2 protects against high levels of L-dopa, which is metabolized into dopamine.⁴

What’s uncertain is how to enhance VMAT2 activity in patients. Miller’s overexpression approach is unlikely to work in the clinic, so “a small molecule that turns on VMAT2 production or increases its activity is what’s needed,” said Sulzer. Toward this goal, Miller’s team is screening for positive allosteric modulators of VMAT2 *in vitro*.

Last year, Sulzer’s team reported fluorescent VMAT2 activity probes that could be useful for drug discovery.⁵

No companies are known to be developing VMAT2 agonists, but several VMAT2 antagonists are marketed or in clinical development for a range of neurological indications.

Another question is whether enhancing VMAT2 activity would work in all cases of PD or just a subset of idiopathic cases. “We don’t know if this would work in the genetic forms of disease,” Sulzer noted. Further studies with postmortem brain tissue from a variety of PD patients using Pifl’s methods could help answer this question.

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