Plant-derived scavengers prowl the body for nerve toxins

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Transgenic tobacco plant is used to produced human butyrylcholinesterase -- a bioscavenger that helps clear acetylcholine from the nervous system. Credit: The Biodesign Institute at Arizona State University

The brain is forever chattering to itself, via electrical impulses sent along its hard-wired neuronal "Ethernet." These e-messages are translated into chemical transmissions, allowing communication across the narrow cleft separating one neuron from another or between neurons and their target cells. Of the many kinds of molecules involved in this lively chemical symposium, acetylcholine is among the most critical, performing a host

of functions in the central and peripheral nervous system. This delicate cholinergic design however is highly vulnerable. It can fall victim to inadvertent or deliberate poisoning by a class of compounds known as organophosphates—chemicals found in a range of pesticides as well as weaponized nerve agents.

Now Tsafrir Mor, a biochemist in the Center for Infectious Diseases and Vaccinology at the Biodesign Institute at Arizona State University has shown that human butyrylcholinesterase (BChE), a so-called bioscavenging molecule, can be produced synthetically—from plants. Further, Mor and his colleagues have demonstrated the effectiveness of plant-derived BChE in protecting against both pesticide and nerve agent organophosphate poisoning.

The group's research, recently reported in the *Proceedings of the National Academy of Science* (*PNAS*), shows promise not only for protecting the nervous system from the effects of organophosphates, but also for gaining a firmer understanding of acetylcholine-linked diseases such as Alzheimer's Dementia and possibly for use against drug overdose and addiction, particularly cocaine. *PNAS* has selected the important paper as an Editor's Choice.

In the developing world, accidental pesticide poisonings are common. Organophosphate compounds are also the method of choice for many suicides in poor, agricultural regions. The development of far more lethal organophosphates engineered to kill humans has continued apace since Nazi Germany invented them and Cold War adversaries, the United States and the Soviet Union refined and stockpiled these agents.

Following the collapse of the USSR, weaponized organophosphate poisons have proliferated, occasionally falling into the hands of rogue states or terrorist organizations, as these lethal nerve toxins are relatively easy and inexpensive to manufacture and store. The threat of a nerve

agent assault on civilians, like the sarin attack in the Tokyo subway system in 1995, perpetrated by the religiously-motivated group Aum Shinrikyo, remains a chilling possibility. The need for effective protection and treatment for organophosphate poisoning is hence a vital concern for public health.

Currently, clinical treatment for exposure to organophosphates involves the use of chemicals like atropine, which can save lives and alleviate acute symptoms, but which fail to address long term neurological effects of such poisoning, which may include muscle weakness, seizures and convulsions, permanent brain defects and social or behavioral symptoms.

Bioscavengers, Mor explains, act as sentries in the body, seeking out and binding with unwanted substances and neutralizing or destroying them. The most heavily studied bioscavengers are the two human cholinesterases—acetylcholinesterase (AChE), which is produced by neurons in the brain and BChE, which is produced mainly by the liver and circulates in blood serum. In addition to their role in defending the body from damaging chemicals, cholinesterases perform a vital housekeeping function, mopping up molecules of acetylcholine, once their signaling tasks are complete.

AChE is a key enzyme bioscavenger that terminates transmission of nerve impulses in the cholinergic synapses of the brain and is also active in the neuromuscular junction, where the axons of motoneurons terminate on muscle cells. As Mor explains, "every time that you move a muscle, the transmission is done through acetylcholine, which is released at the end of the nerve cell and taken up by the receptor on the muscle, causing an influx of ions and contraction of the muscle cell." For this to be accomplished in a coordinated way, the nerve impulse must be cut off almost instantly. This is what the cholinesterases do.

While other neurotransmitters like serotonin are eliminated through

reuptake, cholinesterases remove molecules of acetylcholine by hydrolyzing them. Hydrolysis is a chemical reaction in which a given molecule is split into two parts through the addition of a water molecule. AChE is supremely efficient in its catalytic activity, degrading about 25,000 molecules of acetylcholine per second.

Without a means of rapidly getting rid of acetylcholine molecules once they have performed their signaling duty, they flood the nervous system and in sufficient quantity, produce neuromuscular paralysis, and unregulated muscle contraction, eventually causing death due to respiratory and cardiac collapse. This fact, Mor says, makes the system something of an Achilles heel. Many organisms make use of this cholinergic matrix for both offensive and defensive purposes. Plants produce potent anti-cholinesterases to try to thwart herbivory by insects, which in some cases have evolved mechanisms to circumvent such defenses.

Mammals and birds have developed their own mechanisms for dealing with cholinesterase blocking agents. In humans, a particular gene codes for BChE, a closely related analogue of AChE, but one that circulates in blood, laying in wait to scavenge anti-cholinesterase molecules like those of organophosphate poisons. The effectiveness of BChE in neutralizing potentially deadly organophosphates has made it a highly attractive candidate for protecting against the effects of pesticides or nerve agents, as well as mitigating their effects post-exposure. While AChE occurs in the brain and is therefore tricky to acquire, BChE can be readily extracted from blood and stockpiled for future use.

The problem however is finding enough BChE. To protect a few thousand troops on the battlefield from nerve agent poisoning, the entire nation's blood supply would be required. Further, Mor points to many other applications in medicine that would make the production of a sizeable stockpile of BChE highly desirable. In addition to possible

treatment for cholinergic ailments, BChE could be used post surgery for patients who lack the naturally occurring enzyme and therefore have difficulty recovering from the effects of anesthesia. There is also evidence that BChE may be useful for treating acute cocaine overdose and possibly as a prophylactic that would eliminate cocaine's euphoric effects, making users less likely to seek out the drug. Again, the challenge is producing the enzyme in sufficient quantity.

The solution Mor and his group have come up with is to use transgenic tobacco plants, modified to synthesize human BChE in their leaves. In a series of experiments outlined in the new paper, Mor's group was able to demonstrate successful protection from both pesticide and nerve agent organophosphate poisoning in two animal models. The team was also able to extend the half-life of the plant-derived BChE, more closely replicating the persistence in the bloodstream of naturally occurring BChE, thereby improving its effectiveness. This was accomplished by decorating the outer portion of the enzyme with Polyethylene glycol (PEG).

Mor stresses that much work remains, before synthetic BChE can be applied as a nerve agent antidote or for other clinical purposes. Currently, the plant-derived BChE acts stoichiometrically, meaning that a molecule of the enzyme is needed for every anti-cholinesterase molecule to be degraded. Future work is aimed at developing forms of the enzyme that can act catalytically against organophosphates, which would permit a far lower effective dose of BChE to be used to protect from poisoning or for treatment post-exposure.

More information: Geyer BC, et al. (2010) Plant-derived human butyrylcholinesterase, but not an organophosphorous-compound hydrolyzing variant thereof, protects rodents against nerve agents. *Proc Natl Acad Sci*, in press (available online at www.pnas.org/content/early/2010/11/05/1009021107) .

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