Overcoming inhibitions

Kenneth J. Kellar*

Department of Pharmacology, Georgetown University School of Medicine, Washington, DC 20057

Organophosphorus nerve agents and pesticides (Fig. 1) markedly increase the amount and duration of the action of acetylcholine at all of the synaptic sites where it acts, resulting in overstimulation of critical processes that can lead to incapacitation, muscle paralysis, and death. In a recent issue of PNAS, Albuquerque *et al.* (1) reported that a drug already in use for treating Alzheimer's disease provides remarkable protection against death and neuronal damage from exposure to some of the most potent nerve agents known.

Acetylcholine is a workhorse neurotransmitter. It does the heavy lifting at the neuromuscular junction, where motor axons signal our voluntary muscles to contract by releasing acetylcholine, and it signals the ganglia of our autonomic nervous system, which in turn regulate our blood pressure and heart rate, respiration, digestive processes, visual accommodation, crucial aspects of our sexual function, and other physiological processes. In many cases, these effects are triggered by the release of acetylcholine at the end organ. Acetylcholine even initiates the release of epinephrine from our adrenal glands to prepare us for self-preserving extraordinary activity (the "fight or flight" response). Moreover, acetylcholine is a fundamental neurotransmitter in the CNS, where it is critically involved in functions related to cognition and behavior, in some cases by modulating release of other neurotransmitters, including glutamate, GABA, norepinephrine, and dopamine.

The life cycle of acetylcholine, like that of all neurotransmitters, includes synthesis in cells that have the necessary specialized enzyme(s), release from those cells, activation of its specific receptors on the target tissues, and finally a mechanism to end its action. The action of acetylcholine is ended by the enzyme acetylcholinesterase (AChE), which very rapidly hydrolyzes it to acetate ion and choline. The choline is then transported back into the nerve for reuse in the synthesis of acetylcholine. The rapid enzymatic hydrolysis of acetylcholine is important to maintain the fidelity of its signal, so anything that disrupts that process can have important consequences. In some circumstances, inhibition of AChE can have beneficial effects. For example, in the neuromuscular disease myasthenia gravis, an autoimmune response leads to a failure of the nicotinic acetylcholine receptors in the muscle, which results in inadequate signaling by acetylcholine and consequent muscle weakness. Because inhibition of AChE increases the amount and duration of acetylcholine available to stimulate the receptors that are still functional in the muscle, reversible inhibitors of AChE are useful for both diagnosis and treatment of the disease. Reversible AChE inhibitors also are useful for treating glaucoma, paralysis of the smooth muscle in the intestinal tract and urinary bladder; most recently, they have become a treatment for Alzheimer's disease.

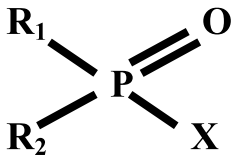

Fig. 1. The general structure of many organophosphorus compounds, which present a threat as nerve agents and in agricultural use as pesticides. All of these compounds have the potential to irreversibly inhibit AChE, which is crucial to normal acetylcholine neurotransmission.

Irreversible inhibitors of AChE, on the other hand, present a very different picture. Most of these inhibitors are organophosphorus chemicals developed initially in agriculture as pesticides in Germany in the 1930s. But their potential for use as weapons was soon realized, and by the end of World War II Germany had produced large stockpiles of the nerve agents soman, sarin, and tabun. Fortunately, these were never used. After World War II, both the U.S. and the Soviet Union manufactured large quantities of these agents and others, as well as the weapon systems to deliver them. Widespread use of these weapons by the postwar superpowers did not occur, but limited use of them by others has been documented. For example, Iraq used nerve agents with devastating effects during its war with Iran in the mid-1980s and again in 1988 against its own Kurdish population in northern Iraq. Moreover, in 1994 and 1995, sarin was used in two terrorist attacks in Japan, resulting in 19 deaths and hundreds of serious injuries, including injuries to those initially exposed and to emergency responders and hospital personnel providing treatment. In addition to these deliberate uses as weapons, accidental exposure of farm workers to organophosphorus agents used as pesticides occurs at an alarming rate worldwide, often, but certainly not exclusively, in developing countries (2, 3). Moreover, some of the pesticides sold for home and garden use contain organophosphorus AChE inhibitors, although their use is restricted and there have been long-running attempts to phase them out (4).

The manifestations of acute poisoning with organophosphorus AChE inhibitors are largely predictable from the physiological changes brought about by acetylcholine acting on its receptors. These receptors are classified as nicotinic or muscarinic, based initially on the discovery of the natural alkaloids that shared with acetylcholine the ability to activate them (5) but now based on the genes that code for them or their substituent subunits. When AChE is inhibited, the actions of acetylcholine at its receptors and thus at the tissues innervated by cholinergic axons are magnified and prolonged. Excessive inhibition of AChE results in widespread combinations of symptoms due to overstimulation of the muscarinic receptors in the eye (miosis is often among the earliest signs), salivary glands, and smooth muscle of the digestive tract as well as bronchial airway constriction (6, 7). Heart rate would be expected to be markedly decreased by overstimulation of the muscarinic receptors in the heart, but bradycardia may be offset by the effects of hypoxia and generalized excitement that lead to increased heart rate (6). Prolongation of acetylcholine actions at the nicotinic receptors in the neuromuscular junction causes muscle twitching and fasciculation, weakness, and finally paralysis, including failure of the muscles of respiration (6, 7). In addition to these effects on the autonomic nervous system and neuromuscular junction, excessive inhibition of AChE depresses CNS respiratory centers, which appears to contribute to respiratory failure (6, 8), so death after acute exposure to AChE inhibitors is probably attributable to a combination of central and peripheral mechanisms. Seizures are another CNS manifestation

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*E-mail: kellark@georgetown.edu.

of AChE inhibition (6, 9). It is not clear whether these seizures are related to overstimulation of acetylcholine pathways in the brain or are sequelae of hypoxia, but in their worst form they can lead to neuronal damage and contribute to death and potentially to disability in the survivors of exposure.

Acute exposure to organophosphorus AChE inhibitors is treated with the muscarinic receptor antagonist atropine in sufficient doses to maintain block of peripheral and CNS receptors, with benzodiazepines to prevent convulsions, and with agents such as pralidoxime (2-PAM), which can prevent the "aging" process, during which organophosphorus compounds phosphorylate the enzyme, rendering it irreversibly inhibited (6, 7). The half-time for this aging process varies from ≈2 min for soman to several hours for other organophosphorus compounds, including sarin and tabun [the compounds used in agriculture and households cause less aging of AChE (10)]. In addition to these treatments for actual exposure, pyridostigmine, a relatively short-acting, reversible AChE inhibitor used in treating myasthenia gravis, has been approved for use as a pretreatment under conditions where there is believed to be a reasonable probability of exposure to organophosphorus agents (for example, pyridostigmine was used prophylactically by U.S. military personnel in the first Persian Gulf War). The rationale for its use as a pretreatment is that a reversible AChE inhibitor can prevent the attachment of the irreversible organophosphorus inhibitor and thus preserve the enzyme until after the danger of exposure to the agent has passed (6). This pretreatment becomes particularly important when the organophosphorus compound in question is soman, because soman-inhibited AChE undergoes the aging process in just minutes.

With that rationale in mind, pyridostigmine appears to be a logical addition as a pretreatment to prevent muscle paralysis. But pyridostigmine is a charged, quaternary amine molecule that doesn't cross the blood–brain barrier, so it doesn't protect the AChE in the CNS. In fact, for many years, the use of reversible AChE inhibitors that

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can cross into the brain to potentially impart protection against the CNS effects of organophosphorus compounds has been considered (11). Physostigmine, a leading candidate, is a reversible inhibitor that readily enters the brain, but it apparently produces incapacitating side effects at doses needed to prevent organophosphorus poisoning (12, 13). The new study by Albuquerque *et al.* (1) makes a potentially crucial contribution toward the solution to this problem.

The Albuquerque *et al.* study (1) investigated the efficacy of galantamine, a reversible AChE inhibitor approved for use in Alzheimer's disease, as a treatment for organophosphorus exposure. In combination with atropine, galantamine demonstrated higher efficacy than pyridostigmine in protecting guinea pigs against the lethal effects of organophosphorus AChE inhibitors. The protection by galantamine was demonstrated against the nerve agents soman and sarin as well as against paraoxon, the active metabolite of parathion, an agricultural pesticide that only recently was phased out of use in the U.S. Perhaps more remarkable, galantamine protected guinea pigs even when administered 5 min after exposure to soman, the organophosphorus agent that causes the fastest aging of AChE. Another exciting finding of these studies is that, although atropine alone did not protect guinea pigs against soman-induced neurodegeneration in brain, galantamine in combination with atropine did, even when administered 5 min after soman. The postexposure protection provided by galantamine in combination with atropine indicates that the "irreversibility" of even a rapid-aging compound like soman should be viewed as relative and perhaps as a challenge to be overcome by further research.

This study (1) very clearly signals that galantamine and perhaps other CNS-acting, reversible AChE inhibitors have great potential as pretreatments before possible exposure to organophosphorus compounds and possibly even as treatments immediately after an exposure. Time and further studies will determine how much these new approaches can diminish the danger of these organophosphorus compounds.

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