Pharmacology of Organophosphates

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The cholinergic nerve fibers, which employ acetylcholine (ACh) as a neurohumoral transmitter, and the results of their activation are listed. The reactions between the enzyme acetylcholinesterase (AChE), its natural substrate, ACh, and the various types of inhibitors are described. The limited therapeutic uses of the anticholinesterase (anti-ChE) agents are considered. The toxicological effects encountered when the anti-ChE agents are employed as insecticides or as chemical warfare (CW) agents are discussed. Certain anti-ChE agents produce also a delayed neurotoxic effect which is apparently unrelated to the inhibition of AChE.

INTRODUCTION

The earliest report of the synthesis of an organophosphate (OP), tetraethylpyrophosphate, was that presented by Phillipe de Clermont to the French Academy of Sciences in 1854. Little further appeared in the literature until nearly 80 years later in 1932, when Lange and Von Kruger described the synthesis of dimethyl- and diethylphosphorofluoridate, and noted that inhalation of their vapors produced dimness of vision and a choking sensation. These observations may have led Gerhard Schrader to the exploration of this class of compounds when he was involved in the development of insecticides for I. G. Farbenindustrie a few years later. One of the earliest OP insecticides synthesized by Schrader, still in widespread use, was parathion (O,O-diethyl O-(4-nitrophenyl)phosphorothioate). Then, shortly before the onset of World War II, his mission was shifted by the authorities from insecticides to chemical warfare (CW) agents. The result was the development of diisopropylphosphorofluoridate (DFP), and then the considerably more potent G agents (tabun, sarin, soman) (reviewed fully in Ref. 1).

The OPs are anticholinesterase (anti-ChE) agents. Their immediate pharmacological and acute toxic effects result from this action. In addition, following chronic exposure many produce another effect characterized as organophosphorus-induced delayed neurotoxicity (OPIDN); the basis of this action is still poorly understood.

PHARMACOLOGY

Early in the course of the present century, acetylcholine (ACh) was identified as the neurohumoral transmitter of cholinergic nerve fibers, by Dale, Loewi and their associates, in parallel with an adrenaline-like compound as the transmitter of adrenergic fibers. The latter was not identified conclusively as norepinephrine until 1946 by von Euler.2

There are four anatomical classes of cholinergic fibers: postganglionic parasympathetic fibers, preganglionic fibers (both sympathetic and parasympathetic), motor fibers to skeletal muscle, and certain fibers within the central nervous system (CNS). All cholinergic fibers contain at their terminals high concentrations of four entities: ACh, the transmitter; choline acetyltransferase (ChAcTr), the enzyme that catalyses the final step in the synthesis of ACh; acetylcholinesterase (AChE), the enzyme that catalyses the hydrolysis of ACh into relatively inactive products, choline and acetic acid; and the sodium-dependent high-affinity choline uptake (SDHACU) system, which enhances the uptake of high concentrations of choline. At postsynaptic sites there are essentially two types of cholinergic receptors: muscarinic (M) and nicotinic (N). These are distributed as shown in Table 1.

The inactivation of AChE by an OP agent or its temporary inhibition by other drugs (see below) results in the accumulation of ACh at all sites of cholinergic transmission. At M receptors this causes enhancement of either excitatory (e.g. bronchoconstriction) or inhibitory (e.g. vasodilatation). At N receptors, the effect is first excitatory (e.g. muscular fasciculation) followed by inhibition (muscular paralysis). It is remarkable that these classifications, proposed by Sir Henry Dale, still held after cholinergic transmission was discovered in the CNS and after several subtypes of M and N receptors were described.

The M receptors are coupled with G proteins,
Figure 1. Binding forces in drug–receptor combinations as illustrated by reaction of acetylcholine (ACh) with acetylcholinesterase (AChE). (1) Ionic (electrovalent) bond: electrostatic attraction between two oppositely charged ions. Quaternary nitrogen of ACh and dissociated free carboxyl group of a dicarboxylic amino acid (aspartic, glutamic) within a protein structure at an anionic site. (bond strength 5 kcal mol⁻¹.) (2) Dipole-induced dipole bond: weak charges induced on carbon atoms by adjacent atoms within the same molecule or by groups of another molecule when the two (i.e. drug and receptor) approach each other within the solution, also known as van der Waals or London forces. (bond strength 0.5 kcal mol⁻¹.) (3) Hydrogen bond: proton (H-nucleus) accepts an electron pair in part from each of two donor atoms (O,N,S). (bond strength 2–5 kcal mol⁻¹.) (4) Covalent bond: pair of electrons shared by two atoms of drug and receptor, respectively, resulting in a very strong irreversible combination, such as alkylation of receptor. (bond strength 100 kcal mol⁻¹; this is considerably weaker with an acetyl group.)

which may activate phospholipase C, resulting in the formation of inositol polyphosphates and, in turn, the release of intracellular Ca²⁺ from the endoplasmic reticulum. Alternatively, coupling with another group of G proteins causes inhibition of adenyl cyclase and activation of K⁺ conductance. The N receptors, in contrast, do not involve second messengers but are essentially ligand-gated ion channels; their activation causes an immediate increase in cation (Na⁺, K⁺) permeability. In the CNS, activation of N receptors causes the same type of rapid response that is characteristic of synaptic transmission elsewhere. On the other hand, the prolonged periods required for the onset and termination of responses following activation of M receptors suggests that in the CNS ACh functions there primarily as a modulator rather than as a transmitter.

**MOLECULAR ASPECTS**

The substrate (ACh) and the various types of inhibitors combine with AChE by essentially the same binding forces; these include all four that commonly participate in various combinations of drugs with their receptors: ionic, dipole-induced dipole, hydrogen bonding, and covalent (Fig. 1). The subsequent steps in the reaction of ACh with AChE are illustrated in line 1 of Fig. 2. It is notable that all four steps require a total of only 80 μs, hence AChE has nearly the highest velocity of any mammalian enzyme. The rate-limiting step is the final hydrolysis of acetylated AChE; its turnover time is approximately 40 μs. Line 2 depicts the reaction between AChE and a truly reversible inhibitor, such as edrophonium. As soon as the local concentration of the inhibitor begins to fall, reversal commences. For practical purposes this means that the effects of an intravenous injection of a bolus containing 10 mg of edrophonium will persist for only about 10 min. This brief effect can be utilized in the initial diagnosis of myasthenia gravis, or in the differential diagnosis between overtreatment (cholinergic crisis) or undertreatment (myasthenic weakness) with an anti-ChE drug such as neostigmine, where the superficial appearance of the two conditions may be quite similar. In line 3 is shown the reaction between AChE and a carbamyl ester inhibitor such as neostigmine (often referred to incorrectly as 'reversible' inhibitors). As seen, the steps are identical with those between AChE and ACh. However the hydrolysis of the carbamylated enzyme requires over a million times longer than the acetylated enzyme. Thus, the effects of a standard dose of neostigmine will persist for 2 h or longer. Phosphorylation of the esteratic site by an OP, as shown in line 4, entails the formation of a highly stable covalent bond; its hydrolysis requires several hours or may be permanent, depending on the nature of the adjacent alkyl or alkoxy groups. The splitting-off of one alkyl group, known as aging, renders the combination of enzyme and OP group unreactivatable by oxime reactivators. As might be deduced from Table 1, the uptake of a sufficient dose of an OP agent can result in effects equivalent to activation of postganglionic parasympathetic fibers, including miosis, ciliary spasm, bradycardia, vasodilation, bronchospasm and increased tracheobronchial secretion, increased tone and motility of the gastrointestinal tract and urinary bladder, lacrimation and sweating, and also stimulation followed by paralysis.
Figure 2. Steps involved in the hydrolysis of acetylcholine (ACh) by acetylcholinesterase (AChE) (I), and in the inhibition of AChE by reversible (II), carbamyl ester (III) and organophosphorus (IV) agents. (I) The substrate, ACh, combines with an active unit of the enzyme to form a complex, by electrostatic attraction between the quaternary N⁺ atom of the choline moiety and the anionic site of the enzyme and by interaction between the electrophilic C atom of the carbonyl group and the nucleophilic serine hydroxyl group (the nucleophilicity of which is enhanced by H-bonding with the histidine imidazole group). Choline is then split off, leaving the acetylated enzyme (K₂). The latter reacts rapidly with water to produce acetic acid and the regenerated active enzyme (K₃). The turnover time is 80 μs (12 000 s⁻¹). (II) Edrophonium combines electrostatically at the anionic site and by H-bonding to the imidazole N atom of histidine at the esteratic site to form an enzyme-inhibitor complex that reverses rapidly upon dilution. (III) Neostigmine and related ammoniumcarbonate or aminocarbamate esters react with the enzyme in the same manner as the substrate does; however, the carbamylated enzyme reacts with water at less than a millionth the rate of the corresponding acetylcholine form to regenerate the active enzyme. (IV) DFP and similar organophosphorus inhibitors react only at the esteratic site to form a phosphorylated enzyme; in the case of diisopropylphosphoryl-AChE, essentially no spontaneous hydrolytic reactivation occurs, as indicated by the square brackets. Heavy, light, and dashed arrows represent extremely rapid, intermediate and extremely slow or insignificant reaction velocities, respectively.
of sympathetic and parasympathetic ganglia, skeletal muscle and certain centers in the CNS. What systems are actually involved at a given dose is apparently dependent on the excess of AChE present over physiological requirements. For example, at the MEP of skeletal muscle and in the CNS there seems to be a ten-fold excess; therefore, over 90% of the AChE must be inactivated before pharmacological effects are observed. In the gut, there is only about a twofold excess; inactivation of 50% causes an increase in motility.

THERAPEUTIC IMPLICATIONS

The effects of anti-ChEs on the eye are shown in Fig. 3. Accumulation of ACh at the two sphincters, the iris sphincter and the ciliary muscle, results in their contraction, causing miosis and ciliary spasm or accommodation for near-vision, respectively. If the intraocular pressure is elevated, as it is in glaucoma, it can be reduced by anti-ChE drugs by improved resorption at the canal of Schlemm; this results from contraction of the iris and of the ciliary spur. Anti-ChE agents can therefore be used in the emergency treatment of acute, narrow-angle glaucoma, and in the long-range therapy of chronic, wide-angle glaucoma. When DFP (Fluoropryl) was initially employed for this purpose during World War II, it appeared to offer a marked advantage over the carbamyl ester inhibitor, phystostigmine, because of its greater potency and longer duration of action. It was then superseded by the quaternary ammonium OP drug eclothiophate (Phospholine), which unlike DFP could be dispensed in aqueous solution. However, during the 1970s it was noted in several clinics that the continued ophthalmic use of long-acting anti-ChE agents (both OPs and others) resulted in the production of lenticular opacities. Therefore, the OPs are now rarely used for this purpose. Similarly, they have not proven advantageous clinically over neostigmine in the other conditions for which the latter has long been employed: myasthenia gravis and atony of the gastrointestinal track and urinary bladder. In the former condition it is not possible to assess accurately the changing dosage requirements; accordingly, the OPs were frequently given in excessive dosage, resulting in intoxication and even death. In the latter conditions there is no advantage in using a long-acting drug, but only increased hazard.

TOXICOLOGY

From a practical standpoint, the OP anti-ChE agents are more significant as toxic than as therapeutic agents. This is because of their current widespread use as insecticides, and the threat of their potential use as chemical warfare (CW) agents. Malathion (O,O-diethyl S-(1,2-dicarbethoxyethyl)phosphorodithioate) is now widely employed in the USA as an agricultural insecticide; its advantage is that it is selectively toxic to insects. However, with sufficient exposure it is potentially lethal to humans. Parathion, which lacks this advantage, is still used in many countries because it is cheaper to manufacture.

The acute lethal action of the OPs and other anti-ChE drugs results from their attack on the respiratory system at several levels: bronchoconstriction and excessive tracheobronchial secretion, paralysis of the diaphragm and other respiratory muscles, and depression of the respiratory center of the CNS. The predominant site varies with the species. Treatment consists in the establishment of an open airway, oxygen via a nasal catheter, atropine (2–20 mg, i.v. or i.m.), artificial respiration and the administration of pralidoxime (1–2 g, i.v., over 2–4 min) or another reactivator. If this regimen is instituted promptly, it should be consistently effective.

Organophosphorus-induced delayed neurotoxicity (OPIDN) appears to be unrelated to the anti-ChE action of these compounds. It is characterized by the sequential development of axonal degeneration, demyelination, and flaccid paralysis after a latent period of approximately 2 weeks following exposure. One theory is that it results from the inactivation of a somewhat elusive enzyme defined as neurotoxic esterase (NTE). Other proposals have been advanced but none is conclusive. Extensive outbreaks of OPIDN, entailing thousands of cases, have followed the adulteration of beverages and cooking oil with triorthocresyl phosphate (TOCP), which is an extremely weak inactivator of AChE. Paraaxon (diethyl 4-nitrophenyl phosphate), a potent anti-ChE agent, does not produce OPIDN, whereas DFP, which inhibits both AChE and NTE, does. The effects of OPIDN may persist for years; there is no effective treatment.
REFERENCES


