
3 Low-Level Nerve Agent Toxicity under Normal and Stressful Conditions

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I. INTRODUCTION

A. NERVE AGENTS

Nerve agents were developed over six decades ago for military use and continue to be a significant threat on the battlefields of the world or as terrorist weapons. Organophosphate (OP) nerve agents (tabun, sarin, soman, and VX) are the most toxic compounds that cause biological effects by inhibiting the enzyme cholinesterase. The first OP nerve agent, tabun (O-ethyl N, N-dimethyl phosphoramidocyanidate) was synthesized by German chemist Dr. Gerhard Schrader in 1936.¹ Later sarin (isopropyl methyl phosphonofluoridate) was synthesized in 1938 followed by soman (pinacolyl methyl phosphonofluoridate) in 1944. A few years after the end of World

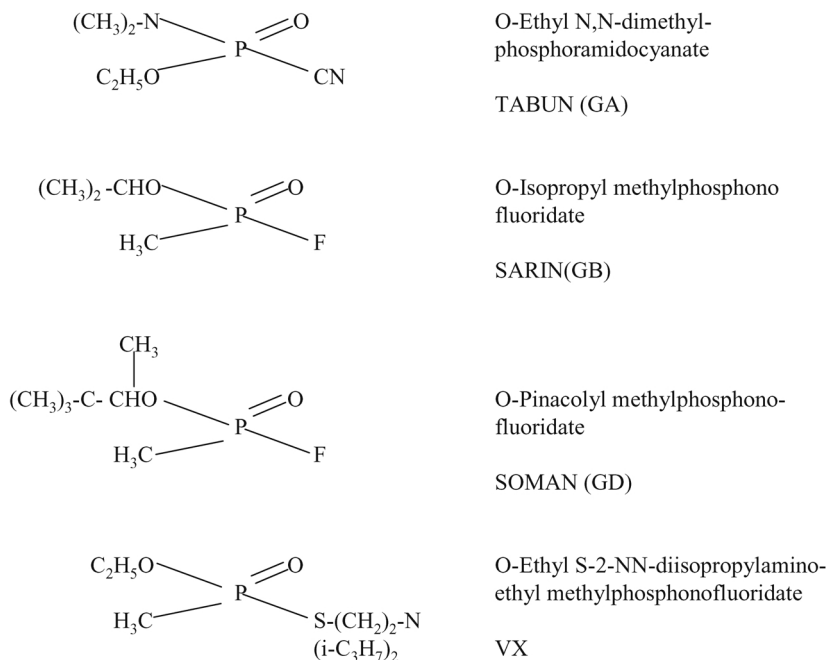


FIGURE 3.1 Chemical structures of organophosphorous nerve agents. (Adapted from Somani et al.²¹²)

War II, Dr. Ranajit Ghosh of England synthesized a nerve agent called VX (O-ethyl-S-2-N,N-diisopropyl amino ethyl methylphosphonothiolate) that was much more potent than sarin. The chemical structures of OP nerve agents are depicted in Figure 3.1.

Among the OP nerve agents, sarin has been used as a chemical warfare agent since its synthesis during World War II. Its use was most recently demonstrated during the Iran-Iraq conflict and during the Gulf War.²⁻⁴ These reports indicate that Gulf War veterans might have been exposed to low doses of sarin. It has been reported that German personnel exposed to nerve agents during World War II suffered neurological problems 5 to 10 years after their last exposures.^{5,6} Furthermore, long-term neurologic and psychiatric abnormalities have also been seen in personnel exposed to sarin in its manufacturing plants.^{7,8} A terrorist attack with sarin gas on March 20, 1995 in Japan and an earlier killing of a Japanese terrorist member by another deadly nerve agent, VX, have attracted the world's attention about the threat to the general world population.^{9,10} These episodes have added new dimensions to the dangers that humanity is facing all over the globe.

Nerve agents are inhaled as vapors or aerosols and, being lipid soluble, immediately enter systemic circulation, resulting in toxic manifestations at muscarinic, nicotinic, and CNS cholinergic sites.¹¹ The acute cholinergic symptoms (tremors, convulsions, salivation, lacrimation, and respiratory failure) are due to the inhibition

of acetylcholinesterase at central, peripheral, and autonomic synapses, resulting in accumulation of acetylcholine at synaptic junctions.¹² The muscarinic effects include ocular (miosis, conjunctival congestion, ciliary spasm, nasal discharge), respiratory (broncho-constriction and increased bronchial secretion), gastrointestinal (anorexia, vomiting, abdominal cramps, diarrhea), percutaneous (sweating and muscular fasciculation), salivation, bradycardia, and hypotension. Nicotinic effects include muscle fasciculations and paralysis. The central nervous system effects include ataxia, confusion, loss of reflexes, slurred speech, headache, anxiety, restlessness, irritability, giddiness, insomnia, convulsions, and coma. The cause of death of persons exposed to nerve agents is generally due to peripheral and central effects leading to respiratory failure.¹² A single acute exposure of nerve gas could cause death within 5 min or in 24 h, depending on the dose, route, and type of organophosphates. If the effective therapy is not started as soon as possible, the organophosphates may cause delayed neuropathy. Convulsions, seizures, and neuropathological lesions are also a significant part of the symptoms of central nervous system toxicity caused by poisoning with nerve agents. The proposed pathway for the nerve agent-induced pathophysiological lesion is depicted in [Figure 3.2](#).

A number of studies have shown that nerve agent-induced neuropathology can be prevented by attenuating the convulsive episodes. Diazepam, a GABA agonist and tranquilizer, was found to be an effective drug for preventing neuropathology when used in conjunction with atropine and oxime in soman-poisoned animals.¹³ A review of toxicological studies of nerve agents indicates vast literature on their anticholinesterase properties¹⁴ and on the determination of acute lethal data, especially lethal dose (LD_{50}) and lethal concentration (LCt_{50}), in various species of animals. A comparison of toxic potencies as inhibitors of acetylcholinesterase for several representative nerve agents is given in [Table 3.1](#). The larger the value, the more potent is the agent.¹⁴ LD_{50} and LCt_{50} of various OP nerve agents by different routes of exposure in human and different species of animals are given in [Table 3.2](#). The LD_{50} values indicate that guinea pig, dogs, cat, monkey, and rabbit are the most susceptible, and that the rat and mouse are the most resistant species for nerve agent intoxication. It should be noted that most studies achieved nerve agent intoxication by intravenous, subcutaneous, and dermal routes of administration in experimental animals.

B. DELAYED NEUROTOXICITY

The chronic, delayed neurotoxic effects (ataxia and paralysis) induced by nerve agents are referred to as organophosphate-induced delayed neurotoxicity (OPIDN), which are due to the inhibition of neuropathy target esterase or neurotoxic esterase (NTE) in the neuronal membrane of the nervous system.^{15–19} OPIDN is a syndrome which is characterized by a delay period of 4–21 days after nerve gas exposure before clinical symptoms (ataxia and paralysis) are manifested.^{17–19} The primary molecular target for the initiation of OPIDN is NTE in the nervous system.^{20,21} NTE is an integral membrane-bound enzyme with a molecular weight of 155 kDa; it has no physiological substrate, but its organophosphorylation and aging in the neuronal tissue are required to trigger the pathogenesis of OPIDN. Phosphorylation (inhibition) and subsequent aging of NTE are depicted in [Figure 3.3](#). The rapid aging of phosphorylated

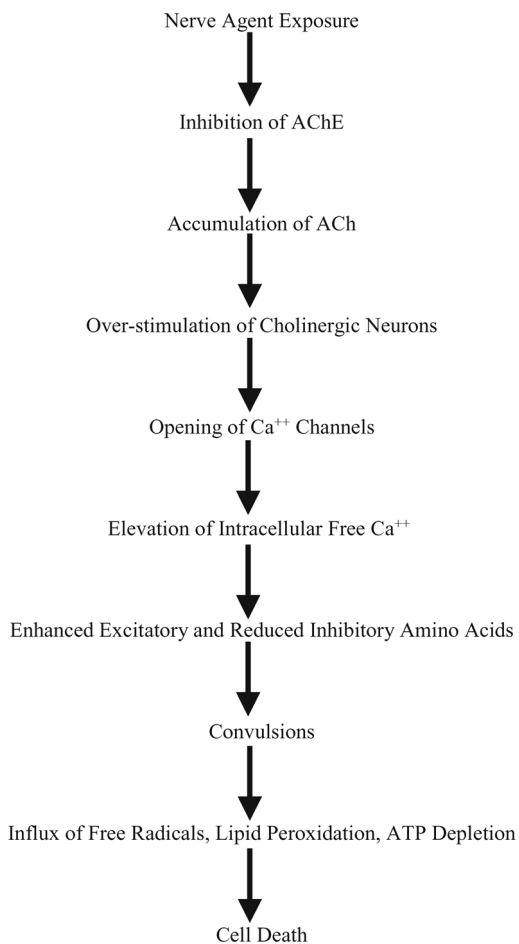


FIGURE 3.2 Proposed pathway for nerve agent-induced central and peripheral pathophysiology.

TABLE 3.1
Potency of Nerve Agents as an
Inhibitor of ChE Enzyme

Nerve Agents	ChE Inhibition Potency
Tabun	8.6
Sarin	8.9
Soman	9.2
VX	8.8

TABLE 3.2

Lethal Dose (LD₅₀) and Lethal Concentration (LC₅₀) Values of Nerve Agents in Human and Different Species of Animals by Different Routes of Administration

Exposure Route	Soman	Sarin	Tabun	VX	Ref.
Species					
<i>Inhalation (mg · min/m³)</i>					
Human		100	200–400	36	189, 190
Monkey		74	187	50	189
Dog		60	320	15	189
Rabbit		120	960	25	189
Guinea Pig	0.101	180		8–30	189, 191
Rat	211	220	450	17	189, 192
Mice		240–310		7–40	189
<i>Dermal (mg/kg)</i>					
Human		24	14–21	0.04–0.14	189, 193, 194, 195
Monkey			9.3	0.065	189
Pig		115.9		0.40	189
Dog		10.8	45	0.054	189
Cat		6.2		0.012	189
Rabbit		4.4	3	0.025	189, 196
Rat		2.5	12.6	0.10	189
Mice		1.0–9.2		0.046	189, 197
<i>Oral (mg/kg)</i>					
Rat		0.10	1.06		189
<i>Intravenous (μg/kg)</i>					
Human		14	14	8	189, 198
Goat		15		5	189
Dog		10	84	6.3	189
Cat		15–18		2.5	189, 199
Rabbit		14.7	63	8.4	189
Guinea Pig		30		4.5	200
Rat		45	70	7.9	189, 194, 201
Mice	42	70–113	311	14.1	98, 189, 202
<i>Subcutaneous (μg/kg)</i>					
Rabbit	29	41.7			203
Guinea Pig	28		119		204
Cat		35			88
Rat	156	158	305	21	205
Mice		190			206
<i>Intramuscular (μg/kg)</i>					
Monkey	3.75				207
Mice	98	179	304		204
Hen		50		30	208, 209
<i>Intraperitoneal (μg/kg)</i>					
Rat		450			150
Mice	440	560			210
<i>Intracerebroventricular (μg/kg)</i>					
Rat	66.4				211

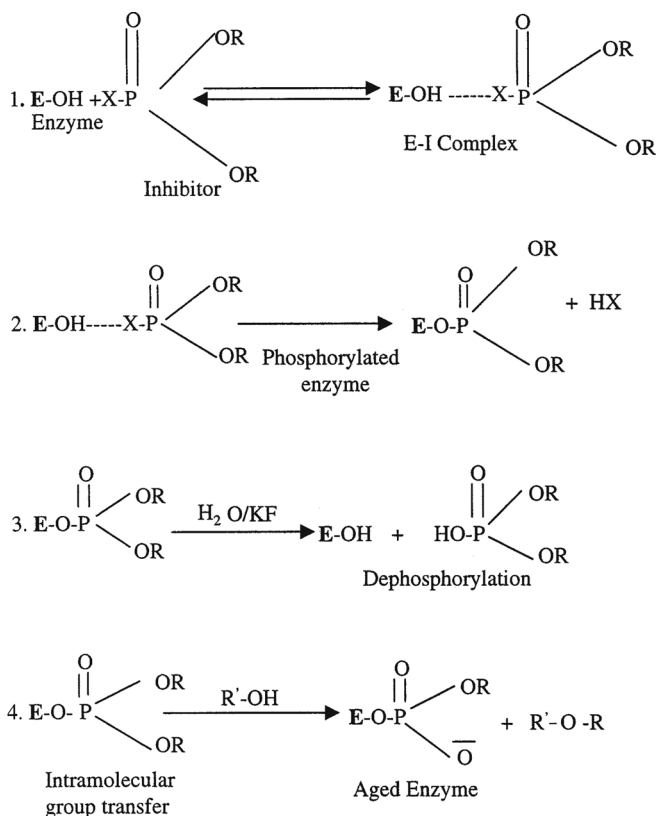


FIGURE 3.3 Interaction of OP nerve agents with enzyme, neurotoxic esterase (E-OH), and inhibitor organophosphate (I): (1) formation of Michaelis complex; (2) phosphorylation of enzyme; (3) reactivation, hydrolysis with H₂O to regenerate the enzyme; (4) aging, the proposed intramolecular transfer of an R group from the phosphate moiety to a receiving group (e.g., R'OH) within the protein structure.

NTE occurs when an alkyl group from a phosphate moiety is intramolecularly transferred to a receiving group within the protein molecule.²² Half-lives of aging for inhibited NTE range from 1 min to 600 min.^{22,23} Aging occurs rapidly with arylalkoxy and linear alkoxy groups attached to a P atom, and slowly with a highly branched alkoxy substitute.²⁴ NTE is defined as phenyl valerate hydrolase which is resistant to inhibition by non-neuropathic organophosphate (paraoxon) and sensitive to inhibition by neuropathic organophosphate (mipafox and DFP). The inhibitors of NTE are classified into two groups. Group A includes phosphates, phosphoramidates, and phosphonates. These are generally irreversible inhibitors and aging is possible. Group B is comprised of sulfonates, phosphonates, and carbamates. These are reversible inhibitors and aging is not possible. Trivalent phosphates such as triphenyl

phosphite and trifluoromethyl ketones are also inhibitors of NTE.^{25,26} Group A inhibitors are also known as agonist and Group B inhibitors as antagonist. Group B inhibitors given prior to Group A inhibitors protect animals against OPIDN.²⁷ When these Group B inhibitors are given after Group A inhibitors, these inhibitors potentiate the OPIDN.²⁸ Recent data show that NTE is comprised of two functional domains: an N-terminal regulatory domain and C-terminal effector domain. The N-terminal domain may bind a cyclic nucleotide and thereby modulate the activity of the C-terminal effector domain.²⁹ The non-esterase function of NTE is important for axonal maintenance and neuron-glial cells signaling. Histopathological changes in spinal cord and peripheral nerves consist of degeneration of axons followed by the demyelination.^{17-19,30,31} NTE-like activity has also been reported to be present in non-nervous tissues such as lymphocytes and platelets of human, hen, and other animal species.^{32,33} Various attempts have been made to use lymphocyte NTE in humans as a biomonitor of OPIDN, but many problems have been encountered.³⁴⁻³⁶ Later platelet NTE as a peripheral biochemical marker has been proposed because platelets can be obtained from blood more easily and without contamination. Platelets are good peripheral models for central neurons and platelet NTE inhibition is well correlated with brain NTE inhibition in human, hen, and mice *in vitro* as well as *in vivo*.^{17,21,32,37} Platelet NTE has now been established as a peripheral biochemical marker for OPIDN.^{17,19,21,33,37} It is suggested that this is an important parameter which can be reliably measured in humans. Hens are very sensitive and have been used as a suitable model to evaluate OPIDN. However, humans are 10 to 100 times more sensitive than hens. Studies in Gulf War veterans by Jamal et al.³⁸ explained the mild impairment of brainstem, spinal cord, and peripheral nerve function. These studies are consistent with the spectrum of OPIDN syndrome. Sarin has been shown to produce delayed neurotoxicity at higher doses in protected hens.^{39,40} However, this report describes the interactive effects of lower doses of sarin, pyridostigmine, and physical stress on biochemical and histopathological changes in tissues of animals.

C. STRESS

The stressful demands of modern military duty include a broad range of activities, especially during wartime. The demanding physical tasks of a combat infantry soldier can be expected to result in significant physical and chemical changes within the body.⁴¹ Notwithstanding this, physiological stress is still to be expected, because of the redistribution of blood flow to serve the demands of active muscle cells⁴² as well as to meet the needs of temperature regulation in the body. In addition, a considerable production of metabolic acids from substrate catabolism will lead to a marked reduction of the intracellular pH.^{43,44} As the time course of a drug in the body may be influenced by exercise dynamics,⁴⁵ it is important to know how physical activity interacts with low-dose nerve agent exposure under combat field conditions. Since Gulf War veterans underwent physical stress (exercise) and possibly were exposed to low doses of sarin, they make an excellent model to answer this. Therefore, the neurotoxic effects of low-dose sarin under conditions that reasonably simulate heavy military duty are discussed in this chapter.

Acute or chronic physical stress is known to influence the cholinergic system in cerebral and peripheral tissues. Acute physical exercise for 1 and 3 h increased cholinesterase (ChE) activity in the blood serum of rats.⁴⁶ In the reticulocytes and young erythrocytes of endurance-trained athletes, AChE activity was higher than that of the control group.⁴⁶ Acute exercise produces a slight elevation of ChE activity in red blood cells (RBC).⁴⁷ Husain and Somani⁴⁸ demonstrated a 55% increase in plasma cholinesterase activity after 30 min of acute exercise in rats. Acute exercise for 10 to 30 min decreased ChE activity in the heart without affecting ChE activity in the diaphragm, and muscle.⁴⁹ However, Tipton et al.⁵⁰ found no significant change in myocardial ChE activity after chronic exercise. Contrary to acute exercise, chronic exercise decreased ChE activity in RBC, heart, diaphragm, and muscle.⁴⁹ Babu et al.⁵¹ have shown that AChE activity decreased in both EDL and soleus muscles 20 min after acute exercise; whereas, the AChE activity decreased in EDL and soleus muscles 24 h after exercise training. This is contrary to Fernandez and Donoso's findings which reported an increase in the G_4 form of AChE in fast twitch muscle due to exercise.⁵² Similarly, a profound increase in the G_4 form of AChE in fast twitch muscle of rat after exercise training has been reported.⁵³ Different intensities of acute exercise stress produced slight decreases in brain AChE activity in rats.⁴⁷ This finding is in agreement with Ryhanen et al.⁵⁴ and is contrary to the findings of Pedzikiewicz et al.⁵⁵ who have shown a slight increase in brain ChE activity after single exercise. Acute exercise for 30 min decreased AChE activity in the striatum, medulla, and cerebral cortex without any change in the hypothalamus and cerebellum of rats.⁴⁸ Chronic exercise stress decreased AChE activity in the brain stem, in cerebral cortex, in striatum, and hippocampus.⁵⁶ These studies indicate that physical stress accelerates the nerve action in the CNS, resulting in an increased amount of acetylcholine in the nerve endings and hence increasing ChE inhibition.

Alterations in the choline acetyl transferase (ChAT) activity (biosynthetic enzyme for ACh) were differentially expressed within subregions of the brain during chronic exercise.⁵⁶ Exercise decreased ChAT activity in the adrenal gland of young rats.⁵⁷ Endurance training decreased ChAT activity in extensor digitorum longus (EDL); whereas, in slow twitch soleus, it increased.⁵¹ Swimming stress in rats has been shown to deplete the ACh content in various brain regions such as hippocampus and cerebral cortex.⁵⁸ Conlay et al.⁵⁹ reported a decrease in the plasma choline levels of marathon runners. Recently, Conlay et al.⁶⁰ have shown that in trained athletes, running a 26 km marathon reduced plasma choline by 40% and decreased ACh release from the neuromuscular junctions by a similar magnitude. The effect of acute exercise (swimming) in rats for 15 min resulted in a decrease in muscarinic cholinergic receptor (mACh) ligand binding in the cerebral cortex and basal ganglia; whereas, it increased in the cerebellum.⁶¹ Chronic exercise has been reported to produce tolerance to muscarinic antagonists in rats.⁶² These studies suggest that acute exercise or stress exerts rapid reversible and selective changes of cholinergic muscarinic receptors.

The cholinergic system is not only modified by physical stress but is also influenced by a variety of other stress factors. Rats exposed to repeated immobilization stress showed diminished ChAT activity in brain basal ganglia.⁶³ ChAT activity also decreased in the cortex, hypothalamus, hippocampus, and the mid-brain of rats after

acute immobilization stress.⁵⁷ Conversely, ChAT activity increased in the rat cerebral cortex after acute and repeated electroshock.⁶⁴ After immobilization, ChAT activity has been reported unchanged in different brain regions: brain stem, striatum, hippocampus, and hypothalamus.^{65,66} Chronic exposure of rats to cold conditions and other stressors has enhanced ChAT activity in basal ganglia, hypothalamus,⁶⁷ and in the medulla.⁵⁷ Acute exposure of rats to cold stress resulted in an increase in ChAT activity in the hippocampus.⁵⁸ Acute immobilization and cold stress have been shown to increase ChAT activity in the adrenal gland of rats; whereas, no change has been observed with chronic cold stress. In addition, Kita et al.⁶⁷ have reported no change in ChAT activity in rat duodenum after repeated cold stress. It is concluded from the above studies that acute and chronic stresses differentially alter ChAT activity in brain regions, thereby regulating the synthesis of the neurotransmitter and altered sensitivity of the cholinergic system. Chronic cold stress to rats has been shown to increase blood butyryl cholinesterase (BChE) activity and decrease the ChE activity in the lung;⁵⁴ whereas, ChE activity decreased in the duodenum.⁶⁷ Repeated exposure of rats to cold stress caused an enhancement of AChE activity in basal ganglia and the hypothalamus.⁶⁷ However, cholinergic parameters in various regions of the brain react differently to altered stress conditions, such as electric shock, cold, and swimming.

It has been demonstrated that the hippocampal cholinergic system is actively involved in stress response. Acute and chronic stress-induced changes in synaptic ACh release and choline uptake (parameter of cholinergic system) have been studied in rat hippocampus.⁶⁸ Acute as well as chronic intermittent immobilization stress increased ACh release; whereas, choline uptake increased after acute stress and decreased after chronic stress. Repeated cold stress has been shown to decrease the total ACh content in basal ganglia and hypothalamus, whereas its amount increased in the duodenum of rat.^{67,69} Similarly, cold stress resulted in a decrease of ACh levels in the hypothalamus and hippocampus of rat.⁵⁸ It has been assumed that the stores of ACh in the hippocampus of a rat that are exposed to stress may become depleted. However, Costa et al.⁷⁰ and Mizukawa et al.⁷¹ failed to find any change in rat ACh after stress. After electric shock stress, the ACh concentration was found to be depleted in brain regions of rat and mice.^{72,73} Following 2 h of mild restrain stress, choline uptake was increased in hippocampus, septum, and frontal cortex of rat.⁷⁴ The administration of chronic electric shock to rats has increased the ACh content in the medulla.⁶⁷

Information from animal and human studies has suggested the stress-induced hyperactivity of central muscarinic mechanisms.^{75,76} Chronic immobilization stress increased muscarinic receptor binding capacity in hippocampal synaptosomes of rats.⁷⁷ Similarly, immobilization stress produced an increase in muscarinic cholinergic (mACh) binding sites in the septum, striatum, hippocampus, and pons, plus medulla oblongata of rats.⁷⁸ Immobilization stress for 30 min increased the concentration of mACh binding sites in the hippocampus of rat.⁷¹ Restrain stress for 10 days induced hypersensitivity of the central cholinergic system in mice, whereas restrain stress for 30 days caused hyposensitivity of the central cholinergic system.⁷⁹ Similarly, shock stress also increased the hypersensitivity of the central acetylcholine

receptors.⁸⁰ It has been demonstrated that hippocampal muscarinic acetylcholine receptor binding increased in rats after chronic intermittent immobilization stress.⁶⁸ It is suggested that acute stress or exercise may enhance the sensitivity of the cholinergic system, whereas chronic exercise or stress decreases the sensitivity of the cholinergic system.

II. CHOLINERGIC TOXICITY

The cholinergic system is the primary target of OP nerve agent intoxication. The cholinergic system consists primarily of synthetic (choline acetyltransferase) and degradative (acetylcholinesterase) enzymes for the neurotransmitter acetylcholine and its receptors, muscarinic and nicotinic. Nerve agents inhibit AChE activity resulting in accumulation of excess ACh at vital cholinergic sites, thereby causing toxic manifestations. Nerve gases exert their toxic effects by phosphorylating the serine hydroxyl group at the active site of the enzyme AChE, producing irreversible inhibition of the enzyme with consequent elevation of acetylcholine levels. Acetylcholine accumulates at the peripheral and central synapses, leading to cholinergic manifestations. There is depression of the respiratory center in the brain followed by peripheral neuromuscular blockade, causing respiratory paralysis and death.⁸¹ The toxic effects of these nerve agents are dependent on their stability, rate of absorption by various routes, distribution, ability to cross the blood-brain barrier, rate of reaction with AChE and selectivity for reaction with the enzyme at specific foci, and their behavior once attached at the enzyme active site. The mechanism of action of AChE and its inhibition by a nerve agent is depicted in [Figure 3.4](#).

The active site of AChE enzyme consists of two subsites, anionic and esteratic sites. The anionic site is represented by a glutamate ion. The esteratic site has been shown to incorporate a serine moiety and histidine as well as tyrosine residue.⁸² A hydrophobic area at the active site is shown in [Figure 3.4A](#). The normal catalytic functioning of AChE enzyme has been depicted in [Figure 3.4B](#). After acting at the cholinergic receptor, ACh forms a reversible complex with the active site of the enzyme AChE. Next the acetyl group is transferred from the ACh molecule to the serine hydroxyl, thus forming acetylated enzyme and releasing choline. This is followed by a rate-limiting hydrolysis of the acetate ester group with a half-life of 42 s, producing acetate anion, which, in turn, provides regenerated enzyme that can be utilized to hydrolyze another molecule of ACh. The high percentage of released choline is transported back into the nerve ending for reconversion to ACh and storage. OP nerve agents bind rapidly with AChE enzyme protein. Soman reacts with AChE completely within minutes of administration to animals. The inhibition of AChE by OP nerve agent is depicted in [Figure 3.4C](#). It is conceivable that a proton on the imidazolium ion forms a partial bond to the “onyl” oxygen (or sulphur) attached to phosphorus. At the same time, the hydrogen bonding of the tyrosine phenolic hydroxyl to the glutamate anion, and of the serine hydroxyl to the phenolic oxygen, increases electron density at the serine oxygen, which facilitates the nucleophilic attack on phosphorus and displacement of the leaving groups X (F or CN).⁸³ In sharp contrast to the rapid

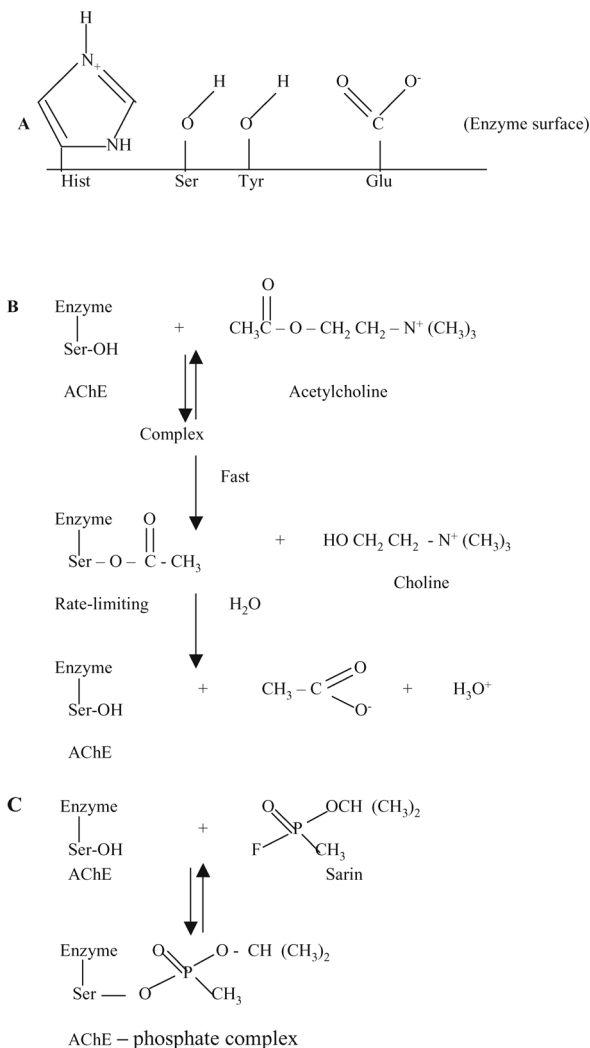


FIGURE 3.4 Mechanism of action of acetylcholinesterase inhibition: (A) Structure of AChE; (B) normal functioning of AChE; (C) inhibition of AChE by nerve agent sarin. (Adapted from Somani et al.²¹²)

hydrolysis of the acetylated enzyme, hydrolysis of phosphorylated enzyme is extremely slow, with a half-life of hours to days. Therefore, the enzyme is effectively inactivated and prevented from carrying out its catalytic function of hydrolyzing ACh. The enzyme molecule phosphorylated with the nerve agent undergoes the aging process (dealkylation) within a few minutes.⁸⁴ The aged enzyme is no longer reactivated with nucleophilic compounds such as oximes. The physiological, biochemical, and histopathological effects due to cholinesterase inhibition induced by low-dose

nerve agents in animals and humans are described below. Behavioral effects including performance in human and animals exposed to low-level nerve agents are described in another chapter in this volume.

Rats exposed to low doses (one ninth the LD_{50}) of sarin produced alterations in motor coordination/balance.⁸⁵ Cholinesterase inhibition induced by continuous infusion of PYR (for at least 3 days before soman exposure and continuing through the exposure period) had little effect on the toxicity of repeated soman exposure in the rodents.⁸⁶ More importantly, there was no deleterious effects of PYR pretreatment and concomitant low-level exposure to soman. The cumulative effects of repeated soman exposure on serum ChE and the relatively insignificant impact of additional PYR exposure are illustrated by the convergence of ChE inhibition levels by the fifth day, regardless of treatment condition.⁸⁷ Low doses of sarin ($3.5 \mu\text{g/kg}$ for 10 days or $7.0 \mu\text{g/kg}$ for 5 days, s.c.) and soman ($2.5 \mu\text{g/kg}$ for 10 days or $5.0 \mu\text{g/kg}$ for 5 days) resulted in a depression of mechano receptors, conduction velocities of muscle spindle, and mechano receptor afferents in cats.⁸⁸ These authors suggested that alteration in muscle spindle function was either due to inhibition of AChE in the muscle or due to direct effects of sarin or soman on the afferents. Rats exposed to low-level sarin (0.2 and 0.4 mg/kg) through inhalation route (1 h singly or 1 h each day for 5 days or for 10 days repeatedly) caused changes in physiological parameters such as respiration rate, core body temperature, and motor activity.⁸⁹ In recent experiments, mice were exercised for 10 weeks and pyridostigmine and/or sarin administered during the fifth and sixth weeks. Exercise parameters such as respiratory exchange ratio (RER) were recorded during exercise. Respiratory exchange ratio (VCO_2/VO_2) decreased significantly at the end of the 5th week of exercise (1 week of dosing) as compared to the 4th week in both the exercise groups, treated with sarin or sarin plus pyridostigmine bromide (PB), respectively (Figure 3.5). Thereafter, a steady increase in RER values was observed with incremental exercise up to the 10th week. However, the patterns of increase in RER values were different in the sarin plus exercise group compared to the sarin plus PB plus exercise group, indicating the interactive effects of PB with low-dose sarin and physical stress.

A. BIOCHEMICAL EFFECTS

The inhibition of AChE activity in nerve tissues of animals at different times after exposure to low-dose nerve agents are depicted in Table 3.3. In most studies, subcutaneous, intravenous, inhalation, and oral routes of exposure were used. AChE activity in whole brain, spinal cord, and brain regions such as cerebral cortex, corpus striatum, medulla, and cerebellum was significantly decreased in rats,^{90–94} mice,^{17,95–98} and hens,⁴⁰ hours, days, and weeks after low-dose exposure to nerve agents such as soman, sarin, and tabun.

The cholinergic effects related to changes in brain AChE activity were assessed in rats repeatedly exposed to low-dose soman for 5 days. The cholinergic effects before and after each injection were examined in the brain regions such as: (1) frontal cortex, (2) piriform cortex, (3) hypothalamus, (4) hippocampus, (5) thalamus, (6) cerebellum, and (7) neostriatum.⁹⁹ Repeated administration of low-dose soman caused a significant decline in AChE activity in all regions of the brain.^{99,100}

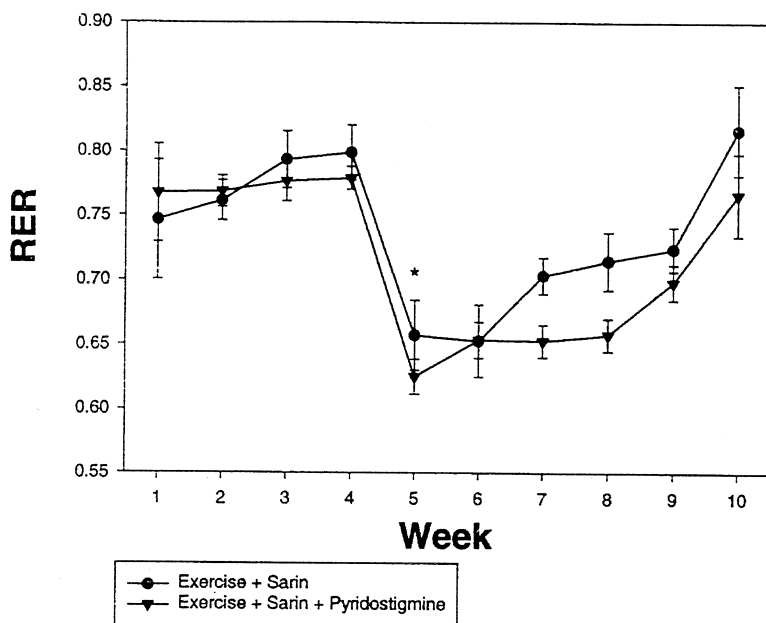


FIGURE 3.5 Effect of exercise training plus sarin (10 $\mu\text{g/kg}$, s.c.) during 5th and 6th weeks; and exercise, sarin, and pyridostigmine (1.2 $\mu\text{g/kg}$, p.o.) during 5th and 6th weeks combination on respiratory exchange ratio (R.E.R.) in mice over a period of 10 weeks. Significant decline in RER observed after the start of pyridostigmine and sarin dosing. Significant * $P < 0.05$.

Exposures to lower levels of soman (approximately 0.3 LD_{50}) in mice⁹⁸ and rats¹⁰¹ caused greater inhibition of cerebellar AChE than either hippocampal or cortical AChE, and less effect on the neostriatum. Soman exposure in these two animal species showed that AChE inhibition in the hippocampus and cortex was equivalent to cerebellar AChE inhibition. The effects of sarin and tabun at 40 and 60% LD_{50} dose levels on AChE activity in different brain regions of mice were studied. The AChE activity was found to be similar to acute high-dose exposures to soman. The hippocampus and cortex were more sensitive, whereas the neostriatum was less sensitive in these studies.⁹⁸ The AChE-rich neostriatum is thus an interesting region neurochemically, being relatively resistant to nerve-agent induced AChE inhibition. It is, however, affected at the same level by soman, sarin, and tabun administered at low doses 50 to 85% of LD_{50} .¹⁰² In another study, tolerance to low-dose soman-induced hypothermia (soman 35 $\mu\text{g/kg}$, s.c. daily for 3 days and then three times weekly for 36 days) was reported by Russell et al.¹⁰³ Body temperature was unaffected during the first 2 days of soman injections and then was reduced after the third injection. The data represent biochemical and physiological correlates of initial resistance of the hypothalamus to chronic low-levels of soman exposure. It seems likely that other processes (such as biogenic amines, other neurotransmitters and their receptors) besides ChE inhibition in the hypothalamus are responsible for tolerance

TABLE 3.3
Effects of Low-Dose Nerve Agents at Different Times on Cholinesterase (ChE) Activity in Nerve Tissues of Animals Under Normal and Stressful Conditions

Species	Nerve Agent Dose ($\mu\text{g/kg}$)	Time	Acetylcholinesterase Activity (% of Control)					Ref.
			Spinal Cord	Brain	Cortex	Striatum	Medulla	
Mice	Sarin 5 mg/m^3 20 min/day for 10 days (Inhalation)	4 days		81				17
Mice	Sarin 85 (s.c.)	1 h			47	39	38	97
Mice	Sarin 85 (s.c.)	1 h			46	41		96
Mice	Sarin 10 (s.c.) $\times 2$ wks	4 wks	89		76	63		95
Mice	Sarin 10 (s.c.) $\times 2$ wks	4 wks	76		81	71		95
Mice	Soman 25 (i.v.) 12.5 (i.v.)	10 min 10 min		56 90				98
Mice	Sarin 60 (i.v.) 40 (i.v.) 20 (i.v.)	10 min 10 min 10 min		24 48 75				98
Mice	Tabun 213 (i.v.) 191 (i.v.) 170 (i.v.) 149 (i.v.)	10 min 10 min 10 min 10 min		31 52 69 69				98
Mice	Soman 37.5 (i.v.)	10 min 4 h 1 day 4 days 1 wk 2 wks		30 27 30 49 55 75				98
Mice	Sarin 80 (i.v.)	10 min 4 h 1 day 4 days 1 wk 2 wks		16 16 33 71 69 89				98

Species	Nerve Agent Dose ($\mu\text{g/kg}$)	Time	Acetylcholinesterase Activity (% of Control)					Ref.
			Spinal Cord	Brain	Cortex	Striatum	Medulla	
Mice	Tabun 213 (i.v.)	10 min		37				98
		4 h		39				
		1 day		50				
		4 days		68				
		1 wk		64				
		2 wks		85				
Rat	Sarin 50 (s.c.)/day for 85 days	24 h				66		94
Rat	Sarin 50.2 mg/m ³ for 15 min	15 min	63		49			92
Rat	Soman 60 (s.c.) 1 \times wk for 6 wks	24 h						93
		after		85				
		2 wks		85				
		4 wks		85				
		6 wks						
Rat	Soman 60 (s.c.) 3 \times wk for 6 wks	24 h						93
		after		60				
		2 wks		42				
		4 wks		25				
		6 wks						
Rat	Soman 25 (s.c.) daily for 85 days	24 h					36	94
		after last dose						
Rat	Soman 60 (s.c.)	1 h			62			90
Rat	Soman 20 (s.c.)	7 days			5	3 (midbrain)	1 (midbrain)	91
Hen	Sarin 61 (p.o.) 70 (p.o.)	24 h		80				40
		24 h		30				
Hen	Soman 3.5 (p.o.) 7.1 (p.o.)			150				40
				60				

Note: Cerebellum of mice showed 40% of control (Sikder et al., 1992) and lung of rat showed 46% of control AChE activity.

development during chronic low-level exposure. A substantial difference has been shown to exist between the recovery of serum ChE and red blood cell (RBC) AChE,¹⁰⁴ which more closely follows the recovery of brain AChE after soman exposure. Similar results reported that RBC AChE activity was significantly inhibited and this inhibition was matched by the responses of whole brain AChE activity.¹⁰³ McDonough et al.¹⁰⁴ compared regional CNS recovery patterns to those of plasma ChE and RBC AChE activity after a 4-week chronic low-dose soman administration protocol. These data indicated slow recovery patterns for RBC, cortex, hippocampus, and neostriatum; whereas, those of midbrain, brainstem, and cerebellum were faster. Plasma ChE activity showed a very fast recovery within 24 to 48 h after the last soman injection. Thus, the earlier discussion suggests that RBC AChE activity may serve as a useful clinical marker for the nerve agent-induced CNS AChE inhibition, but not that of serum ChE. Repeated administration of tabun (70 $\mu\text{g/kg}$, i.m.) for 90 days in hens significantly depressed plasma BuChE activity (44% of control), indicating cholinergic toxicity of tabun in atropine-protected hens.¹⁰⁵ Rats exposed to low-dose sarin (75–300 $\mu\text{g/kg}$, p.o.) for 90 days resulted in a significant inhibition of cholinesterase in plasma and RBC.¹⁰⁶ Low doses of tabun (28 $\mu\text{g/kg}$, p.o.) 5 days per week for 90 days in rats, significantly decreased ChE activity in plasma and RBC.¹⁰⁷ Rats orally exposed to VX at low-dose level (4 $\mu\text{g/kg}$) 5 days per week for 30, 60, and 90 days showed significant inhibition of plasma and RBC ChE activities and slight decrease in body weight.¹⁰⁸ Very low-level sarin inhalation exposure (0.1 and 1.0 $\mu\text{g/m}^3$) for 6 h per day, 5 days per week, for 4–52 weeks to beagle dogs, rats, and mice did not show any adverse toxic side effect in any species at either concentration of sarin.¹⁰⁹ Sarin at a very low dose 1/20 LD₅₀ (10 $\mu\text{g/kg}$, s.c.) daily for 2 weeks resulted in a depression of cholinesterase activity (81, 87, 71, 88, and 68% of control) in plasma, RBC, platelets, sciatic nerve, and triceps muscle, respectively, 4 weeks after the last treatment.⁹⁵ AChE activity in cerebral tissues such as spinal cord, cerebral cortex, and corpus striatum decreased (89, 76, and 63% of control, respectively). It was suggested that the corpus striatum, which has higher basal AChE activity, is more sensitive to sarin exposure. Tabun (70 $\mu\text{g/kg}$, i.m.) for 90 days in atropine-protected hens significantly increased CPK activity (188% of control) in the plasma, indicating muscle damage.¹⁰⁵ The authors suggested that this may be due to increase in acetylcholine followed by mobilization of calcium ions.

The inhibition of cholinesterase in peripheral tissues of animals exposed to low dose nerve agents at different time points is summarized in [Table 3.4](#). In most of the studies, subcutaneous, intramuscular, inhalation, as well as oral routes of exposure were employed. Cholinesterase activity in whole blood and blood constituents, such as plasma, RBC, and platelets, was significantly depressed in humans,¹¹⁰ monkeys,^{111,112} rats,^{18,92,100,113} mice,^{17,18,95–97} and hens^{19,40,105,112} hours and days following low-dose exposure to nerve agents such as soman, sarin, and tabun. AChE activity in sciatic nerve and triceps muscle was decreased 4 weeks after exposure to repeated low-dose sarin in mice.⁹⁵ The inhibition of ChE in plasma, brain, and diaphragm, as well as depression of spontaneous locomotor activity and rectal temperatures, of soman-treated animals had returned to control levels within 24 h, but ChE activity was not fully recovered even after 3 days.¹¹⁴ Acetylcholinesterase inhibited to the

TABLE 3.4
Effects of Low-Dose Nerve Agents at Different Times on Cholinesterase (ChE) Activity in Peripheral Tissues of Various Species of Animals under Normal and Stressful Conditions

Species	Nerve Agent Dose (µg/kg)	Time	Cholinesterase Activity (% of Control)					Ref.
			Plasma/ Serum	RBC	Platelets	Sciatic Nerve	Triceps Muscle	
Mice	Sarin 5 mg/m ³ for 20 min/day for 10 days (Inhalation)	4 days	73 (blood)					17
Mice	Sarin 5 mg/m ³ for 20 min/day for 10 days Inhalation)	4 days		24				18
Mice	Sarin 85 (s.c.)	1 h	40 (blood)					97
Mice	Sarin 85 (s.c.)	1 h	38 (blood)					96
Mice	Sarin 10 (s.c.) × 2 wks	4 wks	81	87	71	88	68	95
Mice	Sarin 10 (s.c.) × 2 wks	4 wks	79	81	58	76	56	95
Rat	Sarin 12.5 mg/m ³ 20 min/day for 10 days (Inhalation)	4 days			29			18
Rat	50.2 mg/m ³ 15 min (Inhalation)	15 min	49 (Blood)					92
Rat	Soman 30 (s.c.) daily for 12 days	Day 5		1.2				113
		Day 12		0.0				
Rat	Soman 39 (s.c.) daily for 5 days	Day 5	14					100

TABLE 3.4 (continued)

Species	Nerve Agent Dose ($\mu\text{g}/\text{kg}$)	Time	Cholinesterase Activity (% of Control)				Ref.
			Plasma/ Serum	RBC	Platelets	Sciatic Nerve	
Marmoset	Soman						111
Monkey	1.75 (i.m.)	1 h	15 (Blood)				
	3.5 (i.m.)	1 h	5 (Blood)				
Monkey	Sarin						112
	2.5 (i.m.)	3 h		64			
	3.0 (i.m.)	3 h		33			
Hen	50 (s.c.) daily for 10 days	4 days			30		19
Hen	Sarin						40
	61 (p.o.)	24 h	40				
	70 (p.o.)	24 h	45				
Hen	Soman						112
	3.5 (p.o.)		64				
	7.1 (p.o.)		33				
Hen	Tabun	2 h	29				105
	70 (i.m.)	6 h	34				
		24 h	54				
		30 days	50				
		60 days	45				
		90 days	44				
Human Volunteer	Sarin						110
	0.5 mg/m^3 for 30 min (Inhalation)	3 h		42			
		3 days		39			

same degree in the corpus striatum and hippocampus 1 h after administration of soman and sarin.¹¹⁵ It was observed that soman and sarin increased the levels of choline and ACh in both striatum and hippocampus with maximal increase at 2 h and recovery of choline levels at 4 h. Drewes and Quian¹¹⁶ showed that the soman-induced increase in brain choline may be secondary to the action of ACh on muscarinic receptors coupled to phosphatidylcholine hydrolysis. Shih¹¹⁷ suggested a possible relationship between elevated choline levels and soman toxicity.

The toxicity of nerve agents may include direct action on nicotinic as well as muscarinic ACh receptors¹¹⁸ when their concentration in circulation rises above micromolar levels.¹¹⁹ At nanomolar levels, their toxicity is the result mainly of their inhibition of AChE. However, at these low concentrations, many OP agents (e.g., soman and VX) may directly affect a small population of muscarinic ACh receptors that have a high affinity for [³H]-cis-methyldioxalane binding. Aas¹²⁰ demonstrated

reduction in the release of ACh from cholinergic nerves in rat bronchi after soman. Long-term inhalation exposure of soman (0.45–0.63 mg/m³) reduced (by approximately 70%) the contraction of bronchi induced by ACh, probably as a result of the reduced number of muscarinic cholinergic receptors.

B. HISTOPATHOLOGICAL EFFECTS

Studies in animals indicate that morphologic changes in the brain may occur after low-dose nerve-agent exposure. In soman-exposed rats,^{121–123} monkeys,^{124,125} and baboons,¹²⁶ in sarin-exposed rats,¹²⁷ and in VX-exposed rats,¹²⁸ neuronal degeneration and necrosis were seen on necropsy as long as 45 weeks after exposure. The neuropathological effects of 5-day repeated soman at daily low doses ranging from 25 to 54 µg/kg were evaluated in rats following survival times of 7 to 35 days after soman exposure. The most sensitive area was noted to be the piriform cortex and the least sensitive, the hypothalamus and neostriatum in both the neurochemical and neuropathological studies. The neostriatum, an extremely rich area for cholinergic function, contains both intrinsic cholinergic neurons and terminals from other nuclei (nigrostriatal dopaminergic pathway) that contain AChE.^{129,130} The nucleus basalis of Meynert in the ventral forebrain, which supplies up to 50% of the cholinergic innervation of frontal and parietal cortices in the rodent,¹³¹ was not damaged even in brains showing the most massive degeneration. Petras^{122,125} reported soman-induced neural degeneration in animals that showed only minor clinical symptoms (fasciculations). Limbic structures (e.g., septum, amygdala, hippocampus) involved with coordinating sensory information of the animal's external environment, as well as that of its viscera with motor function, were heavily affected.

C. CHOLINERGIC TOXICITY UNDER STRESSFUL CONDITIONS

Somani and Husain¹³² have reviewed the effect of physical stress on the cholinergic system indicating that it perturbs the functions of the nervous system. Earlier studies reported that physical stress enhances cholinesterase inhibition due to physostigmine, in central and peripheral tissues of the rat.^{47,133,134} Physical stress is known to induce oxidative stress in the nervous system and increase lipid peroxidation.^{135–137} A correlation between AChE (a membrane-bound enzyme with lipid dependence) inhibition and enhanced lipid peroxidation in specific areas of rat brain following acute and chronic physical stress have been reported.^{48,138} Thus, physical stress influences the membrane lipid peroxidation and membrane-bound enzyme activity which may be related to free radicals. Forced swimming in mice has also been shown to enhance the entry of pyridostigmine (a peripheral reversible cholinesterase inhibitor) across the blood-brain barrier which resulted in inhibition of cerebral AChE activity, enhanced gene expression and cortical functions.¹³⁹ Interaction of pyridostigmine and treadmill exercise resulted in a loss of integrity of the neuromuscular system in rats.¹⁴⁰ Recent reports demonstrated that under physical stress, pyridostigmine enhanced AChE inhibition and increased lipid peroxidation in the triceps muscle of mice 4 weeks after the drug administration.¹⁴¹

The combined effect of physical stress and anticholinesterases on the cholinergic system has not as yet been thoroughly studied. Somani et al.⁵⁶ studied the interaction of a centrally acting anticholinesterase drug physostigmine (PHY), exercise, and the ChAT activity in brain regions of the rat. ChAT activity in the corpus striatum decreased (24, 5, and 8%) due to moderate exercise as well as PHY plus exercise training. Subacute PHY also inhibited brain stem ChAT activity (19%) after 20 min and (22%) after 24 h posttreatment. The hippocampus showed significant decreases in ChAT activity due to PHY plus exercise (28%), but not due to Phy alone. Babu et al.⁵¹ have shown that choline acetyltransferase activity decreased in rats by trained exercise in EDL muscle (32%) and that Phy prolonged this effect even up to 24 h. Soleus muscles showed a small increase of ChAT due to exercise, but Phy plus exercise did not change the activity significantly. No recovery was observed in ChAT activity of EDL in Phy plus trained exercise group even after 24 h. Dube et al.¹³³ reported that the cholinesterase activity in red blood cells of exercised rats that were not exposed to physostigmine increased, while in other tissues the cholinesterase activity decreased slightly. In exercised rats exposed to physostigmine, the cholinesterase activity decreased in 10 to 30 min in red blood cells, brain, heart, diaphragm, and thigh muscles, respectively. Somani and Dube⁴⁹ reported that acute exercise, as well as endurance training, produced a slight decrease in ChE activity of the brain (3 to 9%) at various time points. Acute exercise plus physostigmine showed an increase in ChE inhibition (30% of control) as compared to physostigmine alone (40% of control) at 15 min, which recovered to control level at 60 min. Endurance training plus physostigmine showed a further decrease in ChE activity (48% of control; at 15 min it recovered to 64% of control at 60 min). Somani et al.⁵⁶ demonstrated that AChE activity decreased in the corpus striatum (18%) 20 min after subacute Phy administration and subacute Phy plus acute exercise (19%), or trained exercise (22%). Acetylcholinesterase activity remained at 89, 87, and 90% of control in Phy administered, Phy plus acute exercise, and Phy plus trained exercise, respectively, even after 24 h. The study indicated that Phy, exercise, or the combination of both, decreased AChE activity in a regionally selective pattern.

Besides physical stress, other types of stress including environmental stress have been shown to influence the cholinergic system.^{61,142} The effects of different stresses have been reported in irreversible AChE inhibitors (organophosphate pesticide) intoxication.^{54,143,144} Rynanen et al.⁵⁴ studied the relationship of cold stress and cholinesterase inhibiting organophosphorus compounds to cholinesterase activity in rats. They reported that cholinesterase in the liver of chronically cold-exposed rats (2 weeks) was more sensitive to diisopropyl fluorophosphate (DFP) inhibition when compared to acute cold-exposed rats. Studies have been conducted on the effects of organophosphorous pesticides and exercise on cholinesterase enzymes in rats.¹⁴³ In such studies, parathion-methyl-induced inhibition of serum cholinesterase was less marked 1 h after its termination. The activity of cholinesterase, an enzyme produced in the liver, depends upon a number of endo- and exogenous factors. It may be assumed that increased ChE activity is a secondary effect of hypoxia and the labilization of lysosomal membrane of liver cells after acute exercise. The higher values of serum ChE activity after exercise attenuates the effect of organophosphorus

pesticides on this enzyme; this phenomenon is transient. However, information related to the influence of stress factors on low-dose, nerve-agent-induced cholinergic toxicity is sparse. Rats exposed to restraint stress (5 min/day for 60 days) followed by a single low dose of sarin ($0.1 \text{ LD}_{50} = 10 \text{ } \mu\text{g/kg}$, i.m.) resulted in a significant decrease of nicotinic acetylcholine receptor binding in cortex, brainstem, and mid-brain with no change in cerebellum 24 h after sarin administration.¹⁴⁵ This study further demonstrated that sarin exposure caused up-regulation of M_2 muscarinic ACh receptor binding in midbrain, cortex, and brain stem, with no change in cerebellum, but stress exposure did not alter receptor binding. Plasma cholinesterase activity slightly decreased with sarin and was unaffected with stress exposure. The authors concluded that stress plays a critical role in manifestations of CNS toxicity caused by low-dose sarin exposure. Heat stress did not induce penetration of reversible cholinesterase inhibitor pyridostigmine into the brain of guinea pig.¹⁴⁶ However, exposure to heat stress resulted in a partial inhibition of cerebral AChE activity. Behavioral effects of soman ($20\text{--}160 \text{ } \mu\text{g/kg}$, s.c.) in rats following exposure to different environmental temperatures (-1 , 7 , 15 , 23 , and 31°C) have shown that thermal stress influences soman toxicity.¹⁴⁷ This study concluded that interaction of thermal stress and soman influences motor activity in rats. Rats kept at high environmental temperature (40°C) showed enhanced brain AChE inhibition, hyperglycemia, lactic acidosis, depletion of glycogen in cerebral and peripheral tissues, glycogen phosphorylase and hexokinase activities, and inhibition of succinate dehydrogenase activity when exposed to organophosphorus insecticide diazinon compared to rats kept at normal room temperature.¹⁴⁴ The differences in the toxicity of DFP in inhibiting tissue ChE were observed in experimental animals subjected to a cold environment.¹⁴⁸ Wheeler¹⁴⁹ showed the effect of temperature on soman toxicity in rats. The toxicity of soman increased during exposure to either cold or hot environments and after removal from cold environment. The increased toxicity of soman while in or after removal from a cold environment is believed to be the result of a generalized adrenal cortical stress response. A recent study showed that physical stress enhanced sarin-induced depression of cholinesterase activity in plasma platelets, triceps muscle, sciatic nerve, and corpus striatum of mice.⁹⁵ The effect of cold environmental stress ($+5$ and -5°C) on the toxicity of sarin in mice and rats has been studied.¹⁵⁰ The authors showed that cold temperature sensitized the animals to the inhibition of brain AChE activity by sarin.

III. NON-CHOLINERGIC TOXICITY

In addition to cholinergic toxicity, certain nerve agents at low doses have been reported to induce a long-term neurotoxicity, which is not related to cholinesterase inhibition as mentioned earlier. This noncholinergic toxicity is known as organophosphate-induced delayed neurotoxicity (OPIDN). OPIDN is related to phosphorylation (inhibition) of neuropathy target esterase or neurotoxic esterase (NTE) and subsequent aging of this enzyme. A minimum of 70% NTE inhibition after single exposure and 45% after multiple exposure to organophosphorus nerve agents, and subsequent aging of NTE, is the biochemical prerequisite for the development of

OPIDN.^{18,19,151,152} The main nerve agents (sarin, soman, tabun, and VX) have been shown to inhibit NTE *in vitro* as well as *in vivo*.^{39,153,154} The physiological, biochemical, and histopathological effects of low-dose, nerve-agent-induced delayed neurotoxicity in various species of experimental animals and humans are described in this chapter.

Published studies dealing with delayed neurotoxicity caused by low-level exposure to nerve agents are scanty. However, it has been known since the late 1950s that exposure of normal individuals to low doses of sarin induces abnormalities in central nervous system (CNS) functions.¹⁵⁵ It has also been reported that workers engaged in German chemical warfare production plants showed persistent neurological abnormalities even 10 years after low-level exposure to nerve agents.⁵ Delayed neurotoxic effects have also been reported 6–8 months after sarin exposure to humans in Japan.¹⁵⁶ However, the first reported case of delayed neurotoxicity in animals (sheep) exposed to nerve agents occurred in Skull Valley, Utah.¹⁵⁷ Chickens treated with low-dose tabun (70 $\mu\text{g/kg}$, i.m.) 5 days per week for 90 days did not induce OPIDN behaviorally or histopathologically.¹⁰⁵ Rats treated with low doses of tabun (28 $\mu\text{g/kg}$, i.p.) 5 days per week for 90 days revealed no delayed neurotoxic effects.^{158,159} Antidote-protected chickens treated with VX (40 $\mu\text{g/kg}$, i.m.) for 90–100 days did not show OPIDN behaviorally and histopathologically.¹⁶⁰ Rats exposed to low doses of sarin (0–300 $\mu\text{g/kg}$, p.o.) 5 days per week for 90 days did not induce OPIDN.¹⁰⁷ Repeated intramuscular treatments of tabun (70 $\mu\text{g/kg}$) for 90 days in atropine-protected hens did not cause any delayed neurotoxic symptoms.¹⁰⁵ However, repeated s.c. administration of sarin (50 $\mu\text{g/kg}$) daily for 10 days to hens caused delayed neurotoxic symptoms such as ataxia 4 days after the last dose of sarin.¹⁹ Studies in experimental animals have also shown that low-level sarin (5 mg/m^3 , inhalation) for 20 min daily for 10 days to mice resulted in expression of delayed clinical symptoms such as muscular weakness of the hind limbs, muscle twitching, and mild ataxia 4 days after the last exposure.^{17,18} Studies using very low doses of sarin (10 $\mu\text{g/kg}$, s.c.) daily for 2 weeks in 11 out of 15 mice showed slight to mild muscular weakness in the hind limb and motor incoordination 4 weeks after last sarin administration.⁹⁵ However, there is insufficient information about OPIDN effects at different times after exposure to nerve agents, with different routes of administration.

A. BIOCHEMICAL EFFECTS

Reports on the non-cholinergic biochemical effects such as inhibition of NTE due to low-dose exposure to nerve agents in certain species of animals are scanty. The inhibition of NTE activity in central and peripheral tissues of animals exposed to low-dose nerve agents at different time points are shown in [Table 3.5](#). In most of the studies, subcutaneous, inhalation, and oral routes of exposure were used. NTE activity in platelets, lymphocytes, spinal cord, whole brain, and brain regions, such as cerebral cortex and corpus striatum, was significantly decreased in mice,^{17,95} rats,¹⁸ and hens^{19,40} days and weeks after low-dose exposure to nerve-agent sarin. However, studies have been carried out in protected hens, which are a suitable model for OPIDN evaluation with relatively high doses of nerve agents (at lethal

TABLE 3.5
Effects of Low-Dose Nerve Agents at Different Times on Neurotoxic Esterase (NTE) Activity in Tissues of Animals under Normal and Stressful Conditions

Species	Nerve Agent Dose (μg/kg)	Stress as a Factor	Time	NTE Activity (% of Control)					Ref.
				Platelets	Lymphocytes	Sciatic Nerve	Spinal Cord	Brain	
Mice	Sarin 5 mg/m ³ 20 min/day for 10 days (Inhalation)	Normal	4 days	45			53	41	17
Mice	Sarin 10 (s.c.) × 2 wks	Normal	4 wks	55		82	75	63 (Cortex) 75 (Striatum)	95
Rat	Sarin 12.5 mg/m ³ 20 min/day for 10 days (Inhalation)	Normal	4 days	67			81	65	18
Rat	Sarin 300 (p.o.)	Normal	90 days					85	107
Hen	Sarin 61 (p.o.)	Normal	24 h	67					40
	70 (p.o.)	Normal	24 h	60	80	92			
Hen	Soman 3.5 (p.o.)	Normal	24 h		90	90	86		40
	7.1 (p.o.)	Normal	24 h		70				
Hen	Sarin 50 (s.c.) for 10 days	Normal	4 days	46			62	47	19
Mice	Sarin 10 (s.c.) × 2 wks	Exercise Training	4 wks	42		79	69	58 (Cortex) 72 (Striatum)	95

doses).^{39,40,153,161} Soman at doses of 1.0 and 1.2 mg/kg inhibited spinal cord NTE (67 and 37% of control, respectively) in hens protected with the antidote, atropine.¹⁶¹ Tabun at a dose of 12 mg/kg decreased NTE activity (67% of control) in the spinal cord of protected hens.¹⁶¹ The inhibition of NTE activity in these studies were below threshold levels and suggested the inability of soman and tabun to cause OPIDN. Rats exposed to low-dose sarin (300 μg/kg, p.o.) for 90 days significantly inhibited brain NTE activity (85% of control).¹⁰⁷ Crowell et al.⁴⁰ studied the effects of oral graded

doses of sarin (61, 70, 140, 200, 280, and 400 $\mu\text{g/kg}$) and soman (3.5, 7.1, and 14.2 $\mu\text{g/kg}$) on NTE inhibition in brain, spinal cord, and lymphocytes of hens and showed that sarin decreased lymphocyte NTE activity (33% of control) and brain NTE activity (80–60% of control) whereas, soman did not alter NTE activity in either of the tissues of hens at 24 h after dosing. The authors concluded that sarin might cause a cumulative neurotoxicity but soman appeared to be non-neuropathic.

Female mice exposed to atmospheric sarin ($5 \text{ mg} \cdot \text{m}^{-3}$ for 20 min) daily for 10 days showed significant inhibition of NTE activity in the brain, spinal cord, and platelets (41, 53, and 45% of control, respectively) 4 days after sarin exposure. Results of this study indicate that sarin may induce delayed neurotoxic effects in mice following repeated inhalation exposure.¹⁷ Rats exposed to sarin aerosols (12.5 mg/m^3 for 20 min) daily for 10 days showed significant inhibition of NTE activity in the brain, spinal cord, and platelets (65, 81, and 67% of control, respectively) 4 days after sarin exposure, but the inhibition was below the threshold level.¹⁸ This study concluded that mice are sensitive to delayed neurotoxicity induced by repeated exposure to sarin, whereas rats were insensitive. The delayed neurotoxic effects of the known neurotoxic compound, mipafox, a chemical warfare nerve gas, sarin, and an insecticide, parathion, at low equitoxic doses (0.1 LD_{50}) were compared in hens (more susceptible to OPIDN) after repeated s.c. exposure.¹⁹ Hens treated with mipafox (10 mg/kg , s.c.), sarin (50 $\mu\text{g/kg}$, s.c.), or parathion (1 mg/kg , s.c.) daily for 10 days resulted in significant inhibition of NTE activity in the brain, spinal cord, and platelets 4 days following sarin or mipafox exposures. This study concluded that repeated administration of equitoxic doses of mipafox, sarin, and parathion resulted in severe, moderate, and non-delayed neurotoxic effects, respectively, in hens. In a recent study using a low dose of sarin ($1/20 \text{ LD}_{50} = 10 \mu\text{g/kg}$, s.c.) daily for 2 weeks, it was demonstrated that NTE activity decreased (55, 82, 75, 63, and 75% of control) in platelets, sciatic nerve, spinal cord, cerebral cortex, and corpus striatum, respectively, 4 weeks after the last dose of sarin administration in mice.⁹⁵ Although the inhibition of NTE activity in nervous tissues was below the threshold level, the platelet NTE inhibition was within the threshold limit (45%). This study suggested that platelet NTE inhibition is a more sensitive parameter for assessing OPIDN in experimental animals and humans. However, along with NTE inhibition, clinical symptoms should also be considered.

Organophosphate nerve agents interact with a variety of non-cholinergic enzymes. *In vivo* experiments have shown that nerve agents produce inhibition of succinate dehydrogenase, Na^+K^+ -ATPase, aldolase,¹⁶² Ca^{2+} -ATPase,¹⁶³ tyrosine hydroxylase,¹⁶⁴ and aliesterase.¹⁶⁵ There are reports on the effects of OP nerve agents on non-cholinergic neurotransmitters GABA (gamma amino butyric acid) in the brain,¹⁶⁶ catecholamines,¹⁶⁷ and second messengers, such as cyclic nucleotides.^{168,169} The OP nerve agents produce changes in several neurotransmitters (e.g., dopamine, noradrenaline, and serotonin) in addition to ACh.^{115,166,170–172} These changes may represent a compensatory mechanism in response to overstimulation of the cholinergic system or, in some instances, could result from a direct action of the OP on enzymes relevant to noncholinergic aspects of neurotransmission.¹⁶⁸ Soman, sarin, and tabun inhibited the adenosine receptors' binding of [^3H]-phenylisopropyl adenosine

($[^3\text{H}]\text{L-PIA}$) to the brain membranes in a dose-dependent manner.¹⁷³ Soman was found to be five and nine times more effective than tabun and sarin, respectively, in inhibiting $[^3\text{H}]\text{L-PIA}$ binding. They suggested that nerve agents could interact directly at the A1 adenosine receptors, which could subsequently mediate changes in K^+ permeability of the synaptic membrane, with possible effects on Na^+ and/or Ca^{2+} conductance.

Effects of nerve agents reported to be mediated by hormones include hyperglycemia,¹⁷⁴ hyperlipidemia,¹⁷⁵ increase in cyclic-AMP level,^{169,173} stimulation of protein synthesis,^{176,177} and decrease in brain RNA levels.¹⁷⁸ Kokka et al.¹⁷⁹ studied the time course of the change in temperature and plasma levels of corticosterone, growth hormone, and prolactin following administration of soman. There was an initial rise in corticosterone level after soman administration. The time course of hypothermia after soman did not correlate with the rise in corticosterone.

Spinal cord reflexes were studied by recording the monosynaptic reflexes (MSR), dorsal root reflexes (DRR), polysynaptic reflexes (PSR), and primary afferent depolarization (PAD) due to the effects of nerve agent intoxication. Several investigators^{180,181} reported that the MSR and DRR are especially depressed following OP nerve agent exposure. The OP nerve agents are reported to facilitate the MSR in cats, or depress or abolish it,^{183,184} although the mechanism was found to be unrelated to changes in AChE activity or ACh content of the spinal cord.¹² It has been reported that tabun facilitates PSR and depresses MSR in cat spinal cord. Karczmar¹¹ reported that various mono- and polysynaptic flexor and extensor reflexes vary in their responses to OP compounds owing to variation in the circulatory responses in the spinal cord. Goldstein¹⁸⁵ evaluated subchronic administration of soman and sarin on the spinal MSR and DRR in spinal cord-transected cats. He showed that both agents significantly reduced the area under the MSR and DRR with only minimal changes in the excitability of the potentials. However, none of the nerve agents produced behavioral signs of delayed neurotoxicity. Pretreatment studies of carbamates (physostigmine, pyridostigmine) show that the protective carbamylation of ChE is ineffective against sarin-induced MSR depression.¹⁸⁶ Das Gupta et al.¹⁸⁷ reported that sarin-induced depression of MSR is reversed by thyrotropin-releasing hormone (TRH). They suggested that the beneficial effect of TRH in this situation may involve a noncholinergic mechanism.

B. HISTOPATHOLOGICAL EFFECTS

Studies in experimental animals have shown that repeated low-dose exposure to nerve agents cause histopathological lesions in the nervous system. The delayed neurotoxic lesions in the spinal cord due to low dose repeated inhalation exposure of sarin in two species of rodents, rats and mice, have been reported by Husain et al.¹⁸ Rats exposed to sarin aerosols (12.5 mg/m^3 for 20 min) daily for 10 days showed swollen axons without fragmentation and loss of myelin in a few places of the spinal cord. The axonal degeneration in the spinal cord was not observed in rats exposed to sarin. Mice exposed to sarin aerosols (5 mg/m^3 for 20 min) daily for 10 days showed focal axonal degeneration in the spinal cord.¹⁷ This study concluded that mice are sensitive to

delayed neurotoxicity induced by repeated exposure to sarin, whereas rats are less sensitive.¹⁸ Low doses of sarin (75–300 $\mu\text{g/kg}$, p.o.) for 90 days in rats caused cerebral necrosis.¹⁰⁶ The delayed neurotoxic effects of sarin at low dose (0.1 LD_{50} = 50 $\mu\text{g/kg}$, s.c. daily for 10 days) were studied in the hen.¹⁹ The spinal cords of hens treated with sarin showed moderate axonal degeneration. It is suggested that the repeated exposure of nerve agent, specifically sarin, at low dose may produce OPIDN.

C. NON-CHOLINERGIC TOXICITY UNDER STRESSFUL CONDITIONS

The effects of various types of stress on cholinergic toxicity due to anticholinesterase agents are known. However, the effects of various stress factors on nerve-agent-induced non-cholinergic toxicity are not well documented. The effect of low and high social stress on triorthotolyl phosphate (TOTP)-induced delayed neurotoxicity in hens has been reported by Ehrich and Gross.¹⁸⁸ Low social stress chickens had no competition for food or water and were housed individually in single cages with two automatic water sources and a single feeder. They were exposed to continuous soothing background music and daily socialization with animal caretakers. High social stress chickens had to compete for food, water, and group dominance, and were housed in a group of seven to eight birds per cage with a single automatic water source and a single feeder. These authors showed that clinical signs of OPIDN were less in the low social stress group, unless exposed to a high stress environment 24 h before TOTP administration. NTE activity was less than 20% of control value in all treatment groups. The authors suggested that protection of birds from OPIDN was due to reduction of conversion of TOTP to its active metabolite. A recent report showed that physical stress enhanced the clinical symptoms such as muscle weakness of the hind limb of mice treated with low-dose sarin (1/20th LD_{50} = 10 $\mu\text{g/kg}$) 4 weeks after treatment.⁹⁵ Physical stress enhanced sarin-induced inhibition of NTE in platelets, spinal cord, and cerebral cortex, and increased lipid peroxidation in triceps muscle and spinal cord in mice. Plasma creatine phosphokinase (CPK) activity was also enhanced in mice treated with sarin and exercised on treadmill, indicating neuromuscular effects of the combination. It is suggested that physical stress seems to potentiate the delayed neuro toxicity in subjects exposed to low dose sarin.

IV. SUMMARY

This chapter is a review of low-dose organophosphorus (OP) nerve agents (tabun, sarin, soman, and VX) induced toxicity under normal as well as stressful conditions. This chapter also deals with the interaction of environmental and physical stress on cholinergic as well as non-cholinergic effects induced by low-dose exposure to nerve agents and their potential for additive or synergistic neuropathologic sequelae. These agents exert their major acute toxic effects on the central and peripheral nervous system via acetylcholinesterase (AChE) inhibition. This is a high-affinity, covalent, and irreversible phosphorylation with slow reactivation or dephosphorylation. Aging

occurs within a few minutes to an hour of phosphorylated AChE due to dealkylation. The physiological, biochemical, and histopathological changes due to low-dose exposure to these agents are described in human and experimental animals. These agents induce delayed neurotoxicity in human, hen, and other susceptible animals with a single high dose or repeated low dose which is characterized by a delay period of 4–21 days before clinical symptoms such as muscular weakness of the hind limb and ataxia. The molecular target for delayed neurotoxicity is a membrane-bound enzyme called neuropathy target esterase or neurotoxic esterase (NTE). Phosphorylation of NTE and subsequent aging is required to initiate axonal degeneration followed by demyelination in peripheral nerve and spinal cord. NTE is also distributed in non-nerve tissues, and platelet NTE can be used as a molecular marker for assessing delayed neurotoxicity in humans or animals exposed to neuropathic nerve agents. Delayed neurotoxicity due to low-dose exposure to these agents, specifically sarin, in terms of behavioral, biochemical, and histological changes are described. It is suggested that physical stress seems to potentiate the delayed neurotoxicity caused by low-dose exposure to sarin.

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