

# MECHANISM AND CLINICAL IMPORTANCE OF RESPIRATORY FAILURE INDUCED BY ANTICHOLINESTERASES

Anita Ivošević<sup>1</sup>, Nataša Miletic<sup>2</sup>, Maja Vulovic<sup>3\*</sup>, Zoran Vujkovic<sup>4</sup>, Snjezana Novakovic Bursac<sup>5</sup>, Slavko S. Cetkovic<sup>6</sup>, Ranko Škrbic<sup>7</sup>, and Milos P. Stojiljkovic<sup>2,7</sup>

<sup>1</sup>Department of Internal Medicine, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

<sup>2</sup>Medical Faculty, University of East Sarajevo, Foča, Republic of Srpska, Bosnia & Herzegovina

<sup>3</sup>Department of Anatomy and Forensic Medicine, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

<sup>4</sup>Neurology Clinic, University Clinical Centre of Republic of Srpska, Medical Faculty, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia & Herzegovina

<sup>5</sup>Institute for Physical Medicine and Rehabilitation „Dr Miroslav Zotović“, Banja Luka, Republic of Srpska, Bosnia & Herzegovina

<sup>6</sup>Military Medical Academy, Belgrade, Serbia

<sup>7</sup>Department of Pharmacology, Toxicology & Clinical Pharmacology, Medical Faculty, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia & Herzegovina

## MEHANIZAM I KLINIČKA VAŽNOST RESPIRATORNE INSUFICIJENCIJE IZAZVANE ANTIHOLINESTERAZAMA

Anita Ivošević<sup>1</sup>, Nataša Miletic<sup>2</sup>, Maja Vulovic<sup>3\*</sup>, Zoran Vujkovic<sup>4</sup>, Snjezana Novaković Bursac<sup>5</sup>, Slavko S. Četković<sup>6</sup>, Ranko Škrbic<sup>7</sup>, and Miloš P. Stojiljković<sup>2,7</sup>

<sup>1</sup>Katedra za internu medicinu, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija

<sup>2</sup>Medicinski fakultet, Univerzitet u Istočnom Sarajevu, Foča, Republika Srpska, Bosna i Hercegovina

<sup>3</sup>Institut za anatomiju i sudsku medicinu, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija

<sup>4</sup>Klinika za neurologiju, Univerzitetski klinički centar Republike Srpske, Medicinski fakultet, Univerzitet u Banja Luci, Banja Luka, Republika Srpska, Bosna i Hercegovina

<sup>5</sup>Zavod za fizikalnu medicinu i rehabilitaciju „Dr Miroslav Zotović“, Banja Luka, Republika Srpska, Bosna i Hercegovina

<sup>6</sup>Vojnomedicinska akademija, Beograd, Srbija

<sup>7</sup>Zavod za farmakologiju, toksikologiju i kliničku farmakologiju, Medicinski fakultet, Univerzitet u Banja Luci, Banja Luka, Republika Srpska, Bosna i Hercegovina

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### ABSTRACT

*Respiratory failure is the predominant cause of death in humans and animals poisoned with anticholinesterases. Organophosphorus and carbamate anticholinesterases inhibit acetylcholinesterase irreversibly and reversibly, respectively. Some of them contain a quaternary atom that makes them lipophobic, limiting their action at the periphery, i.e. outside the central nervous system. They impair respiratory function primarily by inducing a desensitization block of nicotinic receptors in the neuromuscular synapse. Lipophilic anticholinesterases inhibit the acetylcholinesterase both in the brain and in other tissues, including respiratory muscles. Their doses needed for cessation of central respiratory drive are significantly less than doses needed for paralysis of the neuromuscular transmission. Antagonist of muscarinic receptors atropine blocks both the central and peripheral muscarinic receptors and effectively antagonizes the central respiratory depression produced by anticholinesterases. To manage the peripheral nicotinic receptor hyperstimulation phenomena, oximes as acetylcholinesterase reactivators are used. Addition of diazepam is useful for treatment of seizures, since they are cholinergic only in their initial phase and can contribute to the occurrence of central respiratory depression. Possible involvement of central nicotinic receptors as well as the other neurotransmitter systems – glutamatergic, opioidergic – necessitates further research of additional antidotes.*

**Keywords:** Anticholinesterase, Acetylcholinesterase, Acetylcholine, Atropine, Oxime, Diazepam, Respiratory depression, Muscarinic receptors, Nicotinic receptors

### SAŽETAK

*Respiratorna insuficijencija je dominantan uzrok smrti kod ljudi i životinja trovanih antiholinesterazama. Organofosforne i karbamatske antiholinesteraze inhibišu acetilholinesterazu ireverzibilno, odnosno reverzibilno. Neke od njih sadrže kvaternarni atom koji ih čini lipofobnim, čime im ograničava delovanje na periferiju, tj. van centralnog nervnog sistema. One oštećuju respiratornu funkciju primarno izazivajući desenzitizujući blok nikotinskih receptora u neuromuskularnoj sinapsi. Lipofilne antiholinesteraze inhibišu acetilholinesterazu i u mozgu i u drugim tkivima, uključujući i respiratorne mišiće. Njihove doze neophodne za prekidanje centralnog respiratornog generatora impulsa su značajno niže od doza potrebnih za paralizaciju neuromuskularne transmisije. Antagonist muskarinskih receptora atropin blokira i centralne i periferne muskarinske receptore i efektivno antagonizuje centralnu respiratornu depresiju izazvanu antiholinesterazama. U cilju kupiranja hiperstimulacije perifernih nikotinskih receptora koriste se oksimi, kao reaktivatori acetilholinesteraze. Dodavanje diazepam je korisno u tretmanu konvulzija, pošto su one holinergičke samo u svojoj početnoj fazi i mogu da doprinesu pojavi respiratorne depresije. Moguća umešanost centralnih nikotinskih receptora, kao i drugih neurotransmiterskih sistema – glutamatergičkog i opioidergičkog – zahteva dalje istraživanje dodatnih antidota.*

**Ključne reči:** Antiholinesteraze, Acetilholinesteraza, Acetilholin, Atropin, Oksim, Diazepam, Respiratorna depresija, Muskarinski receptori, Nikotinski receptori





## ACETYLCHOLINE, ACETYLCHOLINESTERASE AND THEIR PHYSIOLOGICAL FUNCTIONS

Acetylcholine is a neurotransmitter of vital importance in the central nervous system (CNS), but also peripherally, i.e. in the vegetative nervous system (VNS) ganglia and at the endings of the postganglionic parasympathetic fibers, such as heart, smooth muscle cells and exocrine glands (1). A specifically important role of acetylcholine is to mediate transmission at the neuromuscular junction of skeletal muscles (2).

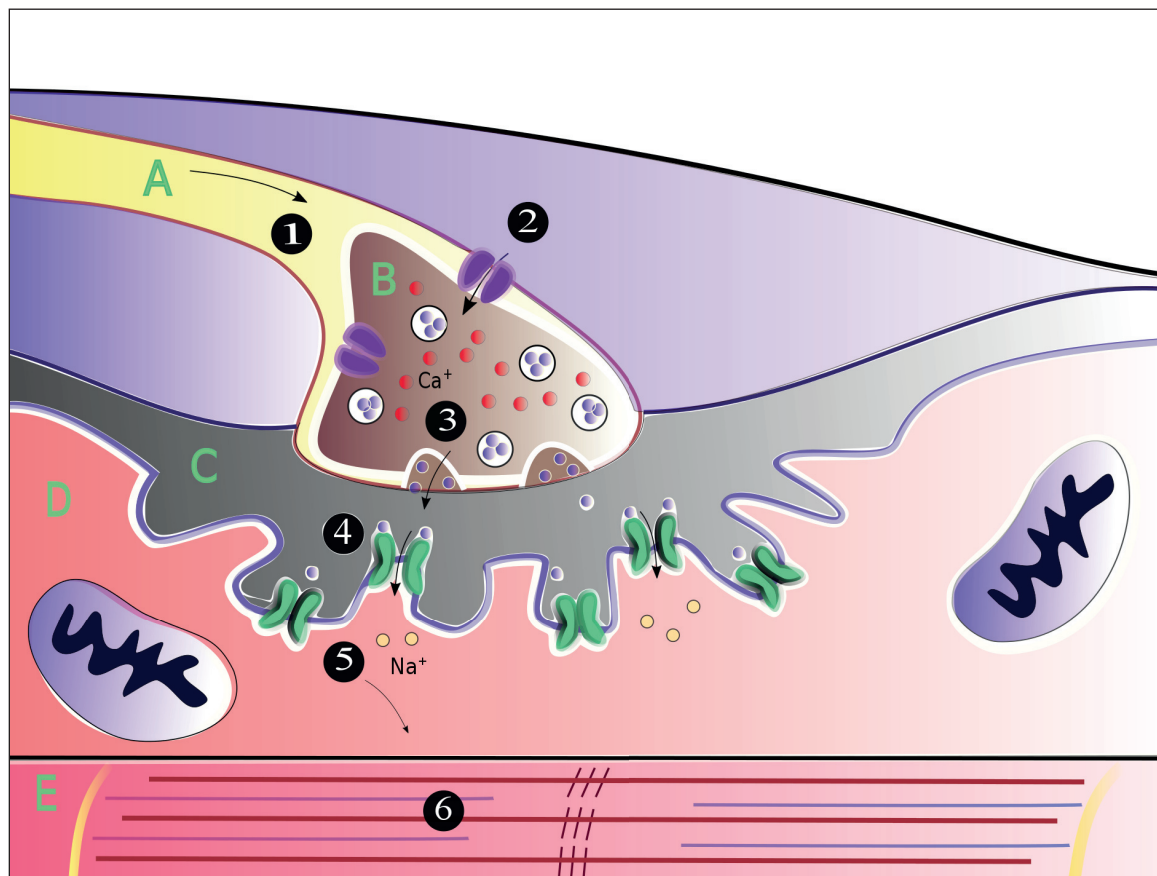
When the action potential reaches the motoneuron ending, it opens the voltage-dependent calcium channels and causes the influx of calcium ions into the nerve ending. As a result, acetylcholine vesicles fuse with the presynaptic membrane and the neurotransmitter is released into the synaptic cleft by exocytosis. After reaching the postsynaptic membrane, acetylcholine binds to nicotinic receptors and opens the sodium channels. The ensuing influx of sodium cations into the skeletal muscle cell triggers the action potential that reaches the myofibrils and causes a muscle contraction (3). All these details are shown in Figure 1.

Acetylcholinesterase (AChE) is an enzyme located in cholinergic synapses within the synaptic cleft. It is very active, which means that it breaks down the molecules of acetylcholine into choline and acetate in split-second assuring thus that there is no surplus of acetylcholine to induce the overstimulation of the cholinergic receptors located at the postsynaptic membrane (2).

While in the CNS the types of cholinergic receptors or cholinceptors through which acetylcholine exerts its action are believed to be both muscarinic and nicotinic, this division is much simpler at the periphery – muscarinic receptors are located at the endings of postganglionic parasympathetic fibers, while nicotinic ones are located in the both sympathetic and parasympathetic ganglia and at the neuromuscular junction (1, 2).

## ANTICHOLINESTERASES AND THEIR MODE OF ACTION

Acetylcholinesterase (AChE) inhibitors or anticholinesterases comprise various chemical entities whose com-



**Figure 1.** Schematic presentation of neuromuscular junction. (1) Nerve impulse reaches the nerve ending. (2) It opens the voltage calcium channels triggering a calcium ion influx. (3) Vesicles with acetylcholine fuse with the presynaptic membrane and the transmitter is excreted into the synaptic cleft. (4) Acetylcholine binds to the postsynaptic nicotinic receptors and opens the sodium ion channels. (5) Sodium ions enter the muscle fiber. (6) Sodium ion influx triggers the action potential in the myofibrils and causes muscle contraction. A = motor neuron axon, B = axon terminal, C = synaptic cleft, D = muscle cell, E = myofibril.



mon characteristic is ability to inhibit AChE, an enzyme crucial for the breakdown of acetylcholine. They can be divided into irreversible and reversible inhibitors, with organophosphates belonging to the former group and carbamates belonging to the latter one. Some of them have a role in medicine as therapeutic agents and are used (i) in treatment of poisonings with anticholinergic drugs - physostigmine, (ii) Alzheimer's disease - rivastigmine, and donepezil, (iii) glaucoma - phospholine (echothiophate), (iv) for antagonizing competitive neuromuscular blockade after the end of the operations - neostigmine and (v) for treatment of myasthenia gravis and (vi) in prophylaxis of the intoxications with nerve agents - pyridostigmine (4-6), while the others are being used as insecticides - parathion, paraoxon, malathion, dichlorvos, carbaryl, carbofuran (7, 8). A special group of organophosphorus anticholinesterases are nerve agents - tabun, sarin, soman and O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX) - that have a potential military use as chemical weapons of mass destruction (2, 9).

The physiological role of AChE is to terminate action of acetylcholine, in order to prevent the overstimulation of the cholinergic receptors. As a consequence, the inhibition of AChE leads to this overstimulation and various phenomena, signs and symptoms occur as a result of this action (10, 11). Clinical picture of anticholinesterase poisoning depends on the route of exposure (12) and on the quantity of the anticholinesterase in the organism, but includes miosis, bronchoconstriction, hypersalivation, bronchorrhoea, skeletal muscle fasciculations, bradycardia, hypotension, seizures and respiratory failure, the latter being the main cause of death (9, 13).

The topic of this mini-review is to elaborate on the mechanism of the anticholinesterase-induced respiratory failure.

## PERIPHERAL VERSUS CENTRAL ANTICHOLINESTERASES

Ever since the organophosphorus nerve agents became known to mankind in the 1940s, a considerable body of literature was published on the clinical picture of intoxications induced by organophosphorus compounds (OPCs) and specifically nerve agents. Cause of death in most of the animal species investigated was respiratory failure (14), although in many publications a considerable attention was drawn to the cardiovascular collapse as an independent factor contributing to a lethal outcome (15).

Anticholinesterases impair the respiratory function in mammals depending on their ability to pass the haematoencephalic or blood-brain barrier (BBB). The ones that contain a quaternary N-atom in their molecules are strongly ionized and hence hydrated and as such cannot pass the BBB, exerting their AChE-inhibiting effect only in the periphery, i.e. outside the CNS. Among the OPCs such examples are phospholine (echothiophate) iodide (16), while the most famous peripherally-acting carbamates are

neostigmine and pyridostigmine, the latter being also used in prophylaxis against nerve agents (17).

The remaining anticholinesterases are, more or less, lipophilic and readily pass the BBB inhibiting thus the brain AChE, as the most important target. Best examples of such molecules are nerve agents tabun, sarin, soman and VX, insecticides dichlorvos (DDVP) and paraoxon (metabolite of parathion) and the oldest known anticholinesterase carbamate, physostigmine, which had become a model-substance in pharmacological research (15, 18). Soman is so highly lipophilic, that it reaches the brain circulation just 1 min after IV injection and completely distributes throughout the brain tissue in only 3 min (19).

## PERIPHERAL COMPONENT OF ANTICHOLINESTERASE-INDUCED RESPIRATORY DEPRESSION

Exclusively peripherally acting anticholinesterases inhibit AChE in peripheral tissues, including bronchi and neuromuscular synapse. The pulmonary consequences include failure of AChE to destruct acetylcholine, leading to overstimulation of muscarinic receptors in smooth muscles of bronchi and in bronchial exocrine glands, leading to bronchoconstriction and bronchorrhoea (20).

Although this pulmonary muscarinic syndrome compromises the alveolar gas exchange and lead to hypoxaemia, the results of significant inhibition of AChE in diaphragm and intercostal muscles is considered more serious and more important for survival, although it depends on the animal species studied (14). Anticholinesterases usually in the beginning induce a slight increase in contractions of diaphragm, but longer-lasting surplus of acetylcholine in the vicinity of nicotinic receptors eventually lead to a Wedensky-type of depolarization block (21). The final outcome is a flaccid paralysis of respiratory muscles and death due to an asphyxia (22). In such cases, peripheral respiratory paralysis usually occurs after the animal was pretreated with atropine, while normal phrenic nerve discharges still can be recorded (21, 23).

## NAUROANATOMY OF RESPIRATORY NEURONS

Respiration is a complex function driven by the groups of cholinergic neurons in the CNS. They can be divided into three groups: dorsal, ventral and pontine (24). Dorsal neurons are located in the medulla, close to Nucleus tractus solitarius and receive sensory signals from the vagal nerve (25). Ventral neurons are located in ventrolateral medulla and are divided into rostral, intermediate and caudal ones. Rostral neurons contain the pre-Bötzinger complex and are very important generator of central respiratory drive. As a matter of fact, pre-Bötzinger complex is a respiratory oscillator. Bilateral injections of OPCs into this group of neurons

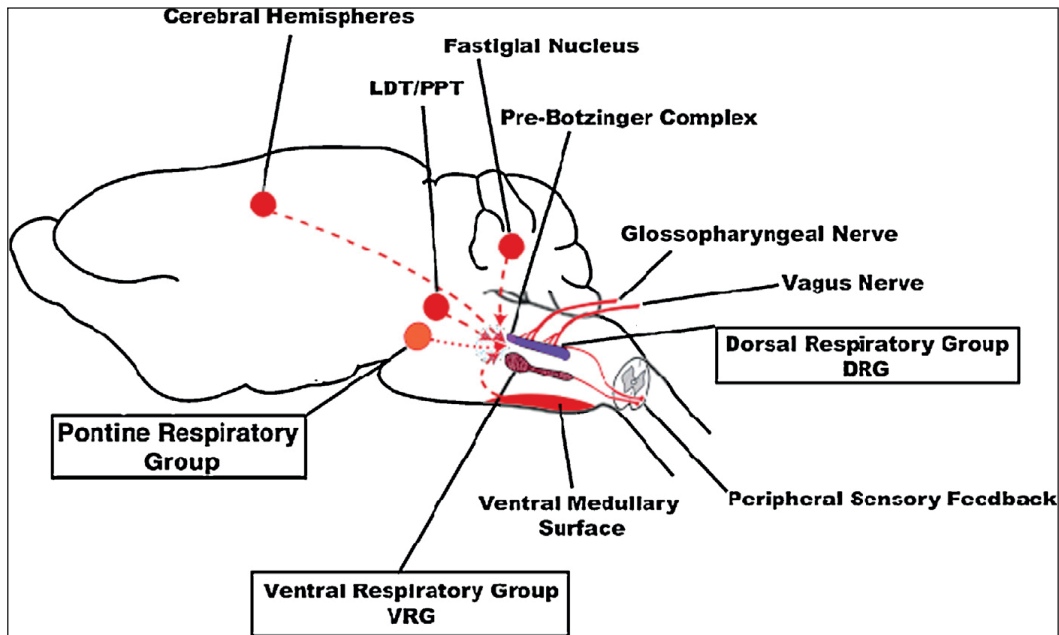


Figure 2. Distribution of neuron groups important for respiration in rat brain and their projections.

induce apnea that can be reversed by atropine (25). According to other authors, ventral respiratory group of neurons consists of five separate subgroups: caudate, intermediate, rostral, pre-Bötzinger and Bötzinger (24). Pontine group is known as pneumotaxic centre. It consists of two groups of neurons – medial parabrachial nucleus and Kölliker-Fuse nucleus (24). Its main function is to switch from inspiration to expiration (25). It is believed that pre-Bötzinger and Bötzinger complexes and rostral ventrolateral medulla contain the neuronal circuits of the respiratory central pattern generator (26). The special details of the distribution of these groups of neurons are shown in Figure 2.

### CENTRAL COMPONENT OF ANTICHOLINESTERASE-INDUCED RESPIRATORY DEPRESSION

Nerve agents have tendency to produce the highest levels of AChE inhibition in the ponto-medullary region where these nuclei are located (27). It automatically means that in these discrete brain regions acetylcholine builds up the most. Indeed, anticholinesterases, depending on the dose injected, first stimulate respiration (21, 22).

Higher doses of anticholinesterases induce respiratory depression. It can be manifested as bradypnoea, prolonged pause between inspirations and expirations and, in most severe cases, as total desynchronisation of the central respiratory stimuli, resulting in chaotic contractions of respiratory muscles that make the respiration inefficient and lead to hypoxaemia and hypercapnia (28).

Local administration of soman into the intermediate part of the ventral surface of medulla oblongata profoundly affected both the respiratory and cardiovascular functions. All these effects were reproduced after replacing soman

with muscarinic receptor agonist oxotremorine and reversed by atropine, implying the involvement of muscarinic mechanisms (29).

At the same time, nicotine does not only act as a powerful poison of respiratory centres, since mecamlamine, a centrally-acting nicotinic receptor antagonist can restore the OPC-induced respiration and exerts significant protection of mice poisoned with soman (30). These and other findings suggest the involvement of both muscarinic and nicotinic receptors in the anticholinesterase-induced central respiratory failure (31).

What is probably more important than the elucidation of the primary receptor pathway of the central anticholinesterase-induced respiratory failure is the fact that most of the authors ascertained that the central respiratory component was endangered even by lower doses of organophosphates and carbamates. For example, in the anaesthetized cat, only 1 LD<sub>50</sub> of soman was needed to cause a central respiratory paralysis, in comparison with 14 LD<sub>50</sub> of soman needed for obtaining the neuromuscular blockade (32). These cats were instrumented in such a way that gastrocnemius muscle was electrically stimulated in situ, allowing thus the peripheral neuromuscular function to be checked irrespectively of the discharges from the CNS, which were at the same time monitored in the proximal part of the phrenic nerve. In this experiment, Rickett et al (32) found that a 14-fold higher dose of soman was needed to block the sciatic nerve-gastrocnemius preparation in situ, than to cause a central cessation of phrenic nerve discharges.

This central versus peripheral ratio varied, depending on both the animal species and the anticholinesterase used. Under the same conditions, dose capable of causing central and peripheral respiratory blockade in guinea pigs





were 38 and 400 mcg/kg IV for soman (ratio 1:10) and 82 and 650 mcg/kg IV for sarin (ratio 1:8) (33). It was shown that VX that has the slowest onset of action, has roughly the equal potential for central and peripheral AChE inhibition and respiratory impairment (18, 34). The same applies to tetraethyl pyrophosphate (TEPP) poisoning in cats (22). As already mentioned, soman is a typical example of predominantly centrally acting anticholinesterase nerve agents, where the activity of respiratory centre is impaired first, followed by the neuromuscular transmission and pulmonary muscarinic syndrome, which is of definitely least clinical significance (14). At the same time, TEPP and sarin in rabbits first affect the neuromuscular transmission and then cause a respiratory arrest (21).

### THERAPEUTIC REGIMENS FOR ANTICHOLIN- ESTERASE-INDUCED RESPIRATORY DEPRESSION

From the therapeutic point of view, pulmonary muscarinic syndrome can be easily treated with muscarinic receptor antagonist atropine (35), which remains without any effect on nicotinic receptors of the neuromuscular junction even when applied in very high doses (21, 36). Additional proof that bronchoconstriction, bronchorrhoea and bradycardia are of a purely peripheral nature consists in a finding that they can be effectively treated even with N-methyl atropine, a quaternary derivative of atropine that cannot pass the BBB (33).

Use of a ganglionic blocker pentamethonium (C5) or a classical neuromuscular nicotinic receptor antagonist d-tubocurarine assures protection against endogenous acetylcholine-induced toxicity resulting from poisonings with nerve agents (22, 37). However, since in the clinical settings it is not easy to find the right dose of nicotinic receptor antagonists that would not be paralyzing per se, peripheral nicotinic receptors are usually treated with oximes, as AChE reactivators (2, 36). They act as chelators that remove the inhibitor from the active centre of AChE and thus restore the enzyme's activity (11, 38).

Therapeutic implications of centrally acting anticholinesterases include use of atropine or even more lipophilic antimuscarinic agent scopolamine. In a vast range of doses (0.5-10 mg/kg) and routes of administration (IV, IM, SC), atropine eliminates signs of central respiratory depression induced by nerve agents (7, 14, 23), while N-methylatropine remains without any effect even after administration of the 100-fold equimolar doses proving thus the central site of the atropine therapeutic action (7). Atropine 2 mg/kg IV was able to counteract the respiratory arrest and bradycardia induced by microinjections of sarin into the lateral reticular nucleus of the rabbit medulla (39). At the same time, there are some limits to the effectiveness of atropine only regimens against soman-induced respiratory depression in guinea pigs – dose of 10 mg/kg IV is efficacious after administration of 2 LD<sub>50</sub>,

partially effective after 5 LD<sub>50</sub> and totally ineffective after 10 LD<sub>50</sub> of soman (40).

Part of the treatment protocols for patients poisoned with anticholinesterases is treatment with atropine and oxime, in order to manage both the central muscarinic and peripheral muscarinic and nicotinic signs of intoxication, respectively (11, 41). Although not efficient as a reactivator of tabun- or soman-inhibited AChE, pralidoxime, in the form of chloride (2-PAM) or methanesulphonate (P2S) has been the most widely used oxime, effective against sarin and VX, but also against many anticholinesterase insecticides (11). It has been determined that the maintenance of pralidoxime minimum plasma concentration of 4 mg/l is crucial for its therapeutic effect (42). Since it falls below this level after 1.5-2 h after the IV bolus administration of 1 g of 2-PAM, it is recommended that this oxime is administered as a continuous IV infusion at a rate of 0.5 g/h (43). In the open field situation, however, IV route of administration might be too demanding for the health personnel trying to treat a number of seriously poisoned individuals and IM route therefore should be preferred, when a single dose of 500 mg of pralidoxime should be injected. Such application, up to three times and separated with 20-min intervals, should not produce any adverse effects, while the same therapeutic regimen in individuals already taking pralidoxime PO as prophylaxis may induce a reversible visual impairment (44).

It is generally accepted that soman and other centrally-acting AChE inhibitors via cholinergic hyperstimulation start a vicious circle that ends up with glutamatergic excitatory discharges that clinically manifest themselves as seizures and leave the survivors with serious brain damage (45, 46). It is not clear what the relation between the onset of seizures and the occurrence of respiratory depression is in soman-poisoned animals, since most of the cited experiments have been performed under urethane anaesthesia (47). In non-anaesthetised animals, loss of consciousness coincided with seizures and respiratory depression occurred immediately thereafter (33). In epilepsy, a considerable number of patients with seizures developed a central apnoea and hypoxaemia (48) and it is therefore logical to assume that then same applies to the seizures induced by acetylcholinesterase inhibitors. For the same reason, it seems plausible to conclude that administration of diazepam, as anticonvulsant, is beneficial for the treatment of both conditions (11).

Although the efficacy of atropine in preventing the central respiratory depression induced by anticholinesterases suggests the obvious role of muscarinic receptors, nicotinic receptors are likely to be involved, too and this mechanism probably includes some non-cholinergic transmitters, such as endogenous opioids. Indeed, it seems that, in presence of soman and atropine, it is the stimulation of nicotinic presynaptic receptors that induces the liberation of glutamate (49). There is some evidence that this pathway can also represent a link between cholinergic and glutamatergic neurons that in turn exerts control over release of endogenous opioids in the CNS (50).



## CONCLUSION

Only peripherally-acting anticholinesterases induce death by overstimulating with excess of acetylcholine the nicotinic receptors at the neuromuscular junction, while muscarinic pulmonary syndrome is of minor importance for survival. Centrally-acting anticholinesterases inhibit AChE in the pontomedullary region and impair the functioning of the respiratory centre neurons, since they receive numerous cholinergic inputs. Phrenic nerve discharges cease in these cases at doses of anticholinesterases much lower than those needed for inducing peripheral neuromuscular block. The recommended therapy includes atropine that blocks central and peripheral muscarinic receptors and oximes, which reactivate the inhibited AChE mainly outside the CNS, but also diazepam, in order to control seizures. Possible involvement of central nicotinic receptors as well as the other neurotransmitter systems – glutamatergic, opioidergic – necessitates further research of additional antidotes.

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