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Review

Choice of approaches in developing novel medical countermeasures for nerve agent poisoning

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ABSTRACT

During the establishment of a research branch, all relevant matters encountered will be of interest to study. After having acquired a body of basal knowledge, it becomes possible to derive ideas or hypotheses for further elaboration of information. The purpose of the present study was to show that therapies for nerve agent poisoning based on specific neuropharmacological approaches can have greater probability for being successful than treatment regimens based on fragmental research or serendipitous discoveries. By following the guidelines for research in experimental epilepsy, neuronal target areas for nerve agents have been identified through lesion studies, and critical receptors for pharmacological treatment have been specified through microinfusion studies of rats. Subsequent experimentations have shown that the results achieved from microinfusion studies are transferable to systemic administration. It is demonstrated that a treatment regimen developed through the novel approach is more efficacious than regimens derived from conventional research on countermeasures. A therapy consisting of HI-6, levetiracetam, and procyclidine that has been worked out along the new lines, exerts powerful anticonvulsant capacity and appears to have universal utility as a stand-alone therapy against soman intoxication in rats. It would be of great interest to examine whether the latter findings can be expanded to other animal species than rats and other classical nerve agents than soman.

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1. Introduction

Organophosphates make up a very large class of chemicals. Several hundreds of organophosphates have been synthesized and produced commercially worldwide since the Second World War. The majority of these compounds are used as pesticides, whereas some are used as parasiticides in veterinary medicine, others are

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used as flame retardants and a very few as nerve agents (Gupta, 2006)

Nerve agents are considered to be the most toxic means among all chemical weapons. The nerve agents were originally synthesized during the 1930s in Germany in attempts to achieve more efficient pesticides based on organophosphorus compounds. However, some of these agents turned out to be too potent for their original purpose. Tabun was the first one synthesized followed by sarin, soman, and cyclosarin. When the Allied forces occupied Germany, the code names of GA (the G is for German), GB, GD, and GF were given to tabun, sarin, soman, and cyclosarin, respectively. VX is another type of nerve agent which was originally developed in the UK when searching for new insecticides. The "V" (venomous) series are generally more toxic than the "G" agents. The organophosphorus nerve agents are highly potent irreversible inhibitors of the enzyme acetylcholinesterase (AChE) that hydrolyzes acetylcholine (ACh). Accumulation of ACh in the synaptic cleft results in over-stimulation of muscarinic and nicotinic receptors. This increased cholinergic activity can affect all organ systems. The toxic signs include miosis, hypersalivation, respiratory distress, tremor, seizures/convulsions, coma, and death (Taylor, 2001).

Exposure to nerve agents requires immediate medical treatment. For this purpose, military personnel are issued with autoinjectors containing countermeasures for self-administration or "buddy aid". Antidotes against nerve agents are based on drugs acting at the muscarinic receptors and $GABA_A$ receptors (McDonough and Shih, 1997). In addition, partial prophylactic protection against nerve agents can be obtained by the use of reversible AChE inhibitor (pyridostigmine) shielding a portion of AChE from irreversible inhibition by nerve agents prior to nerve agent exposure. Furthermore, reactivation of any unaged AChE by an oxime is regarded as important immediate treatment after nerve agent exposure.

A number of armed forces have based their therapy against nerve agent intoxication on an oxime (obidoxime, 2-PAM, HI-6), an anticholinergic (atropine), and a $GABA_A$ agent (diazepam, avizafone) combined with carbamate (pyridostigmine) pretreatment (Aas, 2003). However, such treatment regimens can reduce immediate lethality, but they do not attenuate the occurrence of nerve agent-induced seizure activity and concomitant convulsions, unless atropine is given early and at a high dose (McDonough and Shih, 1997). Such seizures rapidly progress to status epilepticus, a condition that is strongly associated with mortality and brain damage in experimental animals (Shih et al., 2003). Thus, there is an urgent need to search for novel strategies able to save lives and prevent or terminate nerve agent-induced seizures.

2. Conventional research on medical countermeasures

By the end of World War II, the Allies, Soviet Union, and Germany had stockpiled large amounts of chemical agents. In the USA, studies of the G-series agents and medical countermeasures against these agents were initiated during the late 1940s. Throughout the 1950s and 1960s, great advancements were made in therapeutics of agents that inhibit AChE. Atropine was introduced in the early 1950s, and the oxime 2-PAM was used as an adjunct to reactivate the enzyme (Childs et al., 1955; Wilson and Ginsburg, 1955). The autoinjector was developed to make self-administration of atropine more convenient (Sidell et al., 1974). Autoinjectors containing atropine and oxime were introduced in NATO countries during the 1970s. In the late 1980s, several nations introduced pyridostigmine bromide, a reversible AChE inhibitor, as an effective prophylactic means against nerve agents and in particular against soman that is considered to be the most difficult nerve agent to manage.

The mechanisms underlying the lethal effect of nerve agents are relatively well known. The respiratory center in the brain stem (the ventral respiratory group) is innervated by cholinergic input (Ellenberger and Feldman, 1990; Kubin and Fenik, 2004), and excessive cholinergic stimulation has suppressant effect on respiration (Chang et al., 1990; Woch et al., 2000). Both muscarinic and nicotinic antagonists can protect the respiratory center against cholinergic over-stimulation (Kubin and Fenik, 2004). The functional integrity of the diaphragm is not significantly compromised by soman, unless a very high dose is used, as shown in experiments with cats (Rickett et al., 1986).

Nerve agent-induced cholinergic over-activity leading to seizures is strongly associated with death and brain pathology in surviving guinea pigs (Shih et al., 2003). For this reason, great research efforts have been made to elucidate the underlying mechanisms of nerve agent-induced seizures and critical events that lead to brain damage (McDonough and Shih, 1997). In the latter study, results from 200 studies along with the group's unpublished data were used to redefine a previously proposed model of neurochemical, electrophysiological, and neuropathological changes that occur following nerve agent poisoning. This work was made to provide a theoretical framework in terms of a 3-phase model that may guide future studies to determine the best anticonvulsants to treat nerve agent-evoked seizures. Hence, anticonvulsants can serve as neuroprotectants as well as antidotes or countermeasures. The model presented by McDonough and Shih in 1997 can be divided into the progression of 3 phases. An early cholinergic phase lasting from the time of exposure to about 5 min after onset of seizures is dominated by high cholinergic activity. Then follows a transitional phase of high cholinergic activity and increasing glutamatergic activity and finally a predominantly glutamatergic phase after about 40 min. According to this model, effective anticonvulsant therapies should exert cholinergic and glutamatergic antagonism along with GABAergic agonism. These relatively simple criteria have been used in the search for pharmacological agents with potential anticonvulsant efficacy against nerve agent-induced seizures and death.

Soman has been used as a model of nerve agents in mechanistic and antidote research because of its resistance to standard therapy of atropine and oxime. In addition, soman rapidly undergoes a chemical change (aging process) that makes reactivation of AChE activity by any oxime no longer possible (de Jong and Wolring, 1984). A higher dose of anticonvulsants is required to terminate seizures induced by soman than by other classical nerve agents (tabun, sarin, cyclosarin, VX). This finding suggests that drugs effective against soman will also be effective against other nerve agents (Shih and McDonough, 2000). In the latter study, it was shown that soman, tabun, sarin, cyclosarin, VX, and VR in most cases can evoke seizure activity when given at toxic doses ($2 \times LD_{50}$) in guinea pigs. However, for the sake of comparison and the need to limit the extent of the present study, only the neuropharmacological mechanisms of soman-induced seizures will be addressed.

Soman-induced seizures that have lasted more than 10 min seem to be difficult to terminate unless the countermeasures exert cholinergic and glutamatergic antagonism as well as GABAergic agonism (McDonough and Shih, 1997). In a situation where soman is used against civilians, it will take at least 30 min for first responders to access individuals unprepared for exposure to nerve agent. Furthermore, even soldiers properly provided with protective mask, gloves and clothes may need medical help, because bad training, bad discipline or bad luck can lead to intoxication of nerve agent (cf., Lallement et al., 1999). Thus, it is necessary to search for strategies capable of terminating soman-induced seizures 30–40 min following onset in individuals not given any pretreatment. In our laboratory, we have demonstrated that a triple regimen