(Review)

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

Trond Myhrer, Pål Aas

Norwegian Defence Research Establishment (FFI), Protection and Societal Security Division, P. O. Box 25, NO-2027 Kjeller, Norway

Running title: Nerve agent poisoning and countermeasures

Correspondence:
Pål Aas
Norwegian Defence Research Establishment (FFI)
Protection and Societal Security Division
P O Box 25
NO-2027 Kjeller, Norway
Phone: +47 63 80 78 43
Fax: +47 63 80 75 09
E-mail: pal.aas@ffi.no

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

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.

Keywords: Nerve agents; Seizures; Target areas; Lesion studies; Microinfusion studies; Pharmacological receptors; Countermeasures: Novel strategies

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

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 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

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

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.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

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 (Ellenberg 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

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

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 x 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 consisting of procyclidine (6 mg/kg), diazepam (10 mg/kg), and pentobarbital (30 mg/kg) can effectively terminate soman-induced seizures in rats when administered intraperitoneally 5 min apart 30-40 min following onset, but without

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

preventing some neuropathology in the majority of surviving rats after a lethality rate of 43% within 24 h (Myhrer et al., 2005). A refinement of the triple regimen resulted in a double regimen composed of procyclidine (10 mg/kg) and propofol (50 mg/kg) that can stop soman-evoked seizures when given 30-35 min after onset, but 17% of the rats died within 24 h (Myhrer et al., 2006a). Both the triple and double regimens would need monitoring of vital functions, because pentobarbital and propofol can suppress normal function of the respiratory center in the brainstem. Thus, alternative approaches making it possible to design anticonvulsants predominantly affecting the forebrain will be needed.

The purpose of the present study was to show that pharmacological therapies derived from systematic building of knowledge can have a greater potential for being included in future autoinjectors than treatment regimens based on accidental findings or fragmental research. The ultimate aim should be to develop well tolerated countermeasures that can be effective regardless of the time of application and that can be administered in the field by the soldiers themselves.

3. Screening of anticonvulsants by microinfusions

The tripe and double treatment regimens outlined in Section 2 are based on simple criteria derived from the 3-phase model; viz. cholinergic and glutamatergic antagonism along with GABAergic agonism. No clear-cut specifications are worked out for what subreceptors in what sites of the brain that preferentially should be affected. Without such specifications further pharmacological research on anticonvulsants against nerve agents may take the form of a seemingly endless series of trial and error. The vast number of candidate compounds to choose among can as an example be seen in preclinical epilepsy research. The Antiepileptic Drug Development Program funded by the National Institute of Health (USA) has screened over 31.000 compounds of which 13 are approved for use as antiepileptic drugs (Crepeau and

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Treiman, 2010). For prophylaxis and early treatment, it will be important to link therapies to anatomical substrates involved in triggering seizures after nerve agent exposure. For subsequent treatment, therapies should primarily be aimed at structures involved in the early phase of epileptiform activity and propagation (Myhrer, 2007). In experimental epilepsy, studies have been made to identify brain areas critical for triggering and /or controlling propagated seizure activity. Several target areas have been identified, and it is assumed that the ability of a systemically administered drug to confer seizure protection depends on the drug's relative impact on the defined action sites (Gale, 1988).

3.1. Seizures and anatomical structures

Epileptiform discharges do not spread randomly throughout the brain, but they seem to be generated and propagated by specific anatomical routes (Gale, 1988; Löscher and Ebert, 1996). This suggests that certain propagation pathways may function as common denominators for the development of certain types of epileptiform activity, independent of the specific induction means used. The classification of epileptic seizures is solely based on clinical and EEG descriptive phenomena (Engel and Schwartzkroin, 2006). International classification defines seizures that start in one hemisphere as partial seizures, and those that begin in both hemispheres simultaneously as generalized seizures. Partial seizures commonly evolve. Simple partial seizures can progress to complex partial seizures, and both simple and complex partial seizures can progress to secondarily generalized seizures. Simple partial seizures can have motor, sensory, autonomic, or psychic signs or symptoms, whereas complex seizures are associated with impairment of consciousness (Engel and Schwartzkroin, 2006). Nerve agent-induced seizures seem to be complex partial seizures progressing to secondarily generalized seizures accompanied by convulsions, since tonic-clonic convulsions in rats can be triggered by unilateral microinfusion of VX into the amygdala (McDonough et al., 1987).

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Among models of complex partial seizures, kindling is probably the most widely used. Kindling is characterized by the progressive development of electrographic and behavioral seizure activity following the spaced, repeated application of low-intensity electrical stimulation (McIntyre and Kelly, 2000). When the kindled seizures have become secondarily generalized, the discharge seems to propagate so widely that it is difficult to determine the relative importance of each structure involved (Sato et al., 1990). The amygdala and piriform cortex are very sensitive to kindling along with the anterior perirhinal cortex (McIntyre et al., 1993). Kindled epileptiform activity appears to propagate to cortical areas and basal ganglia, as well as the brainstem structures. Substantia nigra and various diencephalic structures including the thalamus influence the spread of seizure activity to the forebrain and brainstem. Seizures induced by chemoconvulsants (kaininc acid, pilocarpine) seem to follow similar routes of propagation (Löscher and Ebert, 1996).

Within the rat brain, there are control mechanisms with capacities to attenuate all aspects of convulsive activity. The substantia nigra pars reticulata and the area tempestas have been identified as two critical substrates for the control of experimentally induced seizures (Gale 1988). Microinfusions into the anterior substantia nigra as well as the subthalamic nucleus have been shown to assure anticonvulsant effects (Dybdal and Gale, 2000; Gernert and Löscher 2001). The area tempestas (located in the deep prepiriform cortex) has been defined morphologically and termed the pre-endopiriform nucleus (Ekstrand et al., 2001). Infusion of the $GABA_A$ agonist, muscimol, into the substantia nigra has been demonstrated to attenuate generalized convulsive seizures induced by several mechanisms (Gale 1988). Infusion of muscimol into the area tempestas prevents the appearance of seizures on subsequent microinfusions in the area tempestas of cholinergic agonist (carbachol), glutamatergic agonist (kainic acid), or GABAergic antagonist (bicuculline) (Piredda and Gale 1985).

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

3.2. Critical pharmacological receptors and their anatomical localization

Cholinergic projection systems involved in induction and propagation of seizures caused by nerve agents have previously been outlined (Myhrer, 2007). Areas critical for generation and/or control of seizures can be identified by means of various methods. Nerve agents or related chemoconvulsants can be microinfused into relevant structures for generation of seizures. Alternatively, anticonvulsants can be microinfused into corresponding structures in animals exposed to a convulsant dose of nerve agent. However, for the initial screening of target areas selective lesion of relevant brain structures may be the most pertinent choice. If selective damage to an area ensures anticonvulsant effects against nerve agent intoxication, the area affected may serve as a trigger site for seizures or may make up an important link in the propagation of seizure activity. The constellation of receptor types in the damaged region may provide clues for designing drugs with powerful anticonvulsant properties. Primary structures are those containing large assemblies of cholinergic neurons and areas demonstrated to have control capabilities in experimental epilepsy. Among structures giving rise to cholinergic projections, aspiration lesion of the medial septum causes prevention or increased latency to onset of convulsions (Fig. 1), whereas damage to the nucleus basalis magnocellularis or nucleus accumbens does not have anticonvulsant effects in rats exposed to a convulsant dose of soman (1.6 x LD_{50}) (Myhrer et al., 2007). Rats with aspiration lesion of the seizure controlling substrate area tempestas in the anterior piriform cortex display marked anticonvulsant impact (Fig. 1), whereas such effect is not seen when the substantia nigra is destroyed (Myhrer et al., 2007). Similar lesions made in the perirhinal cortex or posterior piriform cortex produce anticonvulsant efficacy against soman intoxication (Fig. 1), but anticonvulsant impact is not achieved when lesions are made in the entorhinal cortex, hippocampal region, or amygdala (Myhrer et al., 2008a).

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

In our laboratory, we have carried out 6 studies of microinfusion of 18 pharmacological agents with potential anticonvulsant properties into the brain areas that have been identified as target sites for generation of seizures (the area tempestas, medial septum, perirhinal cortex, posterior piriform cortex). The application of identical procedures in these studies justifies direct comparison of results. The results are summarized in Table 1. The muscarinic receptors appear to be critical in all brain regions, but there are distinct differences among other receptor types. Only general muscarinic binding properties are presented; they are not related to specific brain structures. In the area tempestas, cholinergic, and not glutamatergic antagonism is likely the active property of the antiparkinson agents, caramiphen and procyclidine, since the NMDA antagonists ketamine and MK-801 do not have anticonvulsant effects (Myhrer et al., 2008b). The $GABA_A$ modulators muscimol, ethanol, and propofol produce anticonvulsant effects, whereas diazepam and pentobarbital do not (Myhrer et al., 2006c). In the medial septum, only muscarinic receptors seem to be the effective ones, because ketamin and muscimol do not produce anticonvulsant effects (Myhrer et al., 2009). In the perirhinal cortex, both muscarinic and glutamatergic receptors have to be blocked simultaneously (procyclidine) in order to achieve anticonvulsant effect. Neither scopolamine alone nor ketamine alone causes anticonvulsant effects (Myhrer et al., 2010a). However, the scopolamine dose of 1 µg/µl in the latter study was too low, because it has been shown that the lowest dose of scopolamine yielding anticonvulsant impact in the perirhinal cortex in response to soman is 4.59 µg/µl (Skovira et al., 2012). The positive effect of NBQX suggests that AMPA receptors are critical for anticonvulsant impact in the perirhinal cortex (Myhrer et al., 2010a). The central roles of NMDA and AMPA receptors in this area suggest that there is an early increase of glutamatergic activity in the perirhinal cortex. This view receives further support from the findings that modulation of metabotropic glutamate receptors (mGluRs) in the perirhinal cortex also has anticonvulsant effects. Injection of MPEP

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

(antagonizing mGluR5) or DCG-IV (agonistic effect on both mGluR2 and 3) results in marked anticonvulsant efficacy (Myhrer et al., 2010b). In a recent study, double infusions of anticonvulsants were used to reach a larger area of the perirhinal cortex. With such technique, MPEP protected 67 percent of the rats from evolving soman-induced seizures, whereas the percentage for DCG-IV was 83 (Myhrer et al., 2013a). In the posterior piriform cortex, muscarinic and $GABA_A$ receptors seem to be the critical ones. This constellation of receptors has similarity to area tempestas located in the anterior piriform cortex, and might indicate a general feature of the piriform cortex. According to the present results, efficient anticonvulsant pretreatment has to be based on pharmacological agents affecting a number of subreceptors. Also post-exposure treatment will probably profit by using similar agents.

The structures identified as sensitive to microinfusions of anticonvulsants against soman intoxication are outlined in Fig. 1. The same structures can also serve as trigger sites for seizure activity when affected by nerve agent, chemoconvulsant, or kindling (Table 1 in Myhrer, 2010). The anticonvulsant impact factor (percentage of positive effects) for drugs tested in at least 3 of the 4 seizure controlling brain sites identified has been calculated (Fig. 2). As a prophylactic means scopolamine ranks, not surprisingly, on top with an impact factor of 75. It might appear somewhat surprisingly, however, that procyclidine achieves a corresponding rank (75). This result is most likely related to the M1-M4 antagonism of procyclidine, because the impact factor of the NMDA antagonist ketamine is 0. However, when scopolamine or procyclidine is used systemically as prophylactics, they need to be combined with an AChE inhibitor (e.g. physostigmine) to gain optimal anticonvulsant impact. When such combinations are used, physostigmine and procyclidine exert more powerful anticonvulsant potency than the combination of physostigmine and scopolamine (Philippens et al., 2006, 2007). Also caramiphen exerts more efficacious prophylactic effect than scopolamine against soman poisoning (Raveh et al., 1999, 2002). The anticonvulsant impact

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

of muscimol is moderate (50), while that for NBQX and caramiphen is relatively low (33). When the percentage of nonconvulsing rats from both lesion and microinfusion studies (Table 3 in Myhrer, 2010) is used as guidance for selecting the most influential seizure controlling brain sites, the area tempestas and perirhinal cortex emerge as the most prominent ones. Hence, the agents ranked in Fig. 2 play a central role again. Procyclidine exerts anticonvulsant efficacy in both areas (high impact), whereas scopolamine, NBQX, caramiphen, and muscimol yield anticonvulsant effects in 1 of the areas only (low impact).

4. Systemic administration of anticonvulsants screened by microinfusions

Scopolamine will probably have limited use as a post-exposure countermeasure, because the comparatively small time window of the cholinergic phase in the 3-phase model (about 5 min after seizures onset) makes the efficacy of anticholinergics gradually weaker with elapse of time since start of seizures (McDonough and Shih, 1997). On the other hand, procyclidine (impact factor 75) has proved very useful as a post-exposure means when combined with diazepam and pentobarbital or either muscimol, ethanol, or propofol. However, these GABAergic modulators depress respiratory function and can in combination with procyclidine cause a death rate up to 43% (Myhrer et al., 2005, 2006a). To avoid adverse impact on the brainstem, enhancement of procyclidine's excellent anticonvulsant efficacy in the seizure controlling sites of the forebrain would make up a novel and interesting approach. A drug with such enhancing properties has been developed in preclinical epilepsy research. Levetiracetam is an antiepileptic drug that strongly enhances the anticonvulsant effects of compounds affecting either glutamatergic or GABAergic neurotransmission (Kaminski et al., 2009). It has also been reported that levetiracetam may both reduce release of acetylcholine and reduce postsynaptic responsiveness in cholinergic synapses (Oliveira et al., 2005). From

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

the effects reported for levetiracetam, this drug should have the potency to increase the anticonvulsant efficacy of all the drugs presented in Fig. 2.

A comparative assessment of anticonvulsant effects of levetiracetam, procyclidine, muscimol, caramiphen, NBQX, and ketamine or each drug in combination with levetiracetam was made 20 min after onset of soman-evoked seizures (Experiment 1) (Myhrer et al., 2011). In Experiment 2, levetiracetam was combined with either procyclidine or caramiphen and the treatment started 40 min following seizure onset. In an attempt to prolong survival, the rats were pretreated with pyridostigmine in Experiment 1 and with HI-6 in Experiment 2. A single dose of each anticonvulsant previously shown to cause optimal efficacy was applied. One exception was the use of 2 doses with procyclidine and caramiphen in combination with levetiracetam in Experiment 1 to examine impact of nicotinic antagonism on motor dysfunctions after seizure termination. Additionally, mecamylamine was given as adjunct in both experiments. No single drug is able to terminate seizure activity. However, when levetiracetam (50 mg/kg) is combined with either procyclidine (10 mg/kg) or caramiphen (10 mg/kg) complete cessation of seizures is achieved, but the nicotinic antagonist mecamylamine was needed to induce full motor rest in some rats. In the subsequent experiment, rats were pretreated with HI-6 (125 mg/kg), and treatment started 40 min following seizure onset of a soman dose of 1.6 x LD_{50}. Levetiracetam (50 mg/kg) combined with either procyclidine (20 mg/kg) or caramiphen (20 mg/kg) terminates seizure activity, but the survival rate is considerably higher for levetiracetam and procyclidine than levetiracetam and caramiphen. Both therapies can also save the lives of rats that were about to die 5-10 min after seizure onset. Prophylactic use of the combination therapies 20 min before exposure to soman prevents onset of seizures more effectively when levetiracetam is combined with procyclidine than caramiphen (Myhrer et al., 2011). It should be noted, however, that a high dose of caramiphen (100 mg/kg) can provide partial neuroprotection, even when administered 60 min

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

after soman-evoked seizures in rats pretreated with HI-6 and given atropine 1 min after exposure (Figueiredo et al., 2011).

It has been reported that drugs affecting $GABA_A$ or AMPA receptors can have their potencies increased by up to 16-fold and 19-fold, respectively when combined with levetiracetam (Kaminski et al., 2009). Even if levetiracetam potentiated the anticonvulsant efficacy of muscimol and NBQX, it was not measurable in the study carried out by Myhrer et al (2011). Drugs with a single mechanism of action seem to have relatively weak anticonvulsant efficacy when used well after onset of seizures, unless a potent pretreatment is administered (McDonough and Shih, 1997). Termination of soman-induced seizures by trihexyphenidyl (same group of antiparkinson drugs as procyclidine and caramiphen) 20 or 40 min after onset resembles the anticonvulsant effects provided by the combination of scopolamine and MK-801 at the same times. The latter drugs are without effects when administered separately. The anticonvulsant activity of trihexyphenidyl against soman-evoked seizures at the longer seizure durations may be ascribed to mixed anticholinergic and NMDA antagonistic properties of this drug (McDonough et al., 1993). Likewise, the combination of atropine and ketamine has anticonvulsant impact when administered 30-120 min after soman intoxication (Dorandeu et al., 2005). NMDA antagonists can produce lethal interactions on the respiration function in soman poisoned subjects in absence of anticholinergic drugs (McDonough et al., 1993). The multifunction of procyclidine and caramiphen may explain why these drugs proved efficacious in combination with levetiracetam (Myhrer et al., 2011). In combination with levetiracetam procyclidine ensured a markedly higher survival rate than caramiphen. The potent anticonvulsant capacity of procyclidine observed in the study referred to above is in compliance with the high impact of this drug in seizure controlling sites in the forebrain, as shown in microinfusion studies of rats (Fig. 2).

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

5. Relations between microinfusions and systemic administration of drugs or nerve agents

Very powerful anticonvulsant efficacy can be obtained by focal application of drugs in seizure controlling brain sites in rats. $GABA_A$ modulators microinfused into the area tempestas can protect up to 75% of the animals from evolving seizures after systemic administration of a convulsant dose of soman (Myhrer et al., 2008b). Likewise, microinfusions of metabotropic glutamate modulators into the perirhinal cortex can protect up to 83% of the rats against soman-induced seizures (Myhrer et al., 2013a). Such results achieved by focal application of drugs are transferable to systemic use after seizure onset (Myhrer, 2010). However, focal administration of anticonvulsants after seizure onset has modest impact. Repeated infusions of atropine into the medial septum after a comparatively low dose of soman (0.9 x LD_{50}) can finally stop seizure activity (Lallement et al., 1992). Similarly, repeated infusions of atropine into the area tempestas after onset of seizures induced by soman (1.3 x LD_{50}) have evident anticonvulsant impact, but the seizure activity recurs 10-15 min following each injection (Myhrer and Enger, unpublished data). Thus, there are reasons to believe that large parts of the neuronal network must be affected systemically by anticonvulsants to stop ongoing seizures. Successful termination of seizures well after their onset probably requires combination of drugs each with optimal effects in some of the seizure controlling brain areas. However, even high doses of drugs given systemically may probably not achieve similar impact as direct depletion of drugs in seizure controlling areas.

It has been shown that microinfusion of VX, but not soman, into the amygdala induces convulsions and neuropathology in rats (McDonough et al., 1987). In subsequent studies, it has been demonstrated that microinfusion of soman into the perirhinal or posterior piriform cortices triggers seizures in 75% of the rats, whereas 20% of the rats respond with seizures after infusion of soman into the area tempestas (Myhrer et al., 2008b, 2010a). The symptoms

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

are similar to those generated by a convulsant dose of soman given systemically (Myhrer et al., 2010a). These findings suggest that seizure activity per se can evoke signs of intoxication and result in neuronal damage. This mechanism is attributed to hypothalamic autonomic dysfunction caused by seizures regardless of how they are generated, because corresponding reactions are triggered by kindling or by use of other chemoconvulsants than nerve agents (Mraovitch and Calando, 1999). The malfunctions seen in both brain and body appear to be associated with the epileptiform activity in itself and not the level of soman poisoning in general.

Seizure activity elicited by systemic administration of nerve agents is related to lethality and neuropathology (Shih et al., 2003). It takes about 20 min from seizure onset to the first signs of neuropathology usually become evident (Lallement et al., 1994; McDonough et al., 1995). In 2 previous studies, neuropathology has been reported to follow soman intoxication without concomitant overt toxic signs (Hymowitz et al., 1990; Kadar et al., 1992). In a more recent study, predominantly unilateral neuronal damage was found in rats that either seized for 1-6 min or did not seize at all when treatment started 1 min after exposure to relatively high doses of soman (2, 3, 4 x LD_{50}). A possible explanation of this unexpected finding may be that excitotoxic activity occurs in brain areas with the highest hemispheric levels of glutamate (Myhrer et al., 2013b). The finding of asymptomatic development of neuronal damage in response to nerve agent suggests that further treatment can be needed after termination of epileptiform activity. It appears that if the level of soman poisoning is sufficiently high, neuropathology can, indeed, occur without overt signs of convulsions or seizures. This is a condition that has not been reported to follow bilateral focal application of soman. Apparently, neuropathology can be produced in 2 unconventional ways; by microinfusion of soman into selective brain areas and by administration of high doses of

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

soman followed by early treatment resulting in no seizures or only a brief period with seizures.

The 3-phase model was based on results from systemic testing of drugs with anticonvulsant potency against nerve agents and was suggested to serve as a guide for further research efforts (McDonough and Shih, 1997). In the time to pass, supplementary data from microinfusion studies have been achieved. Some of these results indicate that the 3-phase model should be revised. Results from microinfusions into the perirhinal cortex imply that modulation of NMDA, AMPA, or metabotropic glutamate receptors has anticonvulsant effect against soman-induced seizures (Myhrer et al., 2010a, 2010b). Pure modulation of glutamatergic activity by perirhinal infusions of the metabotropic glutamate receptor agonist DCG-IV prevents development of soman-elicited seizures in 83% of the rats (Myhrer et al., 2013a). An increase of glutamatergic activity appears to start already during the initial stage of the cholinergic phase. This notion is in compliance with a large body of evidence from multiple experimental paradigms implying that glutamatergic systems are recruited at an early stage of nerve agent poisoning, e.g., seconds after exposure (Weissman and Raveh, 2008). Early activation of glutamatergic activity seems to occur in the perirhinal cortex, but not in other seizure controlling brain sites like area tempestas, medial septum, and posterior piriform cortex in which anticonvulsant effects against soman intoxication are obtained by modulation of cholinergic or GABAergic receptor activity (Myhrer, 2010). Hence, the perirhinal cortex may be a likely site for initiating the glutamatergic phase of the 3-phase model.

6. General discussion

6. 1. Potential relationship between experimental approaches and utility of the outcome

As described in Section 2, the triple (procyclidine, diazepam, pentobarbital) and double (procyclidine, propofol) regimens originating from our laboratory terminated soman-

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

induced seizures 30-40 min after onset, but the rate of lethality was relatively high (43 and 17%, respectively) (Myhrer et al., 2005, 2006a). These studies were not based on results from mapping of strategic target areas for nerve agents, since such results did not exist at the time when the studies were performed. In retrospect, it can be seen that procyclidine was a good choice in these early studies. The reason for this choice was the evidence presented that procyclidine can serve as a successful prophylactic drug in combination with physostigmine against soman poisoning in rats, guinea pigs, and dogs (Choi et al., 2004; Kim et al., 2002, 2005). Results from subsequent microinfusion studies showed that procyclidine is the most efficacious multifunctional (antiglutamatergic and anticholinergic) of all drugs tested (Myhrer, 2010). In view of results from microinfusions, diazepam exerts anticonvulsant impact in the substantia nigra, but not in the area tempestas. Pentobarbital has neither anticonvulsant action in the substantia nigra nor area tempestas, whereas propofol ensures antiseizure effects in both of these structures (Myhrer et al., 2006a). Both pentobarbital and propofol are used as anesthetics in human medicine, and most anesthetics have depressant effects on the cardio-respiratory system (Kubin and Fenik, 2004). Hence, these GABAergic drugs do not appear to be well suited as anticonvulsants in combination with procyclidine.

We have examined antidotal efficacy of the current autoinjector therapy containing atropine, obidoxime, and diazepam and a novel therapy suggested by several NATO countries consisting of atropine, HI-6, and diazepam/avizafone (Aas, 2003). In the study, we also included an autoinjector therapy suggested by British investigators. The latter therapy is based on the combination of scopolamine, HI-6, and physostigmine that provide effective protection against all classical nerve agents when administered 1 min following exposure to guinea pigs (Wetherell et al., 2006, 2007). The purpose of our study was to make a comparative assessment of anticonvulsant and life preserving capabilities of the regimens consisting of atropine, obidoxime, and diazepam (termed the obidoxime regimen), atropine, HI-6, and

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

avizafone (termed the HI-6 regimen), and scopolamine, HI-6, and physostigmine (termed the physostigmine regimen) in rats intoxicated by various doses of soman (Myhrer et al., 2013b). Each regimen administered 2 times (1 and 5 min after exposure) effectively prevents or terminates epileptiform activity within 10 min. However, the regimens differ markedly in life saving properties with the physostigmine regimen ranking highest followed by the HI-6 and obidoxime regimens. Pretreatment with pyridostigmine increases the potency of the HI-6 regimen (survival, body weight), but no improvement is recorded for the obidoxime regimen. The latter regimen administered 3 times (1, 5, 9 min after exposure) does not compensate for the insufficiency. Among the 3 types of potential autoinjector regimens assessed, the physostigmine regimen emerges as the one with excellent antidotal properties. However, the very narrow time window of the latter regimen (< 10 min) most likely makes it unsuitable for practical use. The HI-6 regimen is far more efficacious than the obidoxime regimen and would make up an effective future therapy when pyridostigmine is used as pretreatment (Myhrer et al., 2013b). The ultimate aim would be to develop a treatment regimen yielding a superior antidotal capacity like the physostigmine regimen, but with high efficacy regardless of the time of application.

More effective countermeasures against nerve agent poisoning are needed, and a prime object should be to develop antidotes to be fitted in an autoinjector that can be applicable at any time after poisoning and that also in an emergency situation can be used as a prophylactic means. In an attempt to achieve these goals, we have carried out a study in which effects of the physostigmine regimen (HI-6, scopolamine, physostigmine) were compared with those obtained by our procyclidine regimen (HI-6, levetiracetam, procyclidine; cf., Section 4) against various doses of soman at different times after intoxication. The prophylactic potencies of the regimens were also examined (Myhrer et al., 2013c). Both regimens administered 2 times (1 and 5 min after exposure to 3, 4, or 5 x LD_{50} of soman)

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

effectively prevent or terminate epileptiform activity within 10 min. Both regimens also protect against death, and the rats recovered well. However, when the regimens were administered 10 and 14 min after a soman dose of 1.6 x LD_{50}, only the procyclidine regimen terminates seizure activity and protects all rats against death. When rats pretreated with pyridostigmine received treatment 20 and 24 min after a soman dose of 1.3 x LD_{50}, only the procyclidine regimen successfully stops seizures and prevents death among all rats. Rats treated with the physostigmine regimen all continued to seize and subsequently died. When the regimens were given as prophylactic treatment 20 min before a soman dose of 1.3 x LD_{50}, both the physostigmine and procyclidine regimens prevent convulsions, and only a brief period of incapacitation is observed in some rats. At 24 h, all rats appeared unaffected by the nerve agent poisoning (Myhrer et al., 2013c). However, the physostigmine regimen does not prevent the development of neuropathology as shown for the procyclidine regimen. This appears unfortunate, inasmuch as physostigmine together with scopolamine (hyoscine) has been suggested as a future prophylactic countermeasure (Scott, 2007; Wetherell, 1994). Since no obvious toxic signs were observed and EEG appeared normal after exposure to soman, the finding of brain damage occurs intriguing. Administration of physostigmine resulting in AChE inhibition may enhance the effects of soman. Alternatively or additionally, the lack of glutamatergic antagonism or GABAergic agonism of the physostigmine regimen may be related to the morphological damage, because the combination of physostigmine and procyclidine protects effectively against soman exposure and no neuropathology is detectable (Myhrer et al., 2004). According to these results, the procyclidine regimen can have potential to serve as an antidotal treatment with universal utility, whereas the application of the physostigmine regimen is restricted to a period of less than 10 min after nerve agent exposure or as a prophylactic treatment. The shortcomings of the latter regimen may be associated with its history of development, which is quiet different from that of the procyclidine regimen.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

The physostigmine regimen (scopolamine, HI-6, physostigmine) was originally worked out as a prophylactic treatment. Pretreatment (6 days) of guinea pigs implanted with mini osmotic pumps containing physostigmine and scopolamime is effective in preventing lethality and reducing incapacitation caused by a range of nerve agents in absence of supporting therapy. It was further noticed that continued delivery (24 h) of the drugs after the nerve agent exposure does not exacerbate the effects of the nerve agents and may be beneficial (Wetherell et al., 2002). In subsequent studies, the same group showed that the combination of physostigmine, scopolamine, and HI-6 given 1 min following nerve agent poisoning provides good protection against the supralethal effects of tabun, sarin, soman, cyclosarin, or VX (Wetherell et al., 2006, 2007). Hence, the physostigmine regimen shown to have excellent antidotal properties when administered 1 min after soman exposure (Myhrer et al., 2013b) is based on an accidental finding. Although scopolamine reached a peak ranking based on microinfusion studies (Fig. 2), the critical composition of countermeasures is not derived from long term strategic work.

The physostigmine regimen and the procyclidine regimen (HI-6, levetiracetam, procyclidine) have been worked out along quite different experimental lines, and they possess very different modes of action. Table 2 shows the pharmacological actions and half-lives in plasma of rats for the components in these 2 regimens. The antidotal efficacy of the physostigmine regimen can primary be associated with physostigmine's ability to shield a portion of AChE before it is inhibited by soman; either prophylactically or within 1 min after soman exposure. AChE inhibited by physostigmine will spontaneously decarbamylate and become physiologically active again in relatively short time, since physostigmine has a short half-life (Table 2). Scopolamine that is much more potent than atropine (Capacio and Shih, 1991), will have a profound anticonvulsant impact during prophylactic administration. HI-6

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

appears to have a life saving effect by protecting respiratory function, both centrally and peripherally during nerve agent poisoning (van Helden et al., 1996).

In contrast to the physostigmine regimen, the procyclidine regimen possesses properties to exert a number of antidotal functions (Table 2). Levetiracetam reduces the release of acetylcholine and glutamate and additionally has GABAmimetic effect (Kaminski et al., 2009). Procyclidine antagonizes both glutamatergic and cholinergic activity (Kim et al., 2002). Thus, in combination with levetiracetam the glutamatergic antagonism of procyclidine can be highly potentiated. As seen from Table 2, the half-lives of levetiracetam and procyclidine are considerably longer than for scopolamine and physostigmine. The procyclidine regimen will probably have the capacity to terminate fully long-lasting seizure activity and prevent incidents of recurring seizures accompanied by excitotoxicity. The brief half-life of the components in the physostigmine regimen will probably require continuous administration, whereas that is not the case for the procyclidine regimen.

The anticonvulsant efficacy of the procyclidine regimen can be exemplified further by comparing it with effects of a newly developed regimen based on the AMPA antagonist LY293558. In rats without any pretreatment, a soman dose of 1.2 x LD_{50} triggers seizures after about 9.5 min. Twenty min following soman exposure (after about 10 min of seizing), LY293558 along with atropine and HI-6 was administered, and the mean time to seizure termination is 36.9 min (Apland et al., 2013). When the procyclidine regimen is administered 20 min following onset of soman-induced seizures, the mean time to seizure termination is 12.7 min (Myhrer et al., 2013d). The time difference in terminating seizure activity between the above studies will probably be decisive for the development of neuropathology.

The results presented in Table 1 make up a foundation for alternative development of pharmacological therapies, but not all combinations derived from microinfusion studies will work equally well. For example, the potency of the metabotropic glutamate modulators DCG-

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

IV and MPEP is excellent in the perirhinal cortex. However, when administered systemically together with procyclidine and HI-6 both combinations yield effective anticonvulsant impact, but they do not reach the same high level as the procyclidine regimen (HI-6, levetiracetam, procyclidine). The multifunctional properties of procyclidine (antiglutamatergic and anticholinergic) seem to profit from the enhancing effects of levetiracetam, whereas the single function of DCG-IV and MPEP (antiglutamatergic) does not result in increased anticonvulsant capacity when combined with levetiracetam and HI-6 (Myhrer et al., 2013d).

6. 2. The capacities of HI-6, levetiracetam, and procyclidine as components in a future therapy

HI-6 is considered by a number of nations to be the most promising broad spectrum oxime against nerve agent intoxication (Aas, 2003). HI-6 has a relatively low toxicity (Clement 1981; Hamilton and Lundy 1989; Kusic et al., 1985; Lundy et al., 2011; van Helden et al., 1992). A disadvantage of HI-6, however, is its lack of stability in aqueous solutions that requires storing as powder in a separate chamber in the autoinjector (Thiermann et al., 1996). It has been a matter of debate whether the content of quaternary nitrogen atoms allows HI-6 to penetrate the blood-brain barrier. However, some studies conclude that at least a certain concentration of HI-6 enters the central nervous system of rats after systemic administration of the oxime (Cassel and Fosbraey, 1996; Ligtenstein and Kossen, 1983). More specifically, in soman intoxicated rats, 9% of HI-6 in plasma enters the brain and 18% during control condition (Cassel et al., 1997).

The process known as aging during which the enzyme is changed to an unreactivatable state, is very fast with soman. With human AChE a half-life of about 2 min has been determined (de Jong and Wolring, 1984). In the rat, the half-time of aging of soman-inhibited erythrocyte AChE is 8.6 min (Talbot et al., 1988). In some reports, reactivation of

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

AChE in rats has been shown to be achieved by HI-6 up to 30 min post-soman in diaphragm (Clement, 1982) and up to 20 min post-soman in the brain of rats that received HI-6 intracerebroventricularly (Sket and Brzin, 1986).

The effects of HI-6 are not solely associated with reactivation of the enzyme. The oxime has been shown to cause recovery of neuronal transmission in the respiratory center possibly by affecting GABAergic mechanisms and to cause recovery of neuromuscular transmission in the diaphragm (van Helden et al., 1996). HI-6 has also been reported to abolish the increase in liver tyrosine aminotransferase activity and decrease the stress-induced changes in plasma corticosterone level following soman (Kassa, 1995). Furthermore, HI-6 produces a marked reduction in the evoked release of acetylcholine following challenge with soman in hippocampal slices (Øydvin et al., 2005). Thus, the effects of a cholinergic antagonist may be enhanced when combined with HI-6. When combining HI-6, atropine, and avizafone as treatment against soman intoxication in rats the survival-assuring potential of HI-6 does not depend on whether HI-6 dichloride or HI-6 dimethanesulfonate is used (Myhrer et al., 2006b). HI-6 dichloride, however, has limited use, because it cannot be dissolved readily when the temperature is below 5º C (Thiermann et al., 1996).

In a review of efficacy data, it is concluded that HI-6 is the best choice presently available for development of a broad spectrum oxime treatment for use against nerve agent poisoning. It has more promising therapeutic effects against all classical nerve agents than other oximes and is clearly superior to all other oximes regarding its broad spectrum reactivation of AChE in both animals and humans (Lundy et al., 2011). Unlike any of the current oxime candidates it has also undergone extensive preclinical toxicology screening and appears very safe as determined in over 200 human subjects (Lundy et al., 2006). An HI-6 salt derivative (HI-6 dimethanesulphonate) with increased water solubility is under development among several NATO nations (Lundy et al., 2011).

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Levetiracetam (Keppra®) is an antiepileptic drug with a unique profile of activity in preclinical models of epilepsy. It is effective against audiogenic and kindled seizures, but not against maximal electroshock and seizures induced by pentylenetetrazol. This preclinical profile correlates well with the broad spectrum clinical efficacy of levetiracetam (Kaminski et al., 2009). Levetiracetam strongly enhances the anticonvulsant effects of compounds affecting either glutamatergic or GABAergic neurotransmission (Kaminski et al., 2009). The distinctive binding site of levetiracetam appears to be the synaptic vesicle protein 2A (SV2A) (Lynch et al., 2004). Although the exact mechanisms are not well known, levetiracetam probably reduces release of glutamate by which the effects of glutamatergic antagonists are highly increased. The protective effects of the NMDA antagonist MK-801 and the AMPA antagonist NBQX can both be enhanced about 18-fold in combination with levetiracetam (Kaminski et al., 2009).

The mechanisms by which levetiracetam can cause enhanced GABAergic neurotransmission occur somewhat enigmatic, because of a lack of explanation for the role of SV2A in modulating transmission release and that the drug does not have affinity for $GABA_A$ receptors (Kaminski et al., 2009). However, more recently it has been found that levetiracetam binds to SV2A and reduces inhibitory currents in a frequency-dependent manner. Although a reduction of inhibitory postsynaptic current trains by levetiracetam may appear as a paradox for an antiepileptic drug, there are multiple reasons that reduced release of GABA could ultimately attenuate pathological discharges (Meehan et al., 2012). The anticonvulsant potency of drugs enhancing GABAergic inhibition (benzodiazepines, phenobarbital) can be increased more than 16-fold (Kaminski et al., 2009).

Effects of levetiracetam on cholinergic synapses do not seem to have received much attention in experimental epilepsy. It has, however, been reported that levetiracetam may both reduce release of acetylcholine and reduce postsynaptic responsiveness in cholinergic

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

synapses (Oliveira et al., 2005). In our laboratory, we have not observed increased cholinergic antagonism of procyclidine when it was combined with levetiracetam in microinfusions into the area tempestas of rats exposed to soman (Myhrer, Enger, and Jonassen, unpublished data). It should be noticed, however, that a very low combination dose could be used in our model, because low dissolvability of both procyclidine and levetiracetam and the small volume infused (1 µl). Hence, a potency of cholinergic antagonism by levetiracetam cannot be precluded if use of higher doses had been feasible. Indeed, in a recent in vitro study, it was demonstrated that levetiracetam binds to SV2A and causes decreased release of acetylcholine (Vogl et al., 2012).

Levetiracetam has been approved in the European Union for monotherapy of partial onset seizures, with or without secondary generalization. However, it is frequently used in combination with other antiepileptic drugs, which is often necessary in drug-resistant patients (De Smedt et al., 2007).

Procyclidine (Kemadrin®) belongs to a group of antiparkinson drugs (benactyzine, biperiden, caramiphen, procyclidine, and trihexyphenidyl) that can mitigate motor symptoms by exerting cholinergic antagonism in parkisonian sufferers and patients with drug-induced extrapyramidal side effects (Rifkin et al., 1978). However, the anticonvulsant properties of antiparkinson drugs (procyclidine and trihexyphenidyl) were soon acknowledged (Millichap et al., 1968). Later on, in the 1980s and 1990s it was revealed that benactyzin, caramiphen, procyclidine, and trihexyphenidyl possess a distinct glutamatergic antagonism in their pharmacological repertoire (Weissman and Raveh, 2008). The multifunctional combination of anticholinergic and antiglutamatergic capacities makes them excellent agents against nerve agent-induced seizures.

The anticonvulsant impact of procyclidine against soman intoxication was initially examined by Shih et al (1999). Then followed systematic studies of procyclidine’s potency as

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

a prophylactic agent by a South Korean group (Cho et al., 2012; Choi et al., 2004; Kim et al., 2002, 2005) and a Dutch group (Philippens et al., 2006, 2007; van Helden et al., 2011). In our laboratory, anticonvulsant effects of procyclidine against soman poisoning have been investigated in studies of prophylactic treatment (Haug et al., 2007; Myhrer et al., 2004), in studies of post-exposure treatment (Myhrer et al., 2003, 2005, 2006a, 2006b, 2011, 2013c, 2013d), and in studies of microinfusions (Myhrer et al., 2006c, 2008b, 2009, 2010a, 2010b, 2013a).

In rodents, procyclidine exerts cholinergic antagonism at both muscarinic (M1, M2, M4) and nicotinic receptors (Gao et al., 1998; Myhrer and Aas, 2010; Waelbroeck et al., 1992). In mice, procyclidine efficiently antagonizes a lethal dose of NMDA (McDonough and Shih, 1995; Raveh et al., 1999). Furthermore, it has been shown that procyclidine inhibits the phencyclidine site at the NMDA receptor very potently (Olney et al., 1987; Reynolds and Miller, 1988) in a concentration-dependent manner (Myhrer et al., 2004). It is likely that glutamatergic antagonism was responsible for the beneficial anticonvulsant effect seen in epileptic patients treated with procyclidine (Millichap et al., 1968).

7. Concluding comments

At the US Army Medical Defense Bioscience Review Meeting in 2006 (Baltimore, USA), a representative from Defense Threat Reduction Agency expressed disapproval with the progress in research on anticonvulsants against nerve agents and ended his presentation by saying “revolutionary ideas are needed”. The notions presented in the present review are not considered as revolutionary, but represent a novel approach in which the guidelines for research in experimental epilepsy have been followed. We have identified neuronal target areas for soman through lesion studies and specified critical receptors for pharmacological treatment through microinfusion studies. In a recent microinfusion study supporting this

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

approach, it was further emphasized the importance of determining the unique pharmacological thresholds for producing anticonvulsant responses in the specific brain structures activated during nerve agent-induced seizures (Skovira et al., 2012). The results acquired by our series of microinfusion studies have been demonstrated to be transferable to systemic administration through the development of the procyclidine regimen.

The knowledge accumulated through the microinfusion studies proved very important. With reference to these data, we urged to search for an antiepileptic able to enhance the excellent anticonvulsant capacities of procyclidine in the forebrain of rats. It may be asked what would have been the chances to hit the procyclidine regimen accidentally. A likely line of action would have been a long series of trial and error with conventional pharmacological screening of potential anticonvulsants prior to the hit. Such screening activity would probably require large resources and become a time consuming enterprise. The research as such, however, would be expected to be of high quality, as it always has been for pharmacological studies in nerve agent research. The results presented in Table 1 are meant to make up a foundation for alternative development of pharmacological therapies. The ultimate purpose should be to develop anticonvulsants that can be fitted in autoinjectors that can be used by the military personnel themselves regardless of the time of injection. It will definitely be an advantage to concentrate on drugs that are already approved for human use. Otherwise, the way to go will be very long.

The current situation is that an adequate stand-alone post-poisoning treatment is not available. As a substitute it has been claimed that it is reassuring and necessary to have an adequate pretreatment in place (van Helden et al., 2011). However, the regimen consisting of HI-6, levetiracetam, and procyclidine can act as a stand-alone treatment as it has been demonstrated in rats. No pretreatment is needed, and the regimen can also be used as a prophylactic treatment without the need for adjuvant therapy. The procyclidine regimen

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

appears to fulfil the usual criteria set, inasmuch as it is effective, acceptable, practicable, and affordable. However, it remains to examine whether the procyclidine regimen works in other species than the rat, and whether it is effective against other classical nerve agents than soman.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgment

Insightful comments on the draft manuscript by Dr. Lily Raveh (Israel Institute for Biological Research) are greatly appreciated.

References

Aas P. Future considerations for the medical management of nerve agent intoxication. Prehosp Disast Med 2003;18:208-216.

Apland JP, Aroniadou-Anderjaska V, Figueiredo TH, Green CE, Swezey R, Yang C, Qashu F, Braga F. Efficacy of the GluK1/AMPA receptor antagonist LY293558 against seizures and neuropathology in a soman-exposure model without pretreatment and its pharmacokinetics after intramuscular administration. J Pharmacol Exp Ther 2013;344:133-140.

Capacio BR, Shih T-M. Anticonvulsant actions of anticholinergic drugs in soman poisoning. Epilepsia 1991;32:604-615.

Cassel GE, Fosbraey P. Measurement of the oxime HI-6 after peripheral administration in tandem with neurotransmitter levels in striatal dialysates: Effects of soman intoxication. J Pharmacol Toxicol Meth1996;35:159-166.

Cassel G, Karlsson L, Waara L, Ang KW, Göransson-Nyberg A. Pharmacokinetics and effects of HI 6 in blood and brain of soman-intoxicated rats: A microdialysis study. Eur J Pharmacol 1997;332:43-52.

Chang F-CT, Foster RE, Beers ET, Rickett DL, Filbert MG. Neurophysiological concomitants of soman-induced respiratory depression in awake, behaving guinea pigs. Toxicol Appl Pharmacol 1990;102:233-250.

Childs AF, Davis DR, Green A et al. The reactivation by oximes and hydroxamic acids of cholinesterase inhibited by organo-phosphorus compounds. Brit J Pharmacol 1955;10:462-465.

Cho Y, Kim W-S, Hur G-H, Ha Y-C. Minimum effective drug concentrations of a transdermal patch system containing procyclidine and physostigmine for prophylaxis against soman poisoning in rhesus monkeys. Env Toxicol Pharmacol 2012;33:1-8.

Choi E-K, Park D, You J-M, Hur G-H, Ha Y-C, Che J-H et al. Protection by sustained release of physostimine and procyclidine of soman poisoning in rats. Eur J Pharmacol 2004;505:83-91.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Clement JG. Toxicology and pharmacology of bispyridinium oximes – Insight into the mechanism of action vs soman poisoning in vivo. Fund Appl Toxicol 1981;1:193- 202.

Clement JG. HI-6: Reactivation of central and peripheral acetylcholinersterase following inhibition by soman, sarin and tabun in vivo in the rat. Biochem Pharmacol 1982; 31:1283-1287.

Crepeau AZ, Treiman DM. Levetiracetam: a comprehensive review. Expert Rev Neurother 2010;10:159-171.

de Jong LP, Wolring GZ. Stereospecific reactivation by some Hagedorn-oximes of acetylcholinesterase from various species including man, inhibited by soman. Biochem Pharmacol 1984;33:1119-1125.

De Smedt T, Raedt R, Vonck K, Boon P. Levetiracetam: part II, the clinical profile of a novel anticonvulsant drug. CNS Drug Rev 2007;23:57-78

Dorandeu F, Carpentier P, Baubichon D, Four E, Bernabé D, Burckhart M-F et al. Efficacy of ketamine-atropine combination in the delayed treatment of soman-induced status epilepticus. Brain Res 2005;1051:164-175.

Dybdal D, Gale K. Postural and anticonvulsant effects of inhibition of the rat subthalamic nucleus. J Neurosci 2000;20:6728-6733.

Ekstrand JJ, Domroese ME, Johnson DMG, Feig SL, Knodel SM, Behan M et al.. A new subdivision of anterior piriform cortex and associated deep nucleus with novel features of interest for olfaction and epilepsy. J Comp Neurol 2001;434:289-307.

Ellenberger HH, Feldman JL. Subnuclear organization of the lateral tegmental field of the rat. I: Nucleus ambiguus and ventral respiratory group. J Comp Neurol 1990;294:202-211.

Engel JJr, Schwartzkroin PA.What should be modeled? In: Pitkänen A, Schwartzkroin PA, Moshé SL, editors. Models of Seizures and Epilepsy. Amsterdam: Elsevier; 2006, p. 1-14.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Figueiredo TH, Aroniadou-Anderjaska V. Qashu F, Apland JP, Pidoplichko V, Stevens D, et al. Neuroprotective efficacy of caramiphen against soman and mechanisms of action. Br J Pharmacol 2011;164:1495-1505.

Gale K. Progression and generalization of seizure discharge: Anatomical and neurochemical substrates. Epilepsia 1988;(Suppl.2)29:S15-S34.

Gao ZG, Liu BY, Cui WY, Li LJ, Fan QH, Liu CG. Anti-nicotinic properties of anticholinergic antiparkinson drugs. J Pharm Pharmacol 1998;50:1299-1305.

Garrigue H, Maurizis JC, Nicolas C, Madelmont JC, Godeneche D, Hulot T. Disposition and metabolism of two acetylcholinesterase reactivators, pyrimidoxime and HI-6, in rats submitted to organophosphate poisoning. Xenobiotica 1990;20:699-709.

Gernert M, Löscher W. Lack of robust anticonvulsant effects of muscimol microinfusions in the anterior substantia nigra of kindled rats. Eur J Pharmacol 2001;432:35-49.

Gupta, R. Classification and uses of organophosphates and carbamates. In: R Gupta, editor. Toxicology of organophosphate and carbamate compounds. Amsterdam: Elsevier; 2006. p. 5-24.

Hamilton MG, Lundy PM. HI-6 therapy of soman and tabun poisoning in primates and rodents. Arch Toxicol 1989;63:144-149.

Haug KH, Myhrer T, Fonnum F. The combination of donepezil and procyclidine protects against soman-induced seizures in rats. Toxicol Appl Pharmacol 2007; 220:156-163.

Hymowitz N, Ploshnick A, Laemle L, Brezenoff H. Effects of repeated administration of soman on scheduled-controlled behavior and brain in the rat. Neurotoxicol Teratol 1990;12:47-56.

Jang E-J, Lee Y-J, Chung S-J, Shim C-K. Nasal absorption of procyclidine in rats and dogs. Arch Pharm Res2001;24:219-223.

Kadar T, Cohen G, Sahar R, Alkalai D, Shapira S. Long-term study of brain lesions following soman, in comparison to DFP and metrazol poisoning. Human Exp Toxicol 1992;11:517-523.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Kaminski RM, Matagne A, Patsalos PN, Klitgaard H. Benefit of combination therapy in epilepsy: A review of preclinical evidence with levetiracetam. Epilepsia 2009;50:387-397.

Kassa J, 1995. Comparison of efficacy of two oximes (HI-6 and obidoxime) in soman posoning in rats. Toxicology 1995;101:167-174.

Kim Y-B, Cheon K-C, Hu G-H, Phi T-S, Choi S-J, Hong D et al. Effects of combinational prophylactics composed of physostigmine and procyclidine on soman-induced lethality, seizures and brain injuries. Env Toxicol Pharmacol 2002;11:15-21.

Kim W-S, Cho Y, Kim J-C, Huang ZZ, Park S-H, Choi E-K et al. Protection by a transdermal patch containing physostigmine and procyclidine of soman poisoning in dogs. Eur J Pharmacol 2005;525:135-142.

Kubin L, Fenik V. Pontine cholinergic mechanisms and their impact on respiratory regulation. Resp Physiol Neurobiol 2004;143:235-249.

Kusic R, Boskovic B, Vojvodic V, Janovicd D. HI-6 in man: Blood levels urinary excretion, and tolerance after intramuscular administration of the oxime to healthy volunteers. Fund Appl Toxicol 1985;5:S89-S97.

Lallement G, Baubichon D, Clarençon D, Gallonier M, Peoc'h M, Carpentier P. Review of the value of gacyclidine (GK-11) as adjuvant medication to conventional treatments of organophosphate poisoning: Primate experiments mimicking various scenarios of military of terrorist attack by soman. Neurotoxicology 1999;20:675-684.

Lallement G, Denoyer M, Collet A, Pernot-Marino I, Baubichon D, Monmaur P, Blanchet G. Changes in hippocampal acetylcholine and glutamate extracellular levels during soman-induced seizures; influence of septal cholinoceptive cells. Neurosci Lett 1992;139:104-107.

Lallement G, Pernot-Marino I, Baubichon D, Burckhart M-F, Carpentier P, Blanchet G. Modulation of soman-induced neuropathology with an anticonvulsant regimen. Neuroreport 1994;5:2265-2268.

Ligtenstein DA, Kossen SP. Kinetic profile in blood and brain of the cholinesterase reactivity oximen HI-6 after intravenous administration to the rat. Toxicol Appl Pharamacol 1983;71:177-183.

Lundy PM, Hamilton MG, Sawyer TW, Mikler J. Comparative protective effects of HI-6 and MMB-4 against organophosphorous nerve agent poisoning. Toxicology 2011;285:90-96.

Lundy PM, Raveh L, Amitai G. Development of the bisquaternary oxime HI-6 toward clinical use in the treatment of organophosphate nerve agent poisoning. Toxicol Rev 2006; 25:231-243.

Lyeth BG, Ray M, Hamm RJ, Schnabel J, Saady JJ, Poklis A et al. Postinjury scopolamine administration in experimental traumatic brain injury. Brain Res 1992;569:281-286.

Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A et al. The synaptic vesicle protein SV2A is the biding site for the antiepileptic drug levetiracetam. Proc Natl Acad Sci USA 2004;101:9861-9866.

Löscher W, Ebert U. Basic mechanisms of seizure propagation: Targets for rational drug design and rational polypharmacy. Epilepsy Res 1996;Supplement 11:17-43.

Löscher W, Hönak D, Rundfeldt C. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. J Pharamacol Exp Therap 1998;284:474-479.

McDonough Jr JH, Dochterman W, Smith CD, Shih T-M. Protection against nerve agent-induced neuropathology, but not cardiac pathology, is associated with the anticonvulsant action of drug treatment. Neurotoxicology 1995;15:123-132.

McDonough Jr JH, McLeod Jr CG, Nipwoda T. Direct microinjection of soman or VX into the amygdala produces repetitive limbic convulsions and neuropathology. Brain Res 1987;435:123-137.

McDonough Jr JH, Shih T-M. Pharmacological modulation of soman-induced seizures. Neurosci Biobehav Rev 1993;17:203-215.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

McDonough Jr, JH, Shih T-M. A study of the N-Methyl-D-Aspartate antagonistic properties of anticholinergic drugs. Pharmacol Biochem Behav 1995; 51:249-253.

McDonough JH Jr, Shih T-M. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. Neurosci Biobehav Rev 1997;21:559-579.

McIntyre DC, Kelly ME. The parahippocampal cortices and kindling. Ann N Y Acad Sci 2000;911:343-354.

McIntyre DC, Kelly ME, Armstrong J N. Kindling in the perirhinal cortex. Brain Res 1993;615:1-6.

Meehan AL, Yang X, Yuan L-L, Rothman SM. Levetiracetam has an activity-dependent effect on inhibitory transmission. Epilepsia 2012;53:469-476.

Millichap JG, Pitchford GL, Millichap MG. Anticonvulsant activity of antiparkinsonism agents. Proc Soc Exp Biol Med 1968;127:1187-1190.

Mraovitch S, Calando Y. Interactions between limbic, thalamo-striatal-cortical, and central autonomic pathways during epileptic seizure progression. J Comp Neurol 1999;411:145-161.

Myhrer T. Neuronal structures involved in the induction and propagation of seizures caused by nerve agents: Implications for medical treatment. Toxicology 2007;239:1-14.

Myhrer T. Identification of neuronal target areas for nerve agents and specification of receptors for pharmacological treatment. Neurotoxicology 2010;31:629-638.

Myhrer T, Aas P. Dissecting neuropharmacological mechanisms of nerve agent-induced seizures in search for target areas for medical countermeasures. In: Weismann BA, Raveh L editors. The neurochemical consequences or organophosphate poisoning in the CNS. Kerala: Transworld Reseach Network; 2010. p. 41-60.

Myhrer T, Andersen JM, Nguyen NHT, Aas P. Soman-induced convulsions in rats terminated with pharmacological agents after 45 min: Neuropathology and cognitive performance. Neurotoxicology 2005;26:39-48.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Myhrer T, Enger S, Aas P. Pharmacological therapies against soman-induced seizures in rats 30 min following onset and anticonvulsant impact. Eur J Pharmacol 2006a; 548:83-89.

Myhrer T, Enger S, Aas P. Anticonvulsant effects of damage to structures involved in seizure induction in rats exposed to soman. Neurotoxicology 2007;28:819-828.

Myhrer T, Enger S, Aas P. Anticonvulsant impact of lesions in the ventrolateral forebrain of rats challenged with soman. Brain Res 2008a;1226:241-247.

Myhrer T, Enger S, Aas P. Anticonvulsant efficacy of drugs with cholinergic and/or glutamatergic antagonism microinfused into area tempestas of rats exposed to soman. Neurochem Research 2008b;33:348-354.

Myhrer T, Enger E, Aas P. Anticonvulsant efficacy of pharmacological agents microinfused into medial septum of rats exposed to soman. J Med CBR Defense 2009;7:1-14.

Myhrer T, Enger S., Aas P. Roles of perirhinal and posterior piriform cortices in control and generation of seizures: A microinfusion study in rats exposed to soman. Neurotoxicology 2010a;31:147-153.

Myhrer T, Enger S, Aas P. Modulators of metabotropic glutamate receptors microinfused into perirhinal cortex: Anticonvulsant effects in rats challenged with soman. Eur J Pharmacol 2010b;636:82-87.

Myhrer T, Enger S, Aas P. The perirhinal cortex of rats: An intricate area for microinfusion of anticonvulsants against soman-induced seizures. Neurotoxicology, 2013a;34:128-134.

Myhrer T, Enger S, Aas P. Determination of anti-convulsant and life-preserving capacities of three types of auto-injector therapies against soman intoxication in rats. Drug Test Analysis 2013b;5:693-701.

Myhrer T, Enger S, Mariussen E, Aas P. Two medical therapies very effective shortly after high levels of soman poisoning in rats, but only one with universal utility. Toxicology 2013c;314:221-228.

Myhrer T, Enger S, Jonassen M,. Aas P. Enhanced efficacy of anticonvulsants when combined with levetiracetam in soman-exposed rats. Neurotoxicology 2011;32:923-930.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Myhrer T, Mariussen E, Enger S, Aas P. Capacities of metabotropic glutamate modulators in counteracting soman-induced seizures in rats. Eur J Pharmacol 2013d;718:253-260

Myhrer T, Nguyen NHT, Andersen JM, Aas P. Protection against soman-induced seizures: relationship among doses of prophylactics, soman, and adjuncts. Toxicol. Appl. Pharmacol. 2004a;196:327-336.

Myhrer T, Nguyen NHT, Enger S, Aas P. Anticonvulsant effects of $GABA_A$ modulators microinfused into area tempestas or substantia nigra in rats exposed to soman. Arch Toxicol 2006c;80:502-507.

Myhrer T, Skymoen LR, Aas P. Pharmacological agents, hippocampal EEG, and anticonvulsant effects on soman-induced seizures in rats. Neurotoxicology 2003;24:357-367.

Oliveira AA, Nogueira CRA, Nascimento VS, Aguiar LMV, Freitas RM, Sousa FCF et al. Evaluation of levetiracetam effects on pilocarpine-induced seizures: Cholinergic muscarinic system involvement. Neurosci Lett 2005;385:184-188.

Olney JW, Price MT, Labruyere J, Salles KS, Frierdich G, Mueller M et al. Anti-parkinson agents are phencyclidine agonists and N-methyl-aspartate antagonists. Eur J Pharmacol 1987;142:319-320.

Philippens I, Jongsma M, Joosen M, Bouwman G, Vanwersch R. Prophylaxis against nerve agent toxicity: Physiological, behavioral, and neuroprotection aspects of current and novel treatments. NATO/HFM, Edinburgh, 2007. p. 16/1-15.

Philippens IHCHM, Jongsma M, Vanwersch RAP. Efficacy of pretreatment and treatment against soman intoxication. In: Dishovsky C et al, editors. Medical treatment of intoxications and decontamination of chemical agents in the area of terrorist attack. Amsterdam: Springer. 2006. p. 113-121.

Piredda S, Gale K. A crucial epileptogenic site in the deep prepiriform cortex. Nature 1985;317:623-625.

Raveh L, Chapman S, Cohen G, Alkalay D, Gilat E, Rabinovitz I, Weissman B A. The involvement of the NMDA receptor complex in the protective effect of anticholinergic drugs against soman poisoning. Neurotoxicology 1999;20:551-560.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Raveh L, Weissman BA, Cohen G, Alkalay D, Rabinovitz I, Sonego H, Brandeis R. Caramiphen and scopolamine prevent soman-induced brain damage and cognitive dysfunction. Neurotoxicology 2002;23:7-17.

Reynolds IJ, Miller RJ. [^{3}H]MK-801 binding to the N-methyl-D-aspartate receptor reveals drug interaction with the zinc and magnesium binding sites. J Pharmacol Exp Therap 1988;24:1025-1031.

Rickett DL, Glenn JF, Beers ET. Central respiratory effects versus neuromuscular actions of nerve agents. Neurotoxicology 1986;7:225-236.

Rifkin A, Quitkin F, Kane J, Struve F, Klein DF. Are prophylactic antiparkinson drugs necessary? A controlled study of procyclidine withdrawal. Arch Gen Psychiatry 1978;35:483-489.

Sato M, Racine RJ, McIntyre DC. Kindling: Basic mechanisms and clinical validity. Electroencephalogr Clin Neurophysiol 1990;76:459-472.

Scott L. 2007. Pretreatment for nerve agent poisoning. In Marrs TC, Maynard RL, Sidell FR, editors. Chemical Warfare Agents: Toxicology and Treatment (2nd Edition). Chichester: John Wiley & Sons, Ltd; 2007. p. 343-353.

Shih T-M, Dunibo SM, McDonough JH. Control of nerve agent-induced seizures is critical for neuroprotection and survival. Toxicol Appl Pharmacol 2003;188:69-80.

Shih T-M, McDonough JH. Efficacy of biperiden and atropine as anticonvulsant treatment for organophosphorus nerve agent intoxication. Arch Toxicol 2000;74:165-172.

Shih T-M, McDonough Jr JH, Koplovitz I. Anticonvulsants for soman-induced seizure activity. J Biomed Sci 1999;6:86-96.

Sidell FR, Markis JE, Groff WA et al. Enhancement of drug absorption after administration by an automatic injector. J Pharmacokin Biopharm 1974;2:197-210.

Sket D, Brzin M. Effects of HI-6, applied into the cerebral ventricles, on the inhibition of brain acetylcholinesterase by soman in rats. Neuropharmacology 1986;25:103-107.

Skovira JW, Shih T-M, McDonough JH. Neuropharmacological specificity of brain structures involved in soman-induced seizures. Neurotoxicology 2012;33:463-468.

Somani SM, Khalique A. Distribution and pharmacokinetics of physostigmine in rat after intramuscular administration. Fundam Appl Toxicol 1986;6:327-334.

Talbot BG, Anderson DR, Harris LW, Yarbrough LW, Lennox WJ. A comparison of in vivo and in vitro rates of aging of soman-inhibited erythrocyte acetylcholinesterase in different animal species. Drug Chem Toxicol 1988;11:289-305.

Taylor P. Anticholinesterase agents. In: Hardman JG, Limbird LE, Gilman AG, editors. The pharmacological basis of therapeutics. New York: McGraw-Hill Companies, Inc.: 2001. p. 175-191.

Thiermann H, Seidl S, Eyer P. HI-6 dimethanesulfonate has better dissolution properties than HI-6 dichloride for application in dry/wet autoinjectors. Int J Pharmaceut 1996;137:167-176.

Van Helden HPM, Busker RW, Melchers BPC, Bruijnzeel PLB. Pharmacological effects of oximes: how relevant are they. Arch Toxicol 1996;70:779-786.

Van Helden HPM, Herman PM, Van der Wiel HJ, De Lang J, Busker RW, Melchers BPC et al. Therapeutic efficacy of HI-6 in soman- poisoned marmoset monkeys. Toxicol Appl Pharmacol 1992;115:50-56.

Van Helden HPM, Joosen MJA, Philippens IHC. Non-enzymatic pretreatment of nerve agent (soman) poisoning: A brief state -of-the -art review. Toxicol Lett 2011;206:35-40.

Vogl C, Mochida S, Wolff C, Whalley BJ, Stephens GJ. The synaptic vesicle glycoprotein 2A ligand levetiracetam inhibits presynaptic Ca^{2+} channels through an intracellular pathway. Molecul Pharmacol 2012; 82:199-208.

Waelbroeck M, Camus J, Tastenoy M, Mutschler E, Strohmann C, Tacke R et al. Stereoselective interaction of procyclidine, hexahydro-difenidol, hexbutinol and oxyphencyclimine, and of related antagonists, with four muscarinic receptors. Eur J Pharmacol 1992; 227:33-42.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Weissman BA, Raveh L. Therapy against organophosphate poisoning: The importance of anticholinergic drugs with antiglutamatergic properties. Toxicol Appl Pharmacol 2008;232:351-358.

Wetherell JR. Continuous administration of low dose rates of physostigmine and hyoscine to guinea-pigs prevents the toxicity and reduces the incapacitation produced by soman poisoning. J Pharm Pharmacol 1994;46:1023-1028.

Wetherell J, Hall T, Passingham S. Physostigmine and hyoscine improves protection against the lethal and incapacitating effects of nerve agent poisoning in the guinea-pig. Neurotoxicology 2002; 23:341-249.

Wetherell J, Price M, Mumford H. A novel approach for medical countermeasures to nerve agent poisoning in the guinea-pig. Neurotoxicology 2006;27:485-491.

Wetherell J, Price M, Mumford H, Armstrong S, Scott L. Development of next generation medical countermeasures to nerve agent poisoning. Toxicology 2007;233:120-127.

Wilson IB and Ginsburg S. A powerful reactivator of alkylphosphate-inhibited acetylcholinesterase. Biochem Biophys Acta 1955;18:168-170.

Woch G, Ogawa H, Davies RO, Kubin L. Behavior of hypoglossal inspiratory premotor neurons during the carbachol-induced, REM sleep-like suppression of upper airway motoneurons. Exp Brain Res 2000;130:508-520.

Øydvin OK, Tansø R, Aas P. Pre-junctional effects of oximes on [^{3}H]-acetylcholine release in rat hippocampal slices during soman intoxication. Eur J Pharmacol 2005;516:227-234.

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Figure legends

Fig. 1 Lateral view of the rat brain showing neuronal target areas for nerve agents and the respiratory center in the brainstem (ventral respiratory group; VRG). Anticonvulsant efficacy was obtained by lesions in the area tempestas (AT), medial septum (MS), piriform cortex (PC), or perirhinal cortex (PRC) (the areas hatched by diagonal lines). Lack of anticonvulsant effect was seen by damaging the nucleus accumbens (NA), nucleus basalis magnocellularis (NBM), hippocampal region (HCR), amygdala (AM), substantia nigra (SN) or entorhinal cortex (EC).

Fig. 2 The figure is based on the results presented in Table 1 and expresses the anticonvulsant impact factor as the percentage of positive effects for drugs tested in at least 3 of the 4 seizure controlling brain sites identified (area tempestas, medial septum, perirhinal cortex, piriform cortex).

Dette er en postprint-versjon / This is a postprint version.
DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011

Table 1

Effects of microinfusion (1 µl) of drugs (µg unless specified) against soman-induced seizures with percentage of nonconvulsing rats in each group. Anticonvulsant efficacy is based on latency (min) to seizure onset or prevention of seizures. A criterion in min was set for nonconvulsing rats so that data from both categories of results could be combined.

Brain	Anticonvulsant effect relative to saline infusion				No effect relative to saline infusion				
region	Drug	Dose	Receptor	Percent	Drug	Dose	Receptor	Percent	Study
Area tempestas	Atropine	100	M1-M5	62	Benactyzine	0.60/2	M1-M2+NMDA	0	Myhrer et al., 2006c, 2008b
	Scopolamine	1	M1-M5	71	Biperiden	0.11/1	M1-M2+NMDA	13	
	Caramiphen	10	M1	13	Trihexyphenidyl	0.12/1	M1-M4+NMDA	0	
	Procyclidine	6	M1-M4	25	Ketamine	50	NMDA	0	
	Muscimol	120 ng	$GABA_A$	75	MK-801	1	NMDA	0	
	Ethanol	0.47 µmol	$GABA_A$	50	NBQX	40	AMPA+KA	0	
	Propofol	20	$GABA_A$	62	Diazepam	5	$GABA_A$	14	
					Pentobarbital	50/100	$GABA_A$	0	
Medial septum	Atropine	100	M1-M5	0	Ketamine	50	NMDA	0	Myhrer et al., 2009
	Scopolamine	1	M1-M5	0	Muscimol	120 ng	$GABA_A$	0	

	Procyclidine	6	M1-M4	0					
Perirhinal cortex	Procyclidine	6	M1-M4+NMDA	0	Scopolamine	1	M1-M5	0	Myhrer et al., 2010a, 2010b
	NBQX	40	AMPA+KA	0	Ketamine	50	NMDA	0	
	MPEP	0.1	mGluR5	25	Caramiphen	10	M1+NMDA	0	
	DCG-IV	1	mGluR2/3	25	Muscimol	120 ng	$GABA_A$	0	
	MPEP *	0.1	mGluR5	67					Myhrer et al., 2013a
	DCG-IV *	1	mGluR2/3	83					
Posterior piriform cortex	Scopolamine	1	M1-M5	0	Procyclidine	6	M1-M4+NMDA	0	Myhrer et al., 2010a
	Muscimol	120 ng	$GABA_A$	0	Caramiphen	10	M1+NMDA	0	
					NBQX	40	AMPA+KA	0	
					Ketamine	50	NMDA	0	

* Double infusions (reaching a larger area of the perirhinal cortex than single infusion). The percentage of nonconvulsing rats was 0 in some groups with anticonvulsant effect, but the latency to seizure onset was significantly higher than for the saline group. Infusion of biperiden (N=8) or diazepam (N=7) into the area tempestas prevented convulsions in 1 rat in each group, but the total score for these groups was not significantly different from the saline-treated group.

Table 2

Mode of action of the various components of the physostigmine regimen (HI-6, scopolamine, physostigmine) and the procyclidine regimen (HI-6, levetiracetam, procyclidine).

Drug	Action	Half-life in plasma in min	Study of half-life
HI-6	Reactivation of acetylcholinesterase Protection of respiration	24	Garrigue et al., 1990
Scopolamine	Cholinergic (muscarinic) antagonism	17	Lyeth et al., 1992
Physostigmine	Inhibition of acetylcholinesterase	17	Somani and Khalique, 1986
Levetiracetam	Reduction of acetylcholine and glutamate release GABAmimetic effect	150	Löscher et al., 1998
Procyclidine	Cholinergic (muscarinic, nicotinic) and glutamatergic (NMDA) antagonism	120	Jang et al., 2001

DOI til publisert versjon / DOI to published version: 10.1016/j.neuro.2014.04.011