Pharmacological Screening of Anticonvulsants against Nerve Agents: Limitations by Conventional Approach

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ABSTRACT

Nerve agents are highly toxic organophosphate compounds that irreversibly inhibit acetylcholinesterase, the enzyme hydrolysing acetylcholine. Accumulation of acetylcholine results in respiratory dysfunction, prolonged seizures, convulsive status epilepticus, and death. The progression of events can be divided into three phases. An early phase lasting from exposure to about 5 minutes after seizure onset is dominated by excessive cholinergic activity followed by a transitional phase of cholinergic and glutamatergic hyperactivity, and finally a predominantly glutamatergic phase after about 40 minutes. Nerve agent induced seizures that have lasted more than 10 min appears to be difficult to terminate, unless the countermeasures exert cholinergic and glutamatergic antagonism as well as GABAergic agonism. However, even medical therapies assuring such effects become gradually less effective by elapse of time (1 h). It will take at least 30 min for emergency personnel to access individuals unprepared for exposure to nerve agent. Even soldiers properly equipped with a protective mask, gloves, and clothes may need medical help, because bad training, bad discipline or bad luck can lead to intoxication by nerve agent. Hence, there is an urgent need for strategies capable of terminating nerve agent-induced seizures well after their onset. A triple regimen consisting of procyclidine (6 mg/kg), diazepam (10 mg/kg), pentobarbital (30 mg/kg) has been shown to terminate effectively soman-evoked seizures in rats when administered 30-40 min following onset. A refinement of the triple regimen resulted in a double regimen being composed of procyclidine (10 mg/kg) and propofol (50 mg/kg) that can stop seizures 30-35 min after they have been triggered by soman. However, both the triple and double regimens would need monitoring of vital functions, because pentobarbital and propofol can suppress normal function of the respiratory centre in the brainstem. Thus, alternative approaches making it possible to design anticonvulsants predominantly affecting the forebrain will be needed. The ultimate aim should be the development of well tolerated anticonvulsants that can be administered by the soldiers themselves.

1.0 INTRODUCTION

Organophosphorus nerve agents are considered to be potential warfare agents and terrorism compounds (for an overview of the Matsumoto and Tokyo incidents in 1994 and 1995, see Yanagisawa et al., 2006). Nerve agents are considered to be the most toxic means among all chemical warfare agents, and they are potent irreversible inhibitors of acetylcholinesterase (AChE) in both the central and peripheral nervous system. Present medical countermeasures against nerve agents are not sufficiently effective, particularly in protecting the brain. Therefore, new and more effective countermeasures must be developed to enable better medical treatment of civilians and military personnel following exposure to nerve agents (Aas, 2003). Neurochemically, the first reaction to exposure to nerve agents is rapid peripheral and central hyperactivation of the cholinergic system (Shih, 1982; Lallement et al., 1992). It has been hypothesized that several neurotransmitter systems become involved sequentially in the initiation and maintenance of

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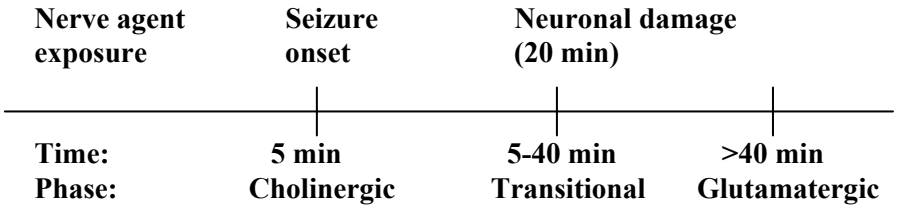
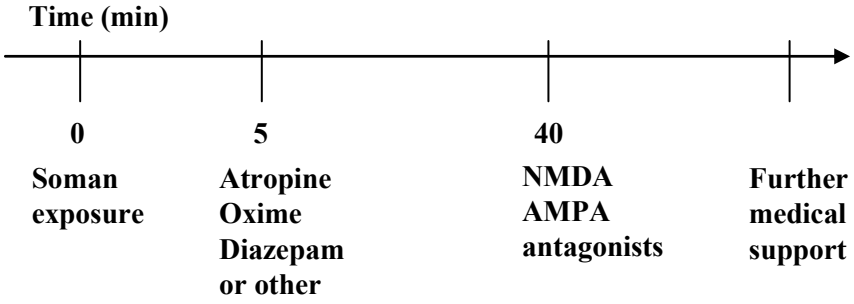
seizures elicited by nerve agents (McDonough and Shih, 1997). The progression of events can conceptually be divided into three phases (fig. 1). An early cholinergic phase lasting from the time of exposure to about 5 min after onset of seizures is dominated by high cholinerigc activity followed by a transitional phase of cholinerigic and glutamatergic hyperactivity and finally a predominantly glutamatergic phase after about 40 min (McDonough and Shih, 1997). In the latter study, a substantial body of evidence has been provided in support of the above model. The three phase model has been suggested to serve as a guide for future research efforts, particularly in the area of developing better treatment regimes against nerve agent intoxications (McDonough and Shih, 1997).

Figure 1. Neuropharmacological 3-phase model of nerve agent intoxication



According to the 3-phase model, efficacious pharmacological countermeasures against nerve agents consists of two categories of treatment; immediate and subsequent therapies. The medical treatment should preferably exert cholinergic and glutamatergic antagonism and GABAergic agonism. Anti-convulsant effects are obtained by drugs acting at the muscarinic receptors, the NMDA/AMPA receptors, and at $GABA_A$ receptors (McDonough and Shih, 1997) (fig. 2). In addition, partial protection against nerve agents can be obtained by the prophylactic use of reversible AChE inhibitors, such as carbamates, shielding a portion of AChE from irreversible inhibition by nerve agents. Furthermore, reactivation of any unaged AChE by an oxime is regarded as important medical treatment after nerve agent exposure.

Figure 2. Challenges and necessary interventions in the medical treatment of nerve gas intoxication

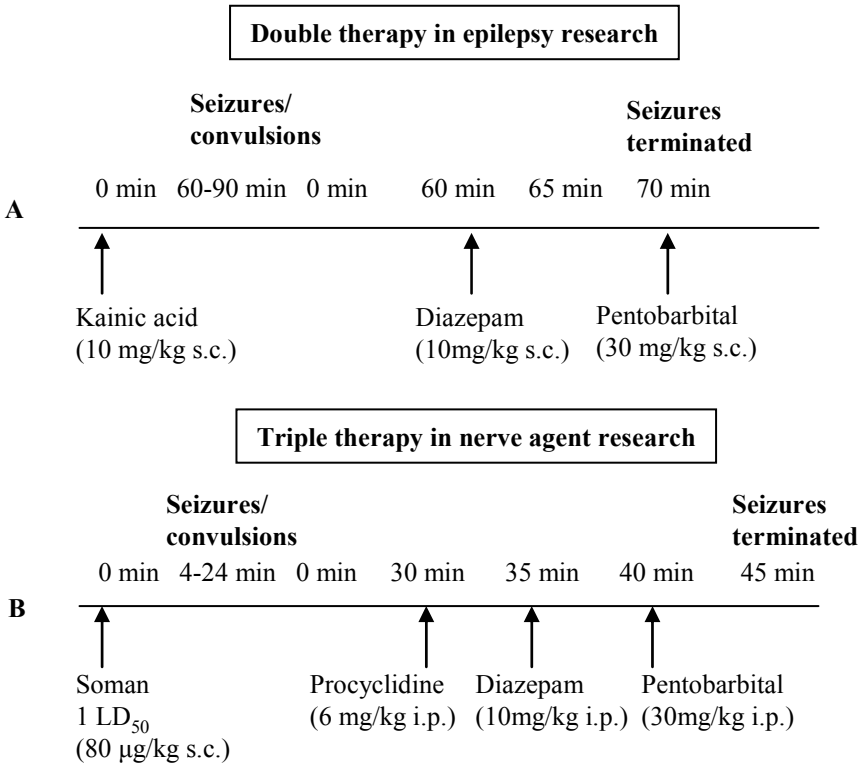


In the first category, drugs with anticholinergic effects will be of preference, whereas drugs with antiglutamatergic effects will be of preference in the second category according to the 3-phase model. Drugs enhancing $GABA_A$ neurotransmission, such as diazepam and other benzodiazepines, are effective during all phases of nerve agent intoxication (McDonough and Shih, 1997). It will take at least 30 min for emergency personnel to access individuals unprepared for exposure to nerve agent. The great challenge in treatment of nerve agent poisoned victims appears to be the cases of long delays before treatment can start. Seizures lasting beyond 40 min are increasingly difficult to terminate (Carpentier et al., 2001; Lallement et al., 1999). Even soldiers properly provided with a protective mask, gloves and protective clothing may need medical assistance, because of intoxication from nerve agents due to, for instance, incorrect use of the protective measures (Lallement et al., 1999).

2.0 RESULTS AND DISCUSSION

It has been 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 (Myhrer et al., 2003, 2006a). A pharmacological possible triple therapy treatment of nerve agent intoxication has been suggested based on a double therapy used in experimental epilepsy (fig. 3).

Figure 3. Pharmacological treatment of nerve agent intoxication. A triple therapy is suggested on the basis of a double therapy used in experimental epilepsy research



In case of soman-induced seizures, a rather complex reaction of pathophysiology is triggered (fig.4).

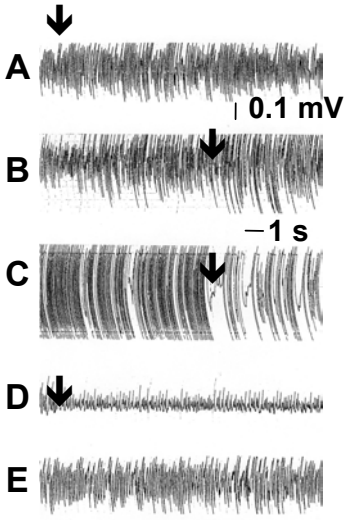
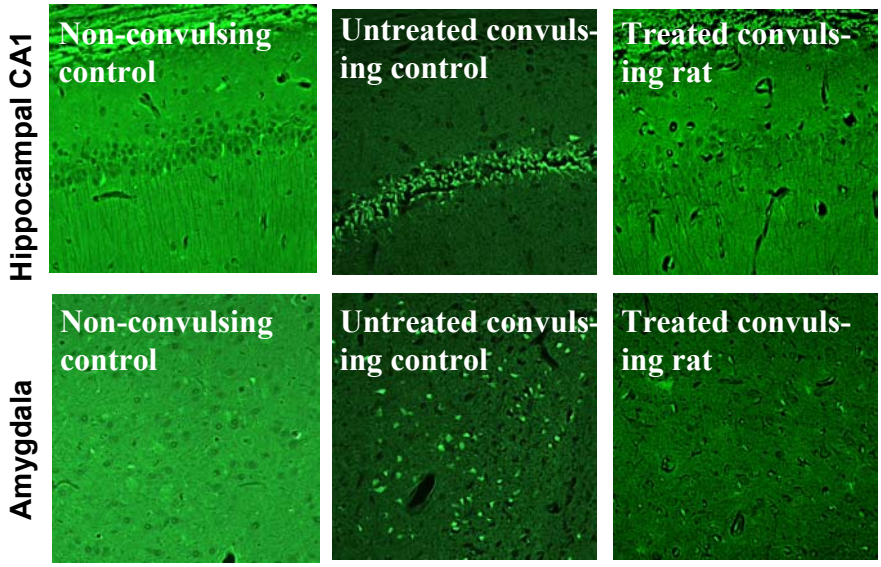


Figure 4. Hippocampal EEG recordings during and after soman poisoning in the rat. A) The rat is walking around 4 min after injection of soman (arrow). B) Seizure activity has started and is accompanied by convulsions 9 min (arrow) after soman challenge. C) The rat has received procyclidine, diazepam and pentobarbital. Three minutes (arrow) after the last injection (45 min since onset of seizures) the seizure activity has declined. D) Five min after the last injection (arrow) (45 min since onset of seizures) the seizure activity has terminated. E) EEG during walking 24 hours following soman exposure.

Potential countermeasures such as anticholinergic, antigutamatergic and GABAergic agonistic effects have been studied in animal experiments. When the seizure activity was terminated 45 min after onset (fig. 3) with procyclidine, diazepam, and pentobarbital, the neuropathology appeared moderate (fig. 5).

Figure 5. Sections stained with Fluro-Jade B (48 hrs following soman exposure (1 LD_{50}, 80µg/kg, s.c.)) show the hippocampal CA1 and amygdala from non-convulsing control rat, untreated convulsing control rat and from rat treated with the anticonvulsant agents procyclidine (6 mg/kg), diazepam (10 mg/kg) and pentobarbital (30 mg/kg).



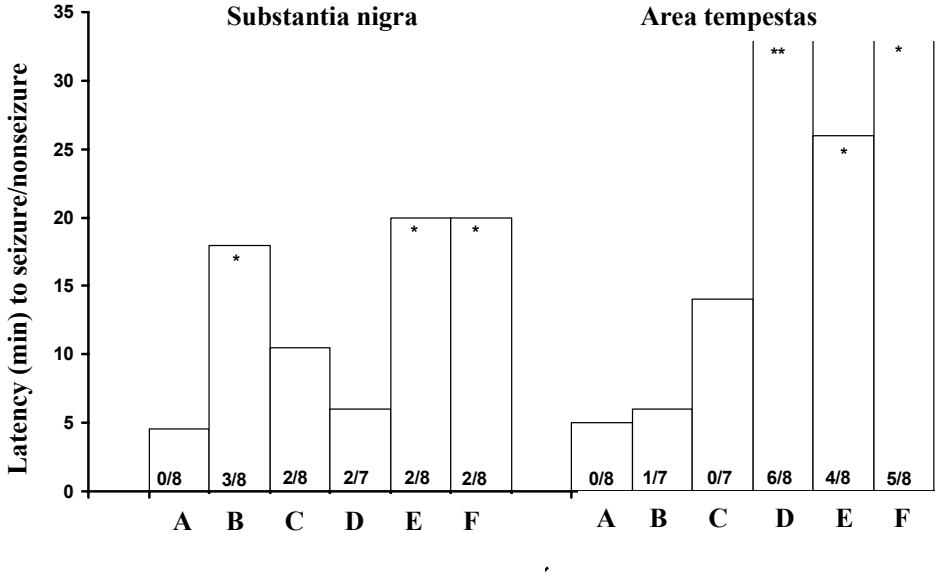
Systemic administration does not allow a clear differentiation between the anticonvulsant properties of $GABA_A$ modulators. For this reason, various $GABA_A$ modulators have previously been micro-infused into seizure controlling substrates in the brain as a screening method for potential systemic administration (Piredda and Gale, 1985; Velíšková,et al., 1998; Gernert and Löscher, 2001).

Within the rat brain, there are control mechanisms capable of attenuating 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). Area tempestas, located in the deep prepiriform cortex, has more recently been defined morphologically and termed the pre-endopiriform nucleus (Ekstrand et al., 2001). Infusion of the $GABA_A$ agonist, muscimol, into substantia nigra has been demonstrated to attenuate generalized convulsive seizures induced by several mechanisms (Gale, 1988). Infusion of muscimol into area tempestas prevents the appearance of seizures on subsequent microinfusions in area tempestas of a cholinergic agonist such as carbachol, glutamatergic agonists as kainic acid, or the GABAergic antagonist bicuculline (Piredda and Gale, 1985).

$GABA_A$ modulating drugs were microinfused bilaterally into the seizure controlling substrates, substantia nigra or area tempestas, of rats subjected to seizures induced systemically by soman (100 µg/kg). The results showed that infusion of ethanol (0.47 µmol) or propofol (20 µg) in both substantia nigra and area tempestas had anticonvulsant effects with prevention of seizures or increased latency to seizures (fig. 6) (Myhrer et al., 2006b). Anticonvulsant effects were also obtained when muscimol (120 ng) was infused

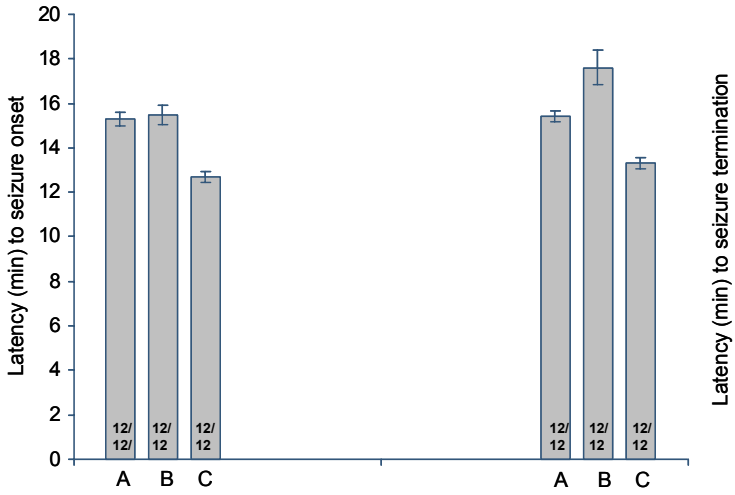
into area tempestas or when diazepam (5 µg) was infused into substantia nigra. Pentobarbital (50 µg) did not attenuate soman-elicited seizures in any of the injection sites (fig.6).

Figure 6. Anticonvulsant effects of bilateral microinfusions (1 µl) of drugs into substantia nigra and area tempestas of rats intoxicated by soman (1.3 LD_{50}, 100 µg/kg s.c.). A) Saline, B) Diazepam (5 µg), C) Pentobarbital (50 µg), D) Muscimol (120 ng), E) Ethanol (0.47 µmol), F) Propofol (20 µg). The numbers given are the number of non-convusing rats and the total number of animals in each experimental group. Significantly different from saline-treated control group. *P<0.05



The anticonvulsant effects of procyclidine with simultaneous injection of propofol, ethanol or muscimol has recently also been studied by systemic administration of the drugs (i.p.) after soman (s.c.) intoxication (Myhrer et al., 2006c). It has been shown that a double regimen consisting of procyclidine (10 mg/kg) and either propofol (50 mg/kg), ethanol (3 mg/kg) or muscimol (20 mg/kg) can effectively terminate soman (100 µg/kg) induced seizures when administered systemically 30-35 min after onset of seizures as for the

Figure 7. Pharmacological treatment of soman-induced (100 µg s.c., 1.3 LD_{50}) seizures 30 min after onset. Experiment A: Procyclidine (10 mg/kg, i.p.) and muscimol (20 mg/kg, i.p.). Experiment B: Procyclidine (10 mg/kg, i.p.) and ethanol (3 mg/kg, i.p.). Experiment C: Procyclidine (10 mg/kg, i.p.) and propofol (50 mg/kg, i.p.). Antiseizure response ratio is shown for each experiment. Mean latencies in min (±S.E.).



above triple regimen (Myhrer et al., 2006c) (fig. 7). Of the three double regimens tested in our studies, the combination of procyclidine and propofol are, following these experiments, considered as the most relevant double regimen to replace the previous triple regimen (procyclidine, diazepam, pentobarbital) against soman-induced seizures. These drugs are also approved for human use, although not together. Procyclidine has been used as an antiparkinsonian drug for decades and is well tolerated in humans (Whiteman et al., 1985). Propofol is used as an anaesthetic drug in human medicine (Jouvert et al., 2002). Muscimol, on the other hand, is not approved for human use (Merck Index, 2001).

3.0 CONCLUSIONS

Microinfusion of GABAergic modulators into substantia nigra or area tempestas can antagonise seizure activity induced systemically by soman. Such pharmacological manipulation of substrates for seizure control may facilitate differentiation between the anticonvulsant capabilities of GABAergic-like drugs. The results from these experiments suggest that muscimol, ethanol, and propofol can act as potent anticonvulsants against nerve agent-induced seizures. In a follow on study we have examined the anticonvulsant impact of each of the three drugs combined with procyclidine and administered systemically. The results showed that all tree combinations could effectively terminate soman-induced (100 µg/kg, s.c.) seizures when administered 30-35 min after onset.

Of the three double regimens tested, the combination of procyclidine and propofol are considered as the most relevant double regimen to replace the previous triple regimen. However, both the triple and double regimens would need monitoring of vital functions, because pentobarbital and propofol can suppress normal function of the respiratory centre in the brainstem. Thus, alternative approaches making it possible to design anticonvulsants predominantly affecting the forebrain will be needed.

Such therapy can be useful for military personnel inadequately protected against nerve agents or civilian individuals unprepared for exposure to such agents. It is very important to control nerve agent-induced seizures at an early stage to avoid neuropathology and cognitive dysfunctions.

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