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Seizure activity post organophosphate exposure

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1. ABSTRACT

Electrographic seizures are a feature of organophosphate anticholinesterase intoxication. Clinical studies of pesticide poisonings suggest that seizures are more common in children than in adults. Since flaccid paralysis, a characteristic sign of organophosphate poisoning, can mask convulsions, the most reliable indicator of seizures is the electroencephalogram, but this has not been widely used in clinical studies. Seizures can rapidly progress to status epilepticus, contributing to mortality and, in survivors, to neuronal damage and neurological impairment. Anticonvulsant drugs can significantly reduce the lethal and toxic effects of these compounds. A benzodiazepine, usually diazepam, is the treatment currently indicated for control of seizures. Animal studies have indicated that the early phase of seizure activity (0-5 min after seizure onset) is purely cholinergic, predominantly involving muscarinic mechanisms. Seizure activity subsequently progresses through mixed cholinergic and noncholinergic modulation (5-40 min) into a final noncholinergic phase. Neuropathology caused by seizures is most likely associated with glutamatergic excitotoxicity. Future prospects for improved treatments include new benzodiazepines, glutamate receptor antagonists, antimuscarinics with additional antiglutamatergic activity and adenosine receptor antagonists.

2. INTRODUCTION

Organophosphorus (OP) anticholinesterases exert their primary toxic effects by preventing the hydrolysis of acetylcholine by acetylcholinesterase (1). The subsequent accumulation of acetylcholine at peripheral cholinergic sites produces the classical muscarinic and nicotinic receptor-mediated signs of anticholinesterase intoxication including salivation, lacrimation, urination, miosis or mydriasis, diarrhoea, bronchospasm, bronchorrhoea, vomiting, muscle fasciculation, sweating, hypertension and tachycardia or bradycardia (1,2). Signs of central nervous system involvement are commonly observed in severe organophosphate poisoning, including respiratory failure, depressed levels of consciousness and seizures (3-5). The seizures can lead to neuronal damage, contributing to mortality and, in the case of survivors, to neurological impairment. The onset of seizures has been attributed to a number of causes, including overstimulation of cholinergic pathways and hypoxia (6).

Although both pesticides and nerve agents act primarily by inhibiting the enzyme acetylcholinesterase, the nerve agents are designed to be more toxic to humans and therefore act more rapidly and have a greater lethality. Consequently, the potential use of such agents in warfare or in terrorism is of considerable concern (7-10). Nevertheless, at least two million people are poisoned by organophosphate pesticides each year in the developing

world (11,12), mainly through self-poisoning. This results in an estimated 200,000 deaths per year (13-15), which accounts for nearly one-third of the world's suicides (16).

Because nerve agents act so rapidly, military therapeutic approaches are based on immediate self or buddy aid using autoinjection devices. In contrast, effective treatment of pesticide poisoning may be delayed because the range of nicotinic and muscarinic signs and symptoms is often incomplete, and may mimic a variety of other conditions, particularly in children (2).

Although the primary cause of death in OP poisoning is respiratory paralysis, both central and peripheral (17-24), other important toxic signs include electrographic seizures in the brain and consequent motor convulsions. It is now recognized that these seizures can rapidly progress to status epilepticus and can contribute to the profound brain damage and cardiac pathology that may develop as a consequence of exposure to these highly toxic compounds (25). Furthermore, seizure activity can cause central apnea (26,27), particularly in children, as well as electrocardiographic changes (28). Treatment with appropriate anticonvulsant drugs can thus significantly reduce the lethal and toxic effects of these compounds.

First-line treatment for organophosphate anticholinesterase intoxication consists of a muscarinic receptor antagonist (usually atropine) combined with an oxime reactivator of organophosphate-inhibited cholinesterase such as pralidoxime or obidoxime (29-31). A benzodiazepine anticonvulsant (usually diazepam) is the treatment currently indicated for control of seizures (29,31).

3. CLINICAL INCIDENCE OF SEIZURES IN OP POISONING

Although there are many thousands of cases of OP pesticide poisoning each year, the majority of these are not well documented and the identity and amount of the OP involved is often unknown. For nerve agents, in contrast, cases of human poisoning are rare. The most notable of these occurred during the Iran-Iraq war and the terrorist attacks in Japan, both of which involved sarin, as well as tabun in the case of Iraq (32,33). There are even fewer examples for other nerve agents, such as soman and VX. Some studies of the effects of low doses have been conducted in volunteers (e.g.(34,35)) and there have been some reported cases of accidental poisoning (e.g. (36-38)), but these are rare. In this context, it should also be remembered that estimates of the human toxicity of OP anticholinesterases are largely derived by extrapolation from animal data.

It is important to differentiate between "seizures", the abnormal electrical activity of the brain, and "convulsions", which are the outward manifestation of the electrical seizure activity. Many reports of OP poisoning in humans have relied on motor convulsions as the indicator of seizure activity; however, convulsions will only be observed if the electrographic seizure activity in the brain results in propagated electrical signals reaching the motor nerves and causing muscle stimulation. In many cases, "silent" seizures can occur, in which the abnormal electrical activity remains confined to the central nervous system and does not cause muscle contraction. This is of particular concern in OP poisoning, in which one of the characteristic effects is flaccid paralysis, which can mask convulsions. The presence or absence of convulsions is therefore an unreliable indicator of seizures, which should be suspected in all cases showing signs of severe central nervous system (CNS) poisoning. The most reliable indicator of seizure activity is the electroencephalogram (EEG), but this has not been widely used in clinical studies.

A study of poisoning in adults (22-42 years old) by three OP insecticides (chlorpyrifos, dimethoate and fenthion) reported that "overt seizures were uncommon for all three compounds" (13). The incidence of seizures ranged from 0% for dimethoate to 5.1% for fenthion and the authors concluded that seizures are uncommon in well oxygenated patients with pesticide poisoning (39); however, these results appear to be based on observations of clinical signs rather than EEG recordings. In an earlier study of 63 patients poisoned with a variety of pesticides, including diazinon, oxydemethon methyl, malathion, ethion and azinphos methyl, the incidence of convulsions was 14% (32). Other studies of OP pesticide poisonings have reported incidences of seizures ranging from 1 to 18% (40-49). One study of 53 cases of severe OP poisoning in Israel reported seizures in 13 cases (24.5%) (50).

From the available data for pesticides, it appears that seizures are more common in children than in adults. In contrast to adults, fasciculations and bradycardia are uncommon following OP and carbamate poisoning in children. Predominant signs and symptoms are associated with the central nervous system (51,52). It has been estimated that the incidence of tonic–clonic seizures averaged 25% in young patients, compared with 2.5% in adults (53,54). For example, a retrospective review by Verhulst *et al.* (2) of case notes for 54 children intoxicated with OP anticholinesterase insecticides, mostly unidentified, documented signs of seizures (tonic-clonic convulsions) in 30% of the patients. This figure is similar to that reported in a different study of 37 infants and children intoxicated with OP or carbamate pesticides, in which 22% of patients had seizures (55). Verhulst *et al.* also found that 26% of the survivors (13 patients) had seizures, compared with 75% of non-survivors (3 patients), although this difference did not reach statistical significance ($p=0.07$). A recent study of 31 children in Israel intoxicated with OPs reported that 12 (38.7%) had seizures (56).

Another study of 16 children poisoned with OPs (parathion, fenthion, malathion and diazinon) found that the predominant signs were related to CNS depression and severe hypotonia in the absence of peripheral muscarinic effects (52). The authors concluded that this clinical presentation differed from that described in adults. The electroencephalographic (EEG) correlate of the victims' stupor is unreported in this study, and it is therefore

impossible to discern how many victims experienced nonconvulsive status epilepticus.

In the case of nerve agents, the data are sparser. There were between 45,000 and 100,000 Iranian chemical casualties in the war with Iraq, of which the majority were apparently caused by nerve agents (57). Treatment of Iranian casualties was reported by Dr Syed Abbas Foroutan, who ran a chemical casualty aid station during the Iran-Iraq war (33). He did not describe the incidence of seizures in these patients, but he did report using diazepam as an anticonvulsant as well as a muscle relaxant. He encountered a few patients who went into "spastic stage" while recovering from severe sarin poisoning and speculated that this might be from central effects of the agent rather than from peripheral cholinergic effects. Since he did not mention using EEG to check these patients, the alternative explanation of non-convulsive seizures remains a possibility.

In the Matsumoto terrorist attack in Japan, seven people died and over 200 sought medical attention (58). Those critically and severely affected by sarin presented with loss of consciousness and generalized seizures (59-65). Following the later Tokyo subway attack, 12 people died and over 5000 civilians presented to medical facilities (58). Only 2.7% of patients admitted to hospital had seizures, compared with 99% who had miosis and 23% with muscle fasciculations (59,66). The concentration of sarin to which most victims were exposed was low, however, and the sarin was diluted with a variety of solvents, such as benzene, petroleum ether and hexane (67).

In considering these reports, it must be remembered that the incidence of seizures is likely to depend strongly on the severity and route of poisoning (68-70). Furthermore, unless EEGs are recorded, there is a strong possibility that many patients could have non-convulsive seizures, masked by the flaccid paralysis typical of OP anticholinesterase poisoning (66,71-74). Indeed, it has been recommended that patients with flaccid paralysis should undergo EEG monitoring because usually generalized seizure activity will not be recognized in such patients (66,72). Although it has been suggested that nerve agents produce a greater incidence of seizures than OP pesticides (39), it is in fact difficult to find sufficient evidence to support this supposition.

4. EXPERIMENTAL MODELS

4.1. Animal models

Clearly, controlled studies of the effects of OP anticholinesterases cannot be carried out in humans. Studies of the mechanisms and pharmacology of OP-induced seizures have therefore been performed using animal models, usually mice (75-81), rats (77,82-85) or guinea-pigs (84,86-88), but occasionally primates (89-94), and the majority of these have used nerve agents to induce seizure activity.

The effects of OPs can vary significantly between species. For example, rats have higher levels than guinea-pigs of carboxylesterases, which bind a proportion of the anticholinesterase and lower its toxicity (95-98). This is particularly significant in studies using carbamate pretreatment to protect a proportion of acetylcholinesterase activity. The guinea-pig has therefore been suggested as a better model than other rodent species for predicting the efficacy of treatments for OP poisoning in primates and humans (99,100): human plasma contains no carboxylesterase (EC 3.1.1.1), in contrast to plasma from other species such as mouse, rat, rabbit, horse and cat (101).

Organophosphate-induced seizures cannot easily be reproduced in naive animals due to the steep dose-toxicity profile shown by irreversible OP anticholinesterases (102). Instead, many studies employ adjuncts (e.g. atropine and oximes) to prevent immediate mortality associated with seizure-producing doses of nerve agents (103-106). The use of these additional treatments may confound interpretation of the anticonvulsant efficacy of drugs tested in these *in vivo* models. An alternative approach which avoids this problem is to use *in vitro* brain slice preparations in which seizure activity can be induced by OPs (102,107-111).

4.2. *In vitro* models

Initial studies to develop a slice model for the study of OP-induced seizures in our laboratory used hippocampal slices from rats (112); however, in our hands the incidence of seizures in slices treated with OPs was too low to provide a useful model. Other studies of rat hippocampal slices treated with anticholinesterases have reported inconsistent results. Although Lebeda and Rutecki consistently saw the induction of epileptiform activity after application of diisopropylphosphorofluoridate (DFP) or soman (108), Cole and Nicoll observed the induction of epileptiform activity in only 20 to 30% of slices after application of the carbamate anticholinesterase physostigmine (107). Another study failed to see any epileptiform activity after application of either DFP or physostigmine, although a secondary population spike was induced (109).

We therefore turned to the guinea-pig hippocampal slice (113), in which we found soman induced epileptiform activity in the CA1 region in approximately 75% of slices. This effect was mimicked by other anticholinesterases (paraoxon, physostigmine and neostigmine), as well as sarin (110), confirming that the epileptiform activity was the result of cholinesterase inhibition, and the soman-induced activity was inhibited by muscarinic antagonists (102). Further evaluation of the model with a range of anticonvulsants demonstrated that soman-induced epileptiform activity could be antagonized by a range of diverse compounds but was resistant to others. All compounds which are known to be effective *in vivo* were also effective inhibitors of seizure activity in the hippocampal slice; however, some compounds which are ineffective *in vivo*, such as phenytoin and fosphenytoin, could inhibit epileptiform activity in the slice. Thus, although the slice model was not fully predictive of the *in vivo* efficacy of the compounds, it did not produce false

negatives (i.e. it did not reject compounds known to be active *in vivo*) and it could therefore be used as a pre-screen to eliminate ineffective compounds before *in vivo* testing, as well as having utility for detailed mechanistic studies.

Clearly, however, the limitations of this model must be recognised. For example, it does not appear to be able to generate ictal-like activity following exposure to OPs. Such activity has been observed in combined hippocampal-entorhinal cortex slice preparations from the rat (114), but this preparation is difficult to achieve in the guinea-pig due to the longer anatomical pathway in that species. Furthermore, other brain structures which are important in seizure activity, such as the piriform cortex and amygdala, are not represented in this model.

5. ETIOLOGY AND CLASSIFICATION OF SEIZURES

Epileptic seizures are classified according to clinical and EEG characteristics (115). Partial seizures are defined as seizures that start in one hemisphere; those that begin in both hemispheres simultaneously are defined as generalized seizures. Simple partial seizures can have motor, sensory, autonomic, or psychic signs or symptoms, whereas complex seizures are associated with impairment of consciousness (115). Simple partial seizures can progress to complex partial seizures, and both simple and complex partial seizures can progress to generalized seizures. Nerve agent-induced seizures seem to be complex partial seizures progressing to generalized seizures accompanied by tonic–clonic convulsions (116). For example, tonic–clonic convulsions in rats can be triggered by unilateral microinfusion of VX into the amygdala (117).

Tonic–clonic seizures involve brainstem structures (118), but only a few animal models of epilepsy have their origin in these structures, such as maximal electroshock, genetically prone animals, and NMDA- or strychnine-induced seizures (119,120). In most animal models of epilepsy, tonic–clonic seizures represent a spread of paroxysmal activity from the forebrain to the brainstem (118). The tonic–clonic seizures are divided into three phases: (1) wild running and bouncing; (2) tonic flexion and extension of forelimbs, hind limbs or both - the tonic extension of hind limbs probably reflects the most intense seizure activity (121) - (3) following the tonic phase, long-lasting clonus of all limbs will occur. Seizures can be initiated either in the forebrain (complex partial seizures) or in the reticular formation of the brainstem (generalized tonic–clonic seizures) (118).

6. PATHWAYS INVOLVED IN OP SEIZURES

An important approach in experimental epilepsy is the identification of brain areas involved in the generation, propagation and control of seizures, in order to identify targets for rational design of antiepileptic drugs (122). Work to identify the brain structures involved in the induction and propagation of nerve agent- induced seizures has recently been reviewed by Myhrer (116).

Eight cholinergic pathways have been defined within the mammalian brain (123), designated Ch1-8 (124). The first four pathways originate in the basal forebrain. Ch1 arises from the medial septal nucleus and provides the cholinergic innervation of the hippocampus. Ch2 originates in the vertical limb of the diagonal band nucleus, which is located below the medial septal nucleus. There is very little cellular delineation between these two nuclei and the cells within this pathway follow much the same trajectory as those in Ch1 towards the hippocampus via the fornix (125-128). Pathway Ch3 arises from within the horizontal limb of the diagonal band and projects via the olfactory tract to the olfactory bulb (126,129). The final basal forebrain pathway, Ch4, originates in the nucleus basalis of Meynert and is the largest of all the cholinergic pathways. This pathway provides almost all of the cholinergic innervation to the cortex (130,131); it is larger in primates and humans than it is in rodents, presumably due to the greater development of the cortex (132).

The pontomesencephalic region of the upper brainstem contains the points of origin of the remaining four pathways. Ch5 is contained within the pedunculopontine nucleus and ascends to innervate the thalamus, zona incerta, habenula, hypothalamus (126), substantia nigra (133) and the rostral aspect of the basal ganglia (134). Ch6 originates within the neighboring laterodorsal tegmental nucleus within the periventricular gray matter and innervates most of the same targets as Ch5 (126,134,135). The Ch5 and Ch6 pathways have descending branches that project caudally into the pons, medulla and the cerebellum (136). The Ch7 pathway projects from the medial habenular nucleus to the interpeduncular nucleus (128). Finally Ch8 originates within the parabigeminal nucleus and projects to the superior colliculus (128,137).

Not all of the cholinergic innervation of the brain is supplied by projection neurones via the pathways described above. In the striatum, the innervation is almost exclusively supplied by cholinergic interneurones contained within the structure (138), although there may be a minor extrinsic component from the Ch6 pathway (125).

Although high doses of nicotine can induce clonic-tonic convulsions in animals (79,139), the nicotinic antagonist mecamylamine has no anticonvulsant efficacy against nerve agents (91,102). It has been suggested, however, that nicotinic activation during soman poisoning may cause a reduction in seizure threshold in the brain stem without contributing to the increased seizure propensity of the hippocampus (140). High densities of nicotinic receptors are found in the interpenduncular nucleus, medial habenula, and thalamic areas related to sensory function, and they are found at much lower densities in the hippocampus (141).

The effectiveness of muscarinic antagonists in preventing seizures, together with the lack of effect of mecamylamine, suggests that muscarinic receptors play a dominant role in nerve agent-induced seizures. Muscarinic receptors in the CNS have recently been reviewed by

Langmead *et al.* (142). The predominant muscarinic receptor in the brain is the M_1 subtype, which is located post-synaptically in the cortex, hippocampus, striatum and thalamus. M_2 receptors are located predominantly in the brainstem and thalamus, but also in the cortex, hippocampus and striatum, where they are found on cholinergic synaptic terminals and are thought to control ACh release. M_3 and M_5 subtypes are expressed at much lower levels than M_1 or M_2: M_3 receptors are found in the cortex and hippocampus, whereas M_5 mAChRs have a very discrete localisation in the substantia nigra. M_4 mAChRs are found in many brain regions including the cortex and hippocampus, but are predominant in the striatum, where they are thought to play a role in controlling dopamine release and locomotor activity. In the rat brain, the highest densities of muscarinic M_1 and M_2 receptors are found in limbic structures and neocortical areas (143). The muscarinic receptors in the piriform cortex, septal area, hippocampal region, and entorhinal cortex may play a pivotal role in seizure induction (144-147).

It has proved difficult to map seizure initiation sites with EEG recordings, due to the very fast propagation of epileptiform activity following the triggering process. Lipp (148) suggested that nerve agent seizure activity in nonhuman primates showed a tendency to begin in the amygdala, but this was not consistently the case. It has since been shown in cats (149) and rats (105,150) that EEG seizures can begin in either cortical or subcortical areas of the brain, there being no consistency between individual animals.

In contrast, microinjection studies have revealed discrete sites within the brain that are sensitive to the convulsant effects of nerve agent. Nanomole quantities of soman or VX microinjected into the basolateral amygdala of rats elicited prolonged seizures and neuropathology virtually identical to that seen following systemic intoxication (117). Atropine pretreatment 10 min before injections of VX into the amygdala prevented the development of seizures. In a later study using this technique, eleven limbic forebrain areas were identified that showed varying degrees of sensitivity to the seizure-inducing effects of directly infused VX (151). The most sensitive areas were the basolateral amygdala, amygdala-cortex transition zone and a restricted portion of the piriform cortex (A-P + 1.2). Two other levels of the piriform cortex (A-P + 3.2 and + 2.2) and the entorhinal cortex showed intermediate sensitivity, and the low sensitivity areas were the dorsal hippocampus, central amygdala nucleus, and another area of the piriform cortex (A-P + 0.2). The anterior and posterior nucleus accumbens were also sensitive to VX, but it was difficult to quantify their sensitivity because injections into these sites were unilateral (91). Except for the dorsal hippocampus, all of the sensitive sites are within the ventrolateral aspects of the forebrain.

The seizures elicited by microinjection of VX into these areas ranged from mild to severe, and prolonged seizure activity typically resulted in some degree of neuropathology, much of which occurred in structures quite distant from the site of injection. Comparable infusions of VX into 13 other brain sites did not produce seizures or neuropathology. These insensitive sites included neuronal structures typically classified as cholinergic (e.g., septum, ventral pallidium, substantia innominata) on the basis of histochemical criteria (co-localization of cholinesterase and choline acetyltransferase (152)). Intracerebroventricular infusions of VX could also elicit prolonged seizures, but substantially higher doses of VX were required and all animals that developed seizures after this treatment also displayed prominent signs of systemic nerve agent intoxication (tremors, fasciculations, prostration and salivation).

These studies showed that there are multiple neural sites within the limbic forebrain that are exceptionally sensitive to the seizure-inducing effects of the nerve agents. This suggests that seizure activity can begin in any one of a number of sites following systemic poisoning, then propagate rapidly to other areas of the forebrain and recruit other neurotransmitter systems into the seizure process (91). Muscarinic receptors at these cholinoceptive sites seem to be critical to the convulsant effect of focal nerve agent, since systemic pretreatment with atropine prevents the development of seizures (McDonough and Shih, 1997).

Repetitive pulsed-train electrical stimulation of several limbic structures in rats will elicit behaviors and seizure states similar to those observed with centrally applied nerve agent (153-156). With VX, however, *status epilepticus* can develop within 20 min of infusion, compared to 30–90 min with electrical stimulation.

An alternative approach to follow the propagation patterns during nerve agent-induced seizures is to use the proto-oncogene c-fos as a marker for activated neuronal cell bodies. An early study using this technique in rats found the densest labelling in the piriform (layers II and III), entorhinal, cingulate, parietal, and temporal cortices after 2 h of soman-induced seizure activity, whereas modest staining was seen in the hippocampus and amygdala (144). A later study observed robust labelling in layer II of piriform cortex and locus coeruleus within 30–45 min of soman injection (157). At 1 h, the staining in these areas became more intense and staining was also seen in layer III of the piriform cortex, the entorhinal cortex, septum, and endopiriform nucleus. By 2 h, c-fos staining was present throughout the cortical areas, thalamus, striatum, and hippocampus. These observations are consistent with patterns of neuronal damage, which is most frequently observed in the piriform cortex, followed by the amygdala, hippocampus, thalamus, cortical areas, and striatum (158).

From studies of experimental epilepsy, two areas of the brain have been identified as seizure controlling or gating sites: substantia nigra and area tempestas, a structure in the piriform cortex. Anticonvulsant drugs affecting cholinergic, glutamatergic, and GABAergic systems are effective in area tempestas, but only those affecting GABAergic systems are effective in substantia nigra (159).

Consistent with this, microinfusions of $GABA_A$ modulators (diazepam, pentobarbital, muscimol, ethanol, and propofol) into substantia nigra or area tempestas have been shown to counteract soman-triggered seizures in rats (160). Microinfusions of anticholinergic drugs into area tempestas 20 min before exposure to a convulsant dose of soman prevented or delayed the onset of seizures, but glutamate antagonists were ineffective (161). The medial septal area (MS) has been identified as a further seizure controlling site in nerve agent poisoning: microinfusions of atropine into this area have anticonvulsant effects in rats exposed to soman (144,145). Consistent with these findings, selective lesions of area tempestas or the MS can prevent soman-induced seizures (162).

Several seizure inducing sites, including area tempestas, have been identified within the piriform cortex (151,161), pointing to this structure as a probable trigger area for nerve agent-induced seizures. Although seizures have not been evoked by microinfusion of nerve agents into MS, hyperactivity can be observed after several injections of VX (117), and wet dog shakes along with frenetic jumping bouts have been seen in rats following an infusion of soman (116). Furthermore, the septal area is known to be an important site for electrical kindling (163).

These findings have led to the hypothesis that cholinergic hyperactivity in response to a convulsant dose of nerve agent takes place initially in the piriform cortex and MS (116). According to this hypothesis, nerve agent poisoning causes increased ACh accumulation from diagonal band cholinergic terminals and protracted muscarinic receptor activation in the piriform cortex, particularly area tempestas (146,164). At the same time, cells in MS activate the hippocampal region by direct septo-hippocampal cholinergic input and by cholinergic input to the entorhinal cortex which in turn activates the glutamatergic perforant path (144,145). The hippocampus then projects through the entorhinal cortex to other cortical areas (165,166). The piriform and entorhinal cortices have been suggested as sites where the cholinergic excitation initiates glutamatergic hyperactivity after soman exposure (145,167).

7. NEUROPHARMACOLOGICAL MECHANISMS OF OP SEIZURES

Research into the mechanisms and treatment of nerve agent-induced seizures has been guided over the last decade by the three-phase model proposed by McDonough & Shih (91). This model proposes that several different neurotransmitter systems become involved sequentially in the initiation and maintenance of seizures induced by OP nerve agents. The hypothesis divides the sequence of events following seizure-inducing doses of nerve agent into three phases. There is an early cholinergic phase, which lasts from the time of exposure to approximately 5 min after seizure onset. This then progresses into a transitional phase of mixed cholinergic and noncholinergic modulation, which lasts until approximately 40 min after seizure onset. At this time, seizure progression enters the final phase, which is predominantly noncholinergic.

This hypothesis was based largely on studies in an animal model using rats pretreated with the oxime HI-6 to enhance survival and challenged with the nerve agent soman (1.6 X LD50) 30 min later (104). The animals were usually prepared with cortical and, at times, depth electrodes to record EEG activity (105). In this model, EEG seizures occurred with a latency of around 4.5 min and were accompanied by consistent behavioral signs. The seizures were most intense during the first hour and then diminished gradually over several hours. The results from this model are supported by those from other studies in rats (147,168-173) and guinea-pigs (174,175).

Following exposure to nerve agent, the earliest neurochemical events detectable in the CNS are the inhibition of AChE and an immediate increase in brain ACh levels (147,174-177). These changes define the cholinergic phase of seizure activity: the increase in ACh is the only notable change in any neurotransmitter that is evident at the time of seizure onset, and is therefore postulated to be the neurochemical event that initiates epileptiform activity in susceptible neural circuits. This activation seems to occur in a dose-related manner: in rats, the latency onset is about 14 min when the dose of soman is 1 x LD50, but this decreases to about 1 min with a dose of 5 x LD50 (69,70). ACh concentrations appear to increase at different rates and to different degrees depending upon the brain area studied, with cortex and limbic areas generally showing the most rapid and largest changes. The changes in brain AChE activity and ACh concentrations are not affected by anticholinergic drugs that antagonize soman-induced convulsions (171).

Changes in other neurotransmitter systems all lag behind seizure onset and these initiating cholinergic events, at either intermediate (5–20 min; norepinephrine, 3,4-dihydoxyphenylacetic acid (DOPAC), homovanilhc acid (HVA), glutamate) or longer times (less than 20 min to hrs; dopamine, 5-hydroxytryptamine, 5- hydroxyindolacetic acid, choline, GABA, aspartate). Most of these secondary neurochemical changes do not take place in animals poisoned with subconvulsant doses of agent or protected from seizure activity with anticholinergic or anticonvulsant drugs, indicating that they occur as a result of the seizure activity.

During the transition phase, from approximately 5 min to 40 min after seizure onset, the excitatory activity spreads rapidly and recruits other neurotransmitter systems. The longer the seizure progresses during this time, the greater is the recruitment of other neurotransmitter systems. Microdialysis studies have reported a rapid increase in extracellular concentrations of the excitatory and potentially neurotoxic amino acid glutamate in the septum, piriform cortex, hippocampal region, and amygdala following the start of soman triggered seizures (178,179). Extracellular concentrations of glutamate increase in limbic regions almost immediately and there is enhanced intracellular Ca^{2+} mobilization and increased cell membrane phosphoinositide (PI) hydrolysis (180,181), mediated by stimulation of M_1, M_2 and M_3 muscarinic receptor subtypes (182). At the same time, depletion of

norepinephrine begins, dopamine turnover increases (resulting in elevations in DOPAC and then HVA) and moderate impairments in GABA function are noted (91,170,171,173-175). Increases in dopamine are seen if the nerve agent challenge elicits pronounced seizures, the largest increases being in the brainstem and cerebellum (91,174). Blocking the activation of cholinergic muscarinic receptors prevents the changes in norepinephrine and dopamine (91,171,175).

After about 40 min of seizure activity, the noncholinergic phase begins, in which noncholinergic neurotransmitter systems predominate. Extracellular concentrations of ACh return toward normal, while marked increases in dopamine and GABA concentrations are observed (168,169,174,175,183,184). Increases in taurine may represent a normal physiological response to the cellular swelling that occurs in the early stages of nerve agent-induced neuropathology (179). Glutamate concentrations remain high in some brain areas and choline concentrations are elevated, possibly indicating disruption of cellular membranes. There is widespread activation of PI metabolism throughout the brain, indicating continued mobilization of intracellular Ca^{2+} (185-187). Late increases in 5-hydroxyindolacetic acid (5-HIAA) and 5-HIAA/5-HT ratios have been observed at 2-4 hours after exposure, indicative of increased 5-HT turnover (91).

Kar & Matin (188) reported reduced brain GABA concentrations in rats that convulsed during intoxication with paraoxon and found that drugs that increased GABA concentrations abolished paraoxon convulsions. They suggested that convulsions induced by paraoxon may be due to a reduction in brain GABA concentrations. Most subsequent studies, however, have reported increases in the concentrations of GABA in the cortex, hippocampus and striatum of rats or guinea pigs following intoxication by nerve agent (168,169,174,175,183) or dimethoate (184). These changes all occurred at delayed times (20 min to several hrs) after the start of seizure activity, and in some cases the changes in GABA were unrelated to the presence or absence of convulsant activity (168), suggesting that nerve agent-induced changes in the brain GABA system are unlikely to be the cause of seizures.

8. NEUROPATHOLOGY

Three main hypotheses have been proposed to account for the neuropathology induced by severe OP poisoning. The excitotoxic hypothesis emphasizes the role of sustained seizure activity as the primary cause for the development of OP- induced neuropathology (104,117,189). A second hypothesis emphasises systemic factors such as oxygenation and blood flow and ascribes the pathology to hypoxic/anoxic ischaemia (190-192). The third hypothesis, proposed by Petras, suggests the possibility that OPs have a direct toxic action on brain neurons (193,194).

There is little experimental evidence to support the hypothesis of direct neurotoxicity. Exposure of cultured hippocampal neurons to soman for prolonged periods of time (24 hr) does not produce neurotoxicity (195), and direct microinjection soman or VX into various brain sites only produces neuropathology if prolonged seizure activity is evoked (117,151).

It might seem reasonable to ascribe the neuropathology to the lack of oxygen reaching the neurons, since respiratory distress is a major feature of severe OP poisoning. Both hypoxia/anoxia/ischemia and prolonged seizure activity are believed to produce neuropathology through the same final mechanism, the prolonged elevation of intracellular calcium ions to neurotoxic levels (196-200); hence, the pathological changes would appear similar for both causes. However, several studies have reported that there are minimal changes in $p0_2$ in blood or brain preceding (201) or during (85,202,203) prolonged nerve agent-induced seizures, and one of these found that brain $p0_2$ levels actually increased during the first hour of soman-induced seizures in rats (85).

Rat hippocampal slices *in vitro* have been used to study the role of hypoxia in the morphological changes produced in neurons by soman (204). Application of soman at a concentration which produced spontaneous epileptiform activity led to morphological changes similar to those observed in hippocampus and other brain sites of animals exposed to repeated low doses of soman (205,206): an irreversible increase in the number of neurons exhibiting indentations in the nuclear envelope and a reversible decrease in the measured nuclear area. Exposure of hippocampal slices to hypoxic conditions (95% N_2, 5% $C0_2$) produced different changes in neuronal morphology: dilated endoplasmic reticulum cistemae, disrupted mitochondria and extensive clumping of heterochromatin.

McLeod and Wall reported swelling of mitochondria and endoplasmic reticulum, as well as clumped nuclear chromatin, in hippocampal pyramidal cells one hour after the onset of soman seizures in rats (207). They concluded that some of the neuropathology was due to hypoxic or ischemic injury, since early mitochondrial swelling is an ultrastructural characteristic of experimentally induced anoxia/ ischemia (208); lesions were also found under light microscopic examination that resembled microinfarcts. A later study, however, found that swelling of the Golgi and endoplasmic reticulum occurred at l–4 hr after soman exposure, before mitochondrial swelling and clumping of nuclear chromatin were observable(209). Such ultrastructural changes are identical to those seen in other models of experimentally-induced seizures (208,210)

Rats show a pronounced hypertensive response to convulsant doses of soman (202,211,212), accompanied by large increases in brain regional blood flow (202,212-214) and regional glucose utilization (189,212,215,216). It has been suggested that nerve agent neurotoxicity may be due to disruption of the microvasculature (hypertension) and/or a relative hypoxia/ischemia due to mismatches between cerebral metabolic demands during seizures and blood flow and/or glucose availability. Increases in blood–brain barrier permeability occur during soman-induced seizures,

particularly during the first hour when blood pressure increases are most pronounced (85,202,217,218), and a relative ischemia (indicated by an increase in cerebral glucose utilization with no increase in blood flow) has been reported in some areas of rat brain that show damage following soman-induced seizures (212). However, no consistent relationship has been found between those brain areas that showed breaches in the blood–brain barrier (85), those areas that developed a relative ischemia (212) and the sites of brain damage following soman exposure. These factors cannot account for the damage to the brain sites most sensitive to soman-induced neuropathology, such as the piriform cortex and the amygdala (158,219). Damage to some thalamic areas, however, may have a vasogenic component (85) and damage to others (CA3 subfield of hippocampus, dentate gyrus, medial thalamic nucleus) may be due to a relative ischemia (212),

Brain edema has also been suggested as a factor that could contribute to neuropathology following sustained seizures. Seizure activity induced by nerve agents or other chemical convulsants (kainic acid, pilocarpine) leads to swelling of astrocytes, and in the case of soman to dilation of cerebral ventricles (220). The resultant edema has been postulated to initiate localized ischemic conditions, especially in temporal lobe structures (221,222). Mannitol, an osmotic diuretic used clinically to reduce brain edema, has been reported to reduce the neuropathological effects of soman-induced seizure activity in rats, but significant neuropathology was still evident throughout the brain, including temporal lobe structures (piriform cortex, amygdala) (223). Mannitol protection against seizure-induced neuropathology may involve mechanisms other than dehydration (223,224), and brain edema per se cannot fully account for development of OP-induced neuropathology.

Some studies have found neuropathology following nerve agent exposure in animals that were not observed behaviorally to have convulsions during the acute phase of poisoning (193,194,209,225-227), and this evidence has been taken to indicate that seizure activity could not totally account for the development of neuropathology following OP exposure. However, none of these studies performed EEG recordings, the absence of seizure activity being inferred from the absence of observable convulsions. Subsequent work has shown that observation of motor activity is not a reliable indicator of central seizure activity and that EEG monitoring is essential (105,150). In fact, in several of these studies, the animals that did not convulse, but had brain lesions, were observed to have had strong persistent whole-body tremors (193,194,209,225-227), a sign of significant central cholinergic intoxication (228). Further, some of the studies reported that treatment with an anticonvulsant reduced or prevented soman-induced acute neural lesions (194,225).

Some of the strongest evidence in favor of an excitotoxic mechanism of OP-induced neuropathology comes from studies with anticonvulsant drugs. Pretreatment with the benzodiazepine diazepam blocks nerve agent-induced seizure activity and prevents the development of brain damage (229-231), and early post-poisoning treatment with this drug prevents or greatly reduces the severity of neuropathology (150,158,219,231,232). Treatment with anticholinergic drugs can also block or terminate OP-induced seizure activity and protect against the development of neuropathology (150,158,219), as can treatment with glutamate receptor antagonists (158,233-237). All of these studies reported that neuropathology was only prevented if the drug treatment stopped seizure activity. Control of seizure activity appears to be essential in protecting against the development of neuropathology following nerve agent intoxication.

This hypothesis is further supported by studies examining the effects of differing durations of soman-induced seizure activity in rats before drug treatment was given to terminate seizure activity (158,235). Animals in which seizures were not terminated showed moderate to severe brain damage on histopathological examination. In contrast, all animals in which seizures were controlled within 10 min exhibited no evidence of brain damage. A low incidence of damage was observed when seizures were controlled after 20 min (10% of the animals were affected and the damage was minimal in severity and/or limited to only a few structures). A higher incidence of neuropathology was seen in animals that experienced 40 min of seizure (79% of the animals; more structures were involved, but damage ratings were minimal to mild). Both studies concluded that around 20 min of seizure activity (i.e. into the mixed cholinergic - non-cholinergic transition phase) was the minimum necessary for producing neurochemical changes sufficient to cause neuropathology, and that the damage process becomes progressively more established after that time (into the non-cholinergic phase). This suggests that the neuropathology caused by nerve agent-evoked seizures is most likely to be associated with glutamatergic excitotoxicity (116).

A later study in rats that developed seizures during soman poisoning reported that the severity of neural damage in cortical areas was related to the levels of EEG delta power recorded 24 hr after exposure; power during the acute seizure did not predict the severity of the lesion (238). Damage was observed consistently in all cortical areas, most severely in the piriform and perirhinal cortices, and in subcortical limbic areas (amygdala, amygdala-piriform transition zone, hippocampus, claustrum) and various thalamic nuclei. Damage was never seen in brainstem structures, cerebellum, spinal cord, or other motor output nuclei.

More recently, Chapman *et al.* studied alterations in neuro-inflammatory markers in the rat brain following intramuscular administration of sarin (239). Significant increases in the levels of the pro-inflammatory cytokine peptides interleukin (IL-1beta and IL-6), tumor necrosis factor-alpha (TNF-alpha) and prostaglandin E_2 (PGE_2) were measured, starting at 2 hours and peaking at 2–24 h following sarin administration. Midazolam was used to limit the duration of seizures to 5 or 30 min. Greater increases in IL-1beta and PGE_2 were found in the hippocampus following 30 min seizure duration.

Furthermore, a second increase in inflammatory markers was observed 30 days following sarin exposure, but only in rats treated following 30 min of seizure activity.

Marked histological damage to the brain was found following 30 min of seizure activity, consisting severe damage to the hippocampus, piriform cortex and some thalamic nuclei. Following 5 min of seizure activity, the damage was confined to minor enlargement of the lateral ventricles in a few animals. The authors concluded that the timing of the anticonvulsive treatment was crucial in modulating the neuro-inflammatory response and the consequent long term brain damage.

9. TREATMENT OF OP SEIZURES

OP-induced seizures differ from other forms of status epilepticus in their response to anticonvulsant drugs (72,240-242). Animal experiments show that many common anticonvulsants, including phenytoin, phenobarbital, lamotrigine, carbamazepine, or valproic acid are ineffective against nerve agent-induced seizures (86,243-245) (although phenytoin and carbamazepine were effective in a hippocampal slice model, see (111)). This may be due to the wide anatomic distribution of the cholinergic system within the brain, which could limit the effectiveness of drugs that depend on reducing the spread of seizure activity (72,240-242). Antimuscarinic drugs show anticonvulsant activity in animal models of nerve agent-induced seizures, but only during the cholinergic phase of seizures (~20 minutes), becoming ineffective as other neurotransmitter systems are recruited (87,246).

Although it has been suggested that seizures are uncommon in well oxygenated patients with OP pesticide poisoning (13,247), and that they seem to be more common with nerve agents such as soman and tabun (248), little evidence is available to support this assertion. There is, however, a strong possibility that many patients could have non-convulsive seizures, masked by the flaccid paralysis typical of OP anticholinesterase poisoning (66,71-74), and it has been recommended that patients with flaccid paralysis should undergo EEG monitoring because of this likelihood (66,72). Whatever the agent, the incidence of seizures is likely to depend strongly on the severity and route of poisoning (68-70,249).

Treatment for OP pesticides and nerve agents is essentially the same: an antimuscarinic (usually atropine), an oxime (e.g. pralidoxime, toxogonin or HI-6) to reactivate inhibited enzyme and a benzodiazepine to prevent or terminate seizure activity (250,251). For military medical countermeasures against nerve agent poisoning, these drugs are incorporated into autoinjection devices for immediate use by a casualty or a buddy (251). The benzodiazepine (usually diazepam) may be contained in a separate autoinjector or incorporated into the same device as the atropine and oxime. In the latter case, a water-soluble prodrug, avizafone, is used. This is a lysine conjugate of diazepam which is rapidly hydrolysed in the body to release the active drug (21,231,252-255). Given sufficient warning, the therapy can be supported by a pretreatment using a reversible carbamate AChE inhibitor (pyridostigmine), the aim being to shield a proportion of AChE from irreversible inhibition by nerve agents (251). In fact, however, in all recent reported exposures (which have involved civilian casualties), treatment - and usually diagnosis - of nerve agent poisoning has been delayed (32,256), resulting in a situation is similar to that faced with pesticide poisoning (257).

Benzodiazepines such as diazepam are the recommended first-line therapy for patients presenting with seizures and other CNS symptoms (29,39,47,248,258-260). In the hospital setting, diazepam is recommended at initial doses of 5 to 10 mg IV for seizure control (74,248). It has been recommended that diazepam should be administered following all severe exposures, even in the absence of convulsions, in case nonconvulsive seizures are present (74). Alternative benzodiazepines include lorazepam and midazolam (74). These drugs act by modulating $GABA_A$ receptor function to enhance synaptic inhibition in the brain (261-263). In treatment of OP pesticide poisoning, diazepam (10 mg given by slow IV injection, repeated as necessary in an adult, up to 30–40 mg per 24 hours) is also recommended for sedation of agitated patients (260).

Due to the possibility that diazepam and other benzodiazepines may exacerbate OP-induced respiratory depression (264), it has been recommended that a patient's airway and mental status should be monitored carefully with this therapy (71); however, animal studies have suggested that diazepam can actually inhibit OP-induced central respiratory depression (265) as well as reducing neural damage (266). Studies in humans are lacking (39): a review of clinical evidence found no randomized clinical trials comparing a benzodiazepine with a placebo or another anticonvulsant, and concluded that it would in fact be unethical to conduct a trial comparing a benzodiazepine with a placebo (267).

A study of a range of benzodiazepines against soman in guinea-pigs showed that midazolam stopped seizures most rapidly and at the lowest blood concentration (87). Nasally administered midazolam has been demonstrated to terminate seizure activity and prevent brain lesions and behavioral deficiencies in rats (268) and guinea-pigs (269) poisoned with sarin. In Israel, the military changed its fielded benzodiazepine to midazolam in 2003 (72,240-242,270). An earlier retrospective study of 19,112 hospital inpatients who received injectable midazolam or diazepam found no increased risk of death for midazolam compared to diazepam for endoscopic, conscious sedative and general anaesthetic procedures (271). In fact, the overall death rate was significantly lower for midazolam than for diazepam (0.76% versus 1.93%, p less than 0.01): this difference remained statistically significant after adjusting for age, sex, comorbidity diagnosis, concomitant drug use, type of medical procedure and hospital size and teaching capability.

10. PROSPECTS FOR IMPROVED TREATMENTS

As discussed earlier, the majority of studies to improve treatment of OP-induced seizures have been directed against nerve agents; relatively few studies have been carried out using pesticides. Due to the rarity of

human cases of nerve agent poisoning, however, the study of antidotes for these compounds relies heavily on animal efficacy and safety data, and on pharmacokinetic studies in humans and animals; clinical data are largely lacking (272). In the absence of pretreatment, or in case of delayed diagnosis and treatment of nerve agent poisoning – as is likely to occur in a terrorist incident (32,256) - the situation may be considered to be similar to that faced with pesticide poisoning and the patients require much the same treatment as those poisoned with pesticides (250). Much of the research on nerve agent-induced seizures is therefore relevant to OP pesticide poisoning; it has also been suggested that clinical trials in pesticide patients could benefit victims of both types of poisoning (257).

Although current treatments for nerve agent poisoning can reduce immediate lethality, they do not fully prevent the occurrence of seizure activity and convulsions, unless atropine is given early and at a high dose (273). The seizures can rapidly progress to status epilepticus, which is strongly associated with mortality and brain damage in experimental animals (91,274). Strategies for improving therapies should therefore aim to prevent or stop the seizure activity (anticonvulsants), or to prevent the processes which lead to subsequent neuronal damage (neuroprotectants).

Work towards improved treatments for nerve agent-induced seizures is largely driven by the three-phase pharmacological model discussed earlier. According to this model, anticholinergic drugs will be effective for immediate therapy, whereas drugs with antiglutamatergic effects will be more effective when therapy is delayed. Drugs enhancing GABAA neurotransmission (e.g. diazepam and other benzodiazepines) are effective during all phases of nerve agent intoxication (75,104,275).

The efficacy of diazepam and other benzodiazepines against organophosphate-induced seizures is well-established, both clinically (29) and in animal studies (103); however, the depressant actions of benzodiazepines on central respiratory drive (29,264) are a potential cause for concern (but see (265)). Furthermore, some animal studies have suggested that OP-induced seizures can recur following apparently successful initial treatment with benzodiazepines (276). An effective anticonvulsant without the respiratory depressant effects would be a significant therapeutic improvement.

Newer benzodiazepines, such as the partial agonist imidazenil, may offer one possible solution. In mice and rats poisoned with DFP, imidazenil had comparable anticonvulsant efficacy to diazepam, but achieved this at doses 5-10 times lower than those which caused sedative and muscle relaxant effects (277,278).

Atropine is currently the antimuscarinic drug of choice for treatment of OP poisoning, with a long history of successful use, but animal studies have suggested that alternative antimuscarinics which have additional antagonistic activity at NMDA receptors (279), such as aprophen, azaprophen, benactyzine, biperiden, procyclidine and trihexyphenidyl are more effective in blocking OP-induced seizure activity (91,171,252,280-282). All of these compounds are antispasmodic and some (biperiden, procyclidine, trihexyphenidyl) are clinically used as antiparkinsonian drugs (91). Biperiden and procyclidine have also been shown to prevent OP-induced seizures when given as pretreatments before DFP and soman (106,283-285). Egyptian troops in 1973 were reported to have been equipped with an antidote containing benactyzine (286), and benactyzine is a component of the Czech military pretreatment Panpal (287).

The antitussive drug caramiphen has been shown to protect against lethality, convulsions, electrographic seizure activity and brain damage in guinea-pigs poisoned with soman (236). Two other antitussives, dextromethorphan and carbetapentane, were less effective against seizures and did not protect against the lethal effects of soman. The superior efficacy of caramiphen was attributed to its stronger anticholinergic properties, but it has since been suggested that its effects on NMDA receptors may be an important component of its protective efficacy (288). Thus, it appears that drugs which combine anticholinergic and antiglutamatergic properties have considerable potential as postexposure therapies against OP poisoning (282).

The evidence for a key role of glutamatergic pathways in the later stages of OP-induced seizure activity, and in the subsequent neuropathology, has stimulated interest in antiglutamatergic drugs as potential therapies. Braitman and Sparenborg demonstrated the efficacy of the NMDA antagonist dizocilpine (MK-801) in attenuating or preventing soman-induced seizures in guinea-pigs (289). Subsequent studies showed that treatment with MK-801 5 minutes after the onset of seizures prevented, arrested or reduced soman-induced seizure activity, convulsions and neuronal necrosis in a dose-dependent manner (237,290). Another NMDA antagonist, TCP (N-[l-(2-thienyl) cyclohexyl]-pipendine, a thienyl analog of phencyclidine) was also found to terminate soman-induced seizures in rats (84,233). The latency to seizure termination was shorter when TCP was given 30, 60 or 90 min after seizure onset than when it was administered as a pretreatment or given 5 min after seizure onset, a property that was also apparent with MK-801 and was consistent with the three-phase model (105,237).

NMDA antagonists have also been tested against the OP pesticide dichlorvos. Experiments in mice showed that both MK-801 and another NMDA antagonist, CPP (3-((RS)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid), when administered alone, blocked convulsions induced by dichlorvos, but not by the carbamate physostigmine (291). When given together with atropine, both NMDA antagonists also increased protection against the lethal effects of dichlorvos, but not against physostigmine. The moderate affinity NMDA antagonist memantine has also been found to alleviate dichlorvos toxicity in rats (292), and to protect against soman-induced seizures when given either prophylactically or 15 min after soman (293).

None of these drugs is approved for clinical use; however, gacyclidine (GK11), another non-competitive antagonist of NMDA receptors derived from phencyclidine, has also been shown to prevent soman-induced seizures in cynomolgous monkys when administered IV at 30 or 45 min after soman (2 or 8 x LD50) and to prevent the neuropathology observed 3 or 5 weeks later (294-296). Neuroprotection by gacyclidine was later demonstrated by magnetic resonance imaging in rats poisoned with soman (78). Gacyclidine underwent clinical trials for use in the treatment of acute spinal cord injury, but development was stopped because it failed to demonstrate long-term benefit on neurological scores; however, there have been reports of the drug offering beneficial long-term effects in patients with traumatic brain injury (297).

There is also some evidence for the involvement of non-NMDA glutamate receptors in nerve agent-induced seizures (298). The AMPA receptor antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulpharnoylbenzoquinoxaline) was found to block the neurotoxic effects of soman exposure in rats, although the animals experienced mild to moderate convulsions for around 90 min (234). When NBQX was given at the same time as soman it blocked seizure onset, and when given 5 min after seizure onset it greatly reduced the intensity of epileptic activity (83). Immediate treatment with atropine, followed by administration of NBQX and TCP 5 or 50 min after the onset of soman-induced seizures, totally arrested seizure activity (82); if atropine treatment was delayed and given together with the other two drugs, then some epileptiform activity remained.

These results suggest that AMPA receptor activation may be critical for the early propagation or maintenance of OP-induced seizure activity; however, a review article by McDonough and Shih (91) reported unpublished data suggesting that NBQX and another AMPA antagonist, GYKI 52466, had minimal effects on soman induced seizures in rats.

A later study of the relation between soman-induced neuropathology and subsequent spatial memory impairments in rats found that, without treatment, neuropathology and learning impairment were observed only in rats which experienced convulsions. When treatment consisting of atropine sulfate, and/or TCP and/or NBQX was administered to intoxicated animals, it was found that only treatment with all three drugs together was able to improve the different parameters of spatial learning, even though the doses given were too low to prevent convulsions of the animals (299). Although there is little information on the role of the metabotropic glutamate receptors in OP induced seizures, one study has reported the involvement of these receptors in the inositol phosphate response after soman intoxication (300). Clearly, further work is needed to understand the roles of the different glutamate receptor subtypes in OP-induced seizures and neurotoxicity, and the therapeutic value of non-NMDA receptor blockade merits further investigation.

Another drug which may afford protection through a glutamatergic mechanism is modafinil, which is licensed as a stimulant. In mice and rats, pretreatment with modafinil protected against soman-induced neuronal damage in the CA1 and CA3 subfields of the hippocampus (301). It was suggested that possible actions of modafinil on the release and uptake of glutamate and/or on NMDA receptors could contribute to this protection.

Calcium channel blockers are among the additional therapies classically used during the treatment of generalized seizures in humans (25). Flunarizine, nifedipine and verapamil have been tested against convulsions induced by soman in rats and mice (75,104,275,302). When used alone, these did not produce any significant protection against convulsions, neuropathology and lethality induced by soman; however, flunarizine showed striking synergistic anticonvulsant effects when given together with diazepam, leading to total disappearance of severe convulsions and substantial reduction of moderate ones (275,302).

It has been suggested that nitric oxide (NO) may be a proconvulsant or a convulsion-promoting factor in poisoning by anticholinesterases, and that a reduction of NO levels may provide more effective protection against seizures (303). There is evidence that delayed apoptotic injury of brain induced by DFP poisoning in rats might be mediated in part through nitric oxide production (304). Treatment of rats with seizure-inducing doses of DFP or the carbamate insecticide carbofuran induced increases of NO (assessed by measuring citrulline as the determinant of NO) in pyriform cortex, amygdala and hippocampus, and there was a concomitant decrease in high-energy phosphates (ATP and phosphocreatine) (305). Pretreatment with an antioxidant, the spin trapping agent N-tert-butyl-alpha-phenylnitrone, prevented DFP- or carbofuran-induced seizures, while the antioxidant vitamin E had no anticonvulsant effect. Both antioxidants, however, significantly prevented the increase of citrulline and the depletion of high-energy phosphates.

Partial agonists of adenosine A1 receptors have been found to possess therapeutic actions against OP poisoning, presumably by reducing the release of ACh (306-312). The A1 agonist cyclopentyladenosine (CPA), and the partial agonists 2-deoxy-CPA and 8-butylamino-CPA, blocked sarin-induced epileptiform activity *in vitro* in the guinea-pig hippocampal brain slice (110). An allosteric enhancer of adenosine A1 receptors, PD 81,723, has been shown to protect against seizures and neuropathology in rats poisoned with DFP (313). In rats poisoned with metamidophos, a single dose of the adenosine A1 receptor agonist, phenylisopropyl adenosine, given immediately after poisoning, prolonged the time to onset of salivation and convulsion and the time to death, but was unable to protect against lethality (314). Adenosine agonists can have significant cardiovascular side effects: an alternative approach would be to raise the intrinsic levels of adenosine by blocking its uptake. A blocker of adenosine uptake, propentofylline, has been shown to inhibit potassium-evoked release of ACh and glutamate in slices of hippocampus *in vitro;* however, neither tonic-clonic convulsions induced by soman, nor the subsequent

hippocampal damage, were reduced in propentofylline-treated mice (81).

In summary, improved antimuscarinics, benzodiazepines and drugs which act on the glutamatergic system appear to offer the best opportunities for improving the treatment of OP-induced seizures. Among the glutamatergic drugs, antagonists of NMDA receptors have shown the clearest advantages; the roles of other glutamate receptors (AMPA/kainate and metabotropic) in OP poisoning are poorly understood. Adenosine receptor agonists have also shown promise against OP-induced seizures. The therapeutic value of other targets, such as calcium channels and nitric oxide pathways, is less clear.

Myhrer has suggested that future research should aim to determine which subtypes of these receptors are most important in OP-induced seizures and in which areas of the brain these should be targeted (116). According to this view, prophylaxis and early treatments should be directed towards anatomical substrates involved in triggering seizures after nerve agent exposure; later treatments should be aimed primarily at structures involved in epileptiform activity and propagation. It could also be added that delayed treatments should be targeted against the processes which lead to neuronal damage. Improved insights into the neuropharmacological mechanisms of OP-induced seizures should also provide a better foundation for the approval of novel anticonvulsants and neuroprotectants.

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