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# Mechanism-based novel antidotes for organophosphate neurotoxicity

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**Abstract**

This article describes current pursuits for developing novel antidotes for organophosphate (OP) intoxication. Recent mechanistic studies of benzodiazepine-resistant seizures have key consequences for victims of OP pesticide and nerve agent attacks. We uncovered why current therapies are not able to stop the OP-induced seizures, status epilepticus (SE), and brain cell death, as well as what type of drug may be more effective. OP exposure downregulates critical inhibitory GABA-A receptors, kills neurons, and causes massive neuroinflammation that induce more neuronal death, which raises the problem of too few benzodiazepine receptors. The loss of inhibitory interneurons creates a self-sustaining seizure circuit and refractory status epilepticus. Thus, there is an urgent need for new, mechanism-based antidotes for OP intoxication. We have discovered neurosteroids as next-generation anticonvulsants superior to midazolam for the treatment of OP poisoning. In 2008, our group was among the first to identify the potential of neurosteroids for SE. Neurosteroids that activate both extrasynaptic and synaptic GABA-A receptors have the potential to stop SE more effectively and safely than benzodiazepines. In addition, neurosteroids confer robust neuroprotection by reducing neuronal injury and neuroinflammation. The synthetic neurosteroid ganaxolone is being considered for advanced development as a future anticonvulsant for nerve agents. Experimental studies showed striking efficacy of ganaxolone and its analogs in OP exposure models. They are also effective in attenuating long-term neuropsychiatric deficits caused by OP exposure. Overall, neurosteroids represent rational anticonvulsants for OP intoxication, even when given late after exposure.

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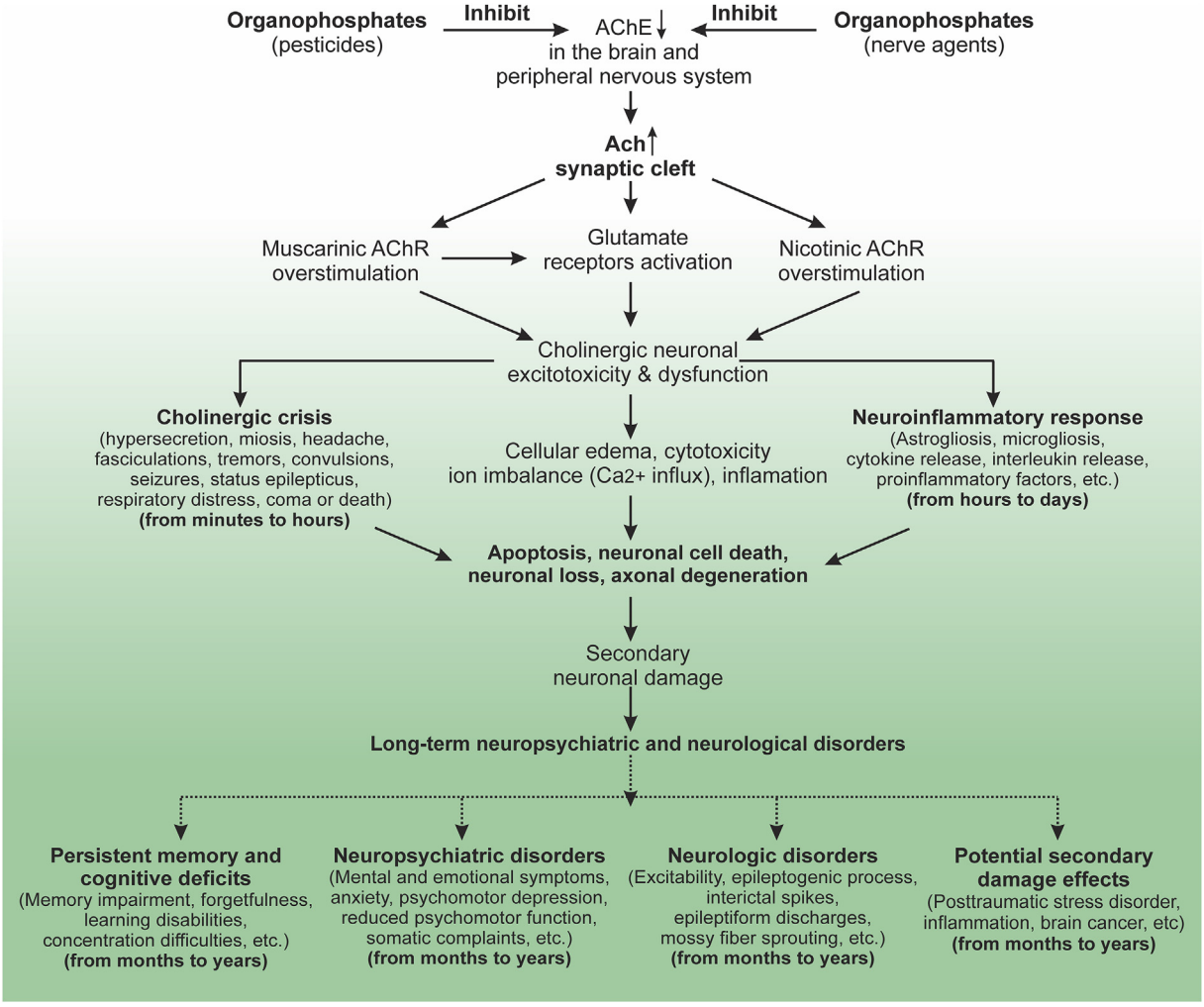
## Introduction

Organophosphate (OP) pesticides and nerve agents produce severe, fast-acting effects on both the body and brain. Nerve agents are among the most toxic of known chemical agents; nerve agents such as tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), and VX are deployed as chemical warfare weapons in combat or as bioterrorism agents against civilians. Chemical attack is becoming a real threat worldwide. In 2013, about 1400 civilians died from the nerve gas sarin attacks in Syria [1]. Over a decade ago, Tokyo civilians were exposed to sarin gas on the subway [2]. These compounds are known to be hazardous in both the liquid and gas form, killing individuals within minutes of exposure. Many countries have the technology to weaponize these deadly agents. Compound known as Novichok is considered among the deadliest chemical weapons ever made and is difficult to identify owing to its poorly defined composition. Novichok, which means 'newcomer' in Russian, is a more dangerous and sophisticated nerve agent than sarin, which has been used in chemical weapon attacks in Syria in 2013, or VX, which was used to assassinate Kim Jong Nam at an airport in Malaysia in 2017. The Novichok exposure incident in Salisbury in England was widely reported in the media. Although the substance was undetectable by standard detection equipment, the patient's response to certain antidotes confirmed that it resembles OPs. In addition, thousands of OP pesticide poisonings occur annually, leading to suicides or agriculture accidents worldwide [3]. OP pesticides such as monocrotophos, parathion, chlorpyrifos, paraoxon, and diisopropylfluorophosphate (DFP) are considered credible threat agents. This article describes a concise overview of current pursuits of mechanistic toxicology and mechanism-based therapies for effective treatment of OP neurotoxicity.

OP pesticides and nerve agents produce lethal neurotoxicity via common mechanisms (Fig. 1), primarily causing neurotoxicity by irreversibly (permanently) inhibiting acetylcholinesterase (AChE); this, in turn, leads to an excessive accumulation of acetylcholine (ACh) in the synaptic cleft in the peripheral and

**Figure 1**




**Schematic illustration of potential cholinergic and noncholinergic mechanisms of organophosphate (OP) neurotoxicity.** OP pesticides and nerve agents produce acute and long-term neurotoxicity. The primary mechanism of action of both classes is irreversible inhibition of acetylcholinesterase (AChE) resulting in accumulation of toxic levels of acetylcholine (ACh) at the synaptic junctions which induces muscarinic ACh receptors (AChR) and nicotinic receptor stimulation and host of other pathways. Muscle fasciculation and cardiac and respiratory distress occur very rapidly after exposure and can ultimately lead to death if left untreated or treated too late. The autonomic nervous system is heavily affected by OPs because the transmission from preganglionic to postganglionic neurons is dependent on ACh. The levels of AChE remain low for several days after OP exposure, but the range of autonomic changes is also dependent on the exposure route. If exposure to OPs is via inhalation, the vapor comes into contact with the eyes and respiratory tract, causing miosis (pupil constriction) and difficulty in breathing, respectively. Furthermore, OPs can rapidly cross the blood–brain barrier (BBB) and induce severe seizures, initially through overstimulation of cholinergic pathways. Seizures can reversibly open the BBB with permeability and trigger a massive inflammation response in the brain. As status epilepticus (SE) progresses, glutamatergic networks are recruited, and several other neurochemical changes may occur. Once this hyperexcitability is initiated, such seizures are difficult to reverse. The secondary events of SE and nonseizure activity, such as neuronal necrosis, cell death, and axonal degeneration, can potentially result in severe brain damage. Thus, survivors of OP exposure suffer from long-term neurological problems including cognitive deficits, anxiety, depression, and epileptic seizures.

central nervous system (CNS). These compounds interfere with brain chemicals that turn neurons and muscles 'on' and 'off.' They inhibit AChE enzyme in plasma, red blood cells, tissues, and the brain [4–6]. In a normal, healthy person, ACh is released at the junction between neurons and muscles, acting as an 'on' switch to allow the brain to contract the muscles. Thus, every time someone wants to walk — or breathe — ACh is released, causing certain muscles to contract and facilitating movement. When the body needs to stop contracting its muscles, AChE acts as the 'off' switch. AChE essentially cleaves the ACh into choline and acetate so that the muscles stop contracting. When an individual is exposed to OP, it blocks AChE. As a result, ACh builds up in massive quantities in the brain and causes widespread nerve excitation and muscle

contraction. Without an 'off' switch, the brain is overly excited, and the muscles in the body begin to continuously contract, lacking the ability to relax (cholinergic crisis). This results in muscle spasms, convulsions, continuous seizures, respiratory arrest, and eventually death [7,8]. If a person is able to survive OP attack, he/she will likely have serious brain damage due to severe secondary neuronal damage (Fig. 1). Besides brain damage, it also affects the peripheral nervous and other systems.

Because OP chemicals are cholinesterase inhibitors, measuring levels of AChE in the blood provides a reliable biomarker of their exposure. The extent of AChE activity also reflects the exposure severity and timeline. OP pesticides and nerve agents present major differences in both duration of neurotoxicity and response to therapy. The initial effects of exposure to these compounds depend on the dose and route of exposure [7,8]. Inhalation (rapid absorption), oral (medium absorption), and dermal (slow absorption) routes are the most common routes of OP exposure. Miosis is an indicative sign of exposure to OP agents. However, the major effect of OPs is on skeletal muscles, producing muscular fasciculation and twitching. On the contrary, exposure to nerve agents (vapor) is initially indicated by rhinorrhea. Such compounds cause bronchoconstriction and increased gland secretions in the airways, oftentimes producing tightness in the chest. Cessation of respiration occurs later after the onset of toxic signs. For both classes of compounds, the severe CNS signs of exposure are loss of consciousness, seizure activity, and apnea. Status epilepticus (SE) occurs within minutes after exposure and may persist for up to 30 min or longer. SE is a life-threatening emergency in which an individual, without regaining consciousness, experiences a prolonged, continuous state of convulsion. If not controlled immediately, this medical emergency could cause tragic consequences, resulting in widespread brain damage or death. Thus, there is an urgent need for a better understanding of the molecular neurotoxicity to design effective countermeasures for OP attacks.

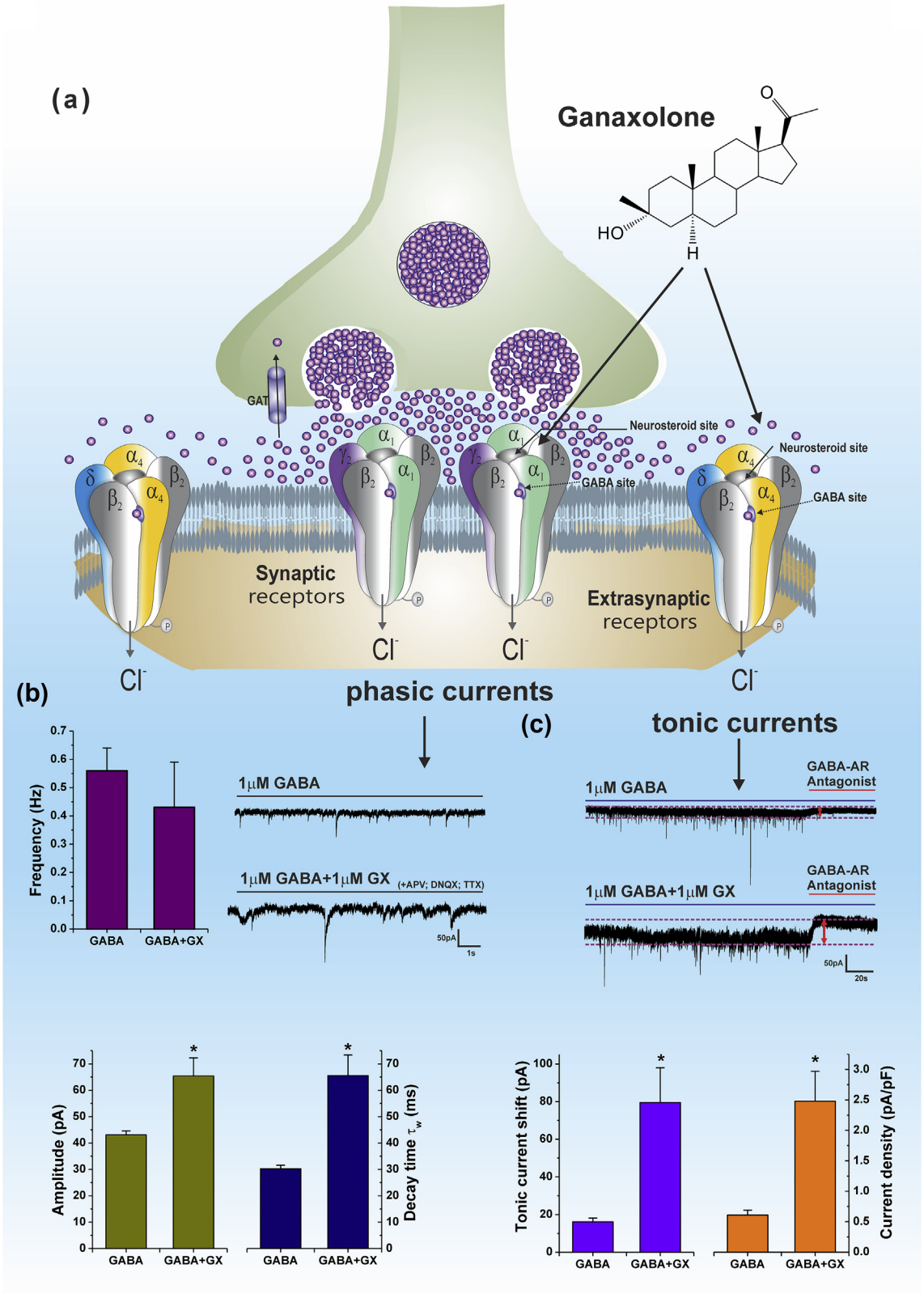
## The current situation

Managing an individual who is intoxicated with OP requires decontamination, ventilation, and administration of antidotes. Decontamination includes the application of reactive skin decontamination lotion, soap and water, and 5% hypochlorate solution. Three drugs — atropine sulfate, pralidoxime chloride (2-PAM), and diazepam — are used to treat OP intoxication [9,10]. This regimen is distributed as CHEMPACKs with autoinjectors for use in case of a chemical attack or accident. Pyridostigmine bromide is used as a protective pretreatment for military personnel at risk of a nerve agent exposure. After exposure, atropine, a muscarinic receptor antagonist, is extremely effective at blocking the effects of excess ACh at peripheral sites. Atropine produces lifesaving effects by decreasing hypersecretions and relieving bronchoconstriction, allowing for easier breathing. However, the nicotinic effects of OPs, such as spasms and fasciculations, will not be improved by atropine. Atropine has a limited effect on the CNS owing to its poor entry into the brain. 2-PAM is an AChE reactivator that can break the agent—enzyme bond to release the free AChE enzyme. Like atropine, 2-PAM has poor penetration into the brain. Hence, despite atropine and 2-PAM therapy, excess ACh remains uncontrolled in the brain, resulting in cholinergic crisis including seizures and SE (Fig. 1).

Currently, there are two drugs used to control OP neurotoxicity. If given promptly after exposure, diazepam and midazolam are benzodiazepine anticonvulsants that can work to prevent OP-induced seizures and brain damage. Both drugs are effective anticonvulsants when given within 30 min of OP exposure but do not have much effect beyond an hour or two after exposure. In the context of chemical warfare and unexpected civilian bioterrorism, this is not a realistic timeline. Thus, the neurotoxic signs of OP exposure, including convulsive seizures and SE, cause profound permanent brain damage that will result in neuronal damage or death (Fig. 1). Brain damage can occur not only by seizure-related excitotoxicity but also via mechanisms independent of seizures such as the activation of microglia, astrocytes, and cellular inflammation [11,12]. The effects of OP intoxication are long-lasting and pose a greater risk of long-term neurologic and cognitive deficits [4,13,14].

Although soldiers often carry antidote kits for personal use in case of a nerve agent attack, civilians have less convenient access to anticonvulsant medications; for many civilians, a trip to the hospital is required to receive the needed medication. The process of getting to the hospital and administering the drugs would most likely take at least 40 min in the majority of scenarios [7,21]. This represents the critical time period, meaning any anticonvulsant antidote for these OP chemical seizures must work after 40 min after exposure. This is the primary goal of the antidote therapy for OP intoxication. However, this timeline is often not practical in many emergencies. Hence, OP-induced neurotoxicity can lead to long-term brain injury and devastating neuropsychiatric dysfunction in survivors of chemical attacks. Five years after the attacks with the nerve agent sarin in Matsumoto and Tokyo, individuals exposed to sarin reported devastating neurological and psychiatric disorders [15—18]. Similarly, thousands of survivors in Syria after sarin exposure may live with the after-effects for the rest of their lives.

**Figure 2**


(a)
Ganaxolone
HO
H
O
GAT
Neurosteroid site
GABA site
Synaptic
receptors
Extrasynaptic
receptors
Cl-
(b)
phasic currents
Frequency (Hz)
GABA
GABA+GX
1μM GABA
1μM GABA+1μM GX (+APV; DNQX; TTX)
50pA
1s
(c)
tonic currents
GABA-AR
Antagonist
1μM GABA
1μM GABA+1μM GX
50pA
20s
Amplitude (pA)
Decay time τw (ms)
Tonic current shift (pA)
Current density (pA/pF)

## Mechanisms of benzodiazepine-refractory seizures

Benzodiazepines are the primary drugs for treatment of SE, but there is strong evidence of refractoriness to diazepam and midazolam [19–21]. We have investigated the mechanistic basis of benzodiazepine-refractory seizures after OP intoxication [22,23]. After DFP exposure in animal models, we examined two major aspects of diazepam and midazolam treatment, which are the most widely used anticonvulsants in OP intoxication. First, we examined how efficiently each drug suppressed seizures and SE. Second, we looked at how efficiently each drug protected against brain damage. The results of both studies showed that diazepam and midazolam were very effective at controlling seizures, neurodegeneration, and neuroinflammation when given 10 min after exposure. However, both medications were completely ineffective when administered at 60 min or 120 min after exposure. Delayed therapy (40 min), a simulation of the practical therapeutic window for first responders or hospital admission, was associated with reduced seizure protection and neuroprotection [22,23]. These results strongly reaffirm the notion that OP-induced seizures and brain damage are progressively resistant to delayed treatment with diazepam or midazolam. This condition is referred to as the benzodiazepine-refractory SE caused by OP exposure [12,23]. Because benzodiazepines are positive allosteric agonists of synaptic GABA-A receptors [24], multiple mechanisms may contribute to their reduced efficacy, including pharmacodynamic (loss or internalization of receptors) rather than a pharmacokinetic mechanism [21,25–27]. It is concluded that benzodiazepines do not control seizures at later time points (after 40 min), but it is not because enough quantities are not reached in the brain. Instead, it is more likely due to the loss of target receptors, the loss of neurons, and damage-induced inflammation. It is likely that OP exposure somehow affects the receptor targets, causing diazepam and midazolam to be unable to find their receptors and diminish the seizure circuits.

Benzodiazepine receptors disappear in more than 50 percent of neurons within 10–20 min of OP-induced seizure onset [26,26]. When benzodiazepines were administered at 40 min after OP exposure, this means that 50% of the benzodiazepine receptors had already vanished or are not functional at the neuronal membrane targets. The administered benzodiazepines bound to the remaining 50% of receptors, but the maximum effect they could produce depended on the number of receptors available, regardless of how high a dose was given. This is why repeated doses of diazepam are needed for partial control of seizures, resulting in sedation, respiratory depression, and tolerance in victims.

Moreover, OP poisoning also kills neurons, which worsens the problem of too few benzodiazepine receptors. Massive brain cell death further exacerbates the problem of a lack of receptors, as demonstrated by massive neurodegeneration of principal neurons and interneurons [22,23]. The cell must be alive for the drug to bind to its target receptors. The benzodiazepine receptors are on the main neurons. However, so many of these neurons are dead such that it further reduces the number of available receptors. The loss of inhibitory interneurons, which apply strong breaks on excessive neuronal excitation and synchronization that manifest into seizures, creates a self-sustaining seizure circuit. Finally, OP poisoning causes persistent inflammation in the brain, as evident in astrogliosis and microgliosis [22,23], which in turn causes more cell death and a loss of more receptors. This forms the mechanistic reason for why benzodiazepines are failing when they are administered later in a field setting.

These studies have provided deeper insights for developing next-generation anticonvulsants that are more effective than benzodiazepines. Benzodiazepine receptors are exclusively present in postsynaptic junctions. However, a new type of GABA-A receptors is present at perisynaptic and extrasynaptic sites (Fig. 2) [33]. Although OP molecules can destroy the 'synaptic' forms comprising benzodiazepine receptors, they do not affect the 'extrasynaptic' forms of GABA-A receptors. These GABA-A receptors should be targets for new drugs because they will not disappear in 10–20 min after OP exposure. However, a reverberating circuit still exists because the neuronal loss leads to neuroinflammation and loss of receptors. By targeting extrasynaptic

**Mechanisms of ganaxolone (GX) at extrasynaptic and synaptic GABA-A receptors in the brain. (a)** As other neurosteroids, ganaxolone enhances the function of extrasynaptic and synaptic GABA-A receptors by binding to 'neurosteroid-binding' sites, which are distinct from the sites for GABA, benzodiazepines, and barbiturates. There are two subtypes of GABA-A receptors: (i) synaptic receptors composed of 2α2βγ subunits, which mediate phasic inhibition in response to action potential-dependent vesicular release of high levels of GABA, and (ii) extrasynaptic receptors composed of 2α2βδ subunits, primarily contributing to tonic inhibition when exposed to low, ambient levels of GABA. GX can bind to both subtypes and enhance the phasic and tonic currents. (**b** and **c**) GX potentiation of phasic current and tonic current in hippocampal granule cells. **(b)** Sample traces, frequency, amplitude, and decay time of miniature postsynaptic currents (mIPSCs) with or without GX. **(c)** Sample traces, current shift, and current density of tonic currents before and after GX. Overall, GX and other neurosteroids can significantly enhance the phasic inhibition and tonic inhibition and thereby promote maximal inhibition. This contributes to their robust anticonvulsant actions, including controlling organophosphate-induced seizures and brain damage. In contrast, benzodiazepines (e.g. midazolam) bind specifically to γ2-containing synaptic receptors and augment phasic inhibition. Benzodiazepines do not bind to the extrasynaptic δ-containing extrasynaptic receptor and do not affect tonic inhibition in the brain. GAT: GABA transporter (GAT); TTX: tetrodotoxin, voltage-gated sodium channel blocker; APV: D-2-amino-5-phosphonopentanoic acid, NMDA receptor blocker; DNQX: 6,7-dinitroquinoxaline-2,3-dione, AMPA receptor blocker.

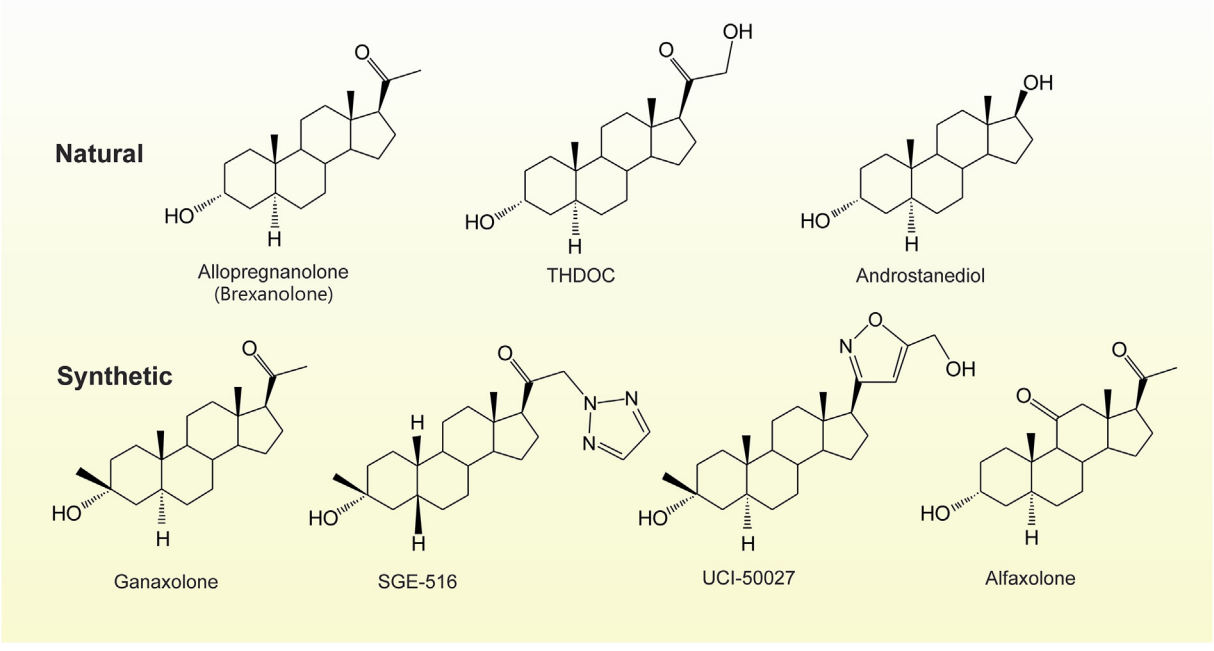
receptors, it is more likely to control seizures and stop neuronal loss, effectively breaking the circuit.

## Neurosteroids as novel anticonvulsant antidotes for OP intoxication

Neurosteroids with a unique mechanism of action have the potential to stop seizures more effectively and safely than benzodiazepines. In addition, neurosteroids may confer neuroprotection as well. This is achieved by shunting the excessive excitability and its exacerbating impact on neuronal injury and neuroinflammation, which are typically associated with OP poisoning. These products are being developed for approval by the U.S. Food and Drug Administration (FDA) and could be revolutionary for both military members and civilian victims of nerve agent attacks [28–30]. The term 'neurosteroid' refers to steroids synthesized within the brain that rapidly alter neuronal excitability through interactions with nongenomic membrane receptors and lack conventional hormonal effects. A variety of neurosteroids are present in the brain, including allopregnanolone (brexanolone), pregnanolone, androstanediol, and allotetrahydrodeoxycorticosterone [31]. They play critical roles in modulating neuronal excitability and neuroplasticity [31].

Neurosteroids act as positive allosteric modulators and direct activators of GABA-A receptors (Fig. 2). They bind to 'neurosteroid binding sites' on the receptor channel, which are distinct from the benzodiazepine and GABA sites [32]. Neurosteroids act on all GABA-A receptor isoforms in the brain. They activate receptor channels primarily via allosteric potentiation of GABA at nanomolar concentrations and through direct activation of the channel at micromolar concentrations [33]. There are distinct categories of GABA-A receptors that are stratified into synaptic and extrasynaptic receptors (Fig. 2). They exhibit different characteristics in their affinity and efficacy to GABA, desensitization rate, and drug sensitivity [32,33]. Synaptic (γ-containing) receptors produce rapid and transient phasic currents in response to the presynaptic release of GABA (at millimolar). However, extrasynaptic (δ-containing) receptors generate persistent, nondesensitizing tonic currents in response to ambient GABA release (at micromolar). Tonic currents contribute to the overall basal tone and shunting inhibition via continuous channel conductance, thereby regulating network excitability and seizure susceptibility. Neurosteroids have a high potency for synaptic receptors that mediate phasic inhibition, whereas they exhibit greater efficacy or sensitivity for extrasynaptic receptors that promote tonic inhibition [32,33]. The net output is maximal inhibitory tone that can effectively shunt hyperexcitability and focal discharges in the brain. Hence, neurosteroids have broad-spectrum anticonvulsant activity and promising clinical potential for treating seizure disorders [34].

**Figure 3**




**Chemical structures of neurosteroids tested in organophosphate (OP) intoxication models.** Natural neurosteroids such as allopregnanolone, also called brexanolone (3α-hydroxy-5α-pregnan- 20-one), tetrahydrodeoxycorticosterone (THDOC; 5α-pregnan-3α,21-diol-20-one), and androstanediol (AD; 5α-androstan-3α,17β-diol) and synthetic neurosteroids such as ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan- 20-one), SGE-516 (3α-hydroxy-3β-methyl-21-(1′,2′,4′-triazol-1′-yl)-19-nor-5β-pregnan-20-one), UCI-50027 (3-[3α-hydroxy-3β-methyl-5α-androstan-17β-yl]-5-(hydroxymethyl)-isoxazole), and alfaxolone (3α-hydroxy-5α-pregnan-11,20-dione) are screened in specific OP exposure models.

Natural neurosteroids such as allopregnanolone (brexanolone) have limited therapeutic utility because after oral administration, these are inactive owing to first-pass metabolism; they exhibit short half-lives and hormonal side effects via metabolism into C3-keto steroids, which can bind to steroid hormone receptors such as the progesterone receptor [31]. Synthetic neurosteroids are designed to overcome these limitations [35–37]. For example, the 3β-methyl substitution in ganaxolone minimizes the metabolic conversion to hormonally active C3-keto forms, rendering it orally active, and providing a longer half-life (4 to 6-fold) than endogenous neurosteroids. Several synthetic compounds are prepared using structure–activity designs (Fig. 3). In addition, the molecular modeling of neurosteroid potentiation of GABA-A receptors has created new opportunities for creating novel neurosteroid analogs for general treatment of seizure conditions, including OP-induced refractory SE [34].

Refractory SE is a hallmark of OP intoxication. Mechanistically, a rapid decline in synaptic GABA-A receptors and consequent reduction in phasic inhibition occurs in the hippocampus during SE [25–27]. These changes may account for the resistance to benzodiazepines in SE [19–23]. To overcome this, we have proposed neurosteroids as novel anticonvulsants against OP-induced SE [28]. Our 'neurosteroid therapy' is based on the premise that extrasynaptic δGABA-A receptors that generate tonic inhibition do not internalize during SE. Therefore, neurosteroids, which activate both extrasynaptic and synaptic receptors, are more effective treatments for SE. In experimental paradigms, SE causes a reduction in synaptic phasic inhibition (owing to internalization or downregulation of synaptic receptors) but not in extrasynaptic tonic inhibition [25]. Because neurosteroids can enhance extrasynaptic inhibition to a greater extent than benzodiazepines [35], they offer a rational treatment strategy for OP exposure as they can maximally enhance inhibition to counteract the sustained seizure activity. We were among the first to identify the anticonvulsant potential of neurosteroids for SE therapy [38–40]. First, we demonstrated the protective effects of neurosteroid in the pilocarpine SE model following a pretreatment protocol in 2008 [38]. Second, we identified the promising efficacy of neurosteroids in treating SE in epilepsy rats in 2008 [39]. Third, we confirmed the protective potential of neurosteroids in a rat pilocarpine-model of refractory SE in 2011 [40]. Pilocarpine is considered a chemical surrogate of OP compounds that share cholinergic neurotoxicity. In these studies, post-exposure treatment with neurosteroids significantly aborted SE and improved survival of animals. Consequently, allopregnanolone, allotetrahydrodeoxycorticosterone, and ganaxolone have been tested in rodent models of cholinergic SE induced by pilocarpine [41–43], the OP pesticide DFP [30], and the nerve agent soman [30,44]. Later, our findings are confirmed by others [41,43,44]. Recently, we further characterized the efficacy of natural neurosteroids (see Fig. 3), synthetic analogs (alfaxolone and ganaxolone), and super analogs (ganaxolone analogs) in OP exposure models including DFP, soman, and VX [30]. Neurosteroids were effective when given 40 min or later after OP exposure in rat models; they produced rapid and effective control of SE and neuronal damage [30]. Overall, neurosteroids are more effective as anticonvulsants and neuroprotectants than midazolam for OP intoxication at doses that are lower than the doses that are associated with adverse effects as evident from multiple preclinical toxicokinetic studies [30].

## Ganaxolone and its analogs as future anticonvulsants for refractory SE

Ganaxolone, the 3β-methylated synthetic analog of allopregnanolone, has been tested extensively in OP exposure models. Recently, we have elucidated the mechanism of action of ganaxolone in native neurons in the brain [36]. Ganaxolone potentiates GABA currents by allosteric potentiation and direct activation of synaptic receptors and extrasynaptic GABA-A receptors [36] (Fig. 2a). It potentiates the GABA-gated phasic currents by significantly increasing the amplitude and prolongation of miniature inhibitory post-synaptic current (mIPSC) decay without altering the frequency of mIPSC in the hippocampus (Fig. 2b) and other brain regions. The unique mechanism of ganaxolone on GABA-gated tonic currents is characterized in a hippocampal slice preparation, in which synapses and dendritic connections remain functional [36]. It strikingly enhances the tonic current for the entire duration of its application with little rundown (Fig. 2c), as evident from the persistent tonic current measured as the shift in mean conductance before and after application of the GABA-A receptor antagonist gabazine [36,45].

Ganaxolone has a unique advantage over midazolam in that tolerance does not appear to occur with extended use [46,48]. In preclinical models, ganaxolone causes mild side effects such as sedation and hypoactivity, which are comparable with that of the benzodiazepine midazolam [31–36]. Among the synthetic neurosteroids, ganaxolone has been well studied; the mechanism of action, anticonvulsant profile, pharmacokinetics, and safety profile have been well documented [46–50]. Hence, ganaxolone makes an excellent practical option for development as a medical countermeasure for OP intoxication.

We have developed an intramuscular (IM) formulation of ganaxolone [30]. The product has demonstrated the desirable features of efficient absorption and rapid distribution to the brain. Levels of ganaxolone in plasma

**Table 1** A summary of experimental efficacy studies of ganaxolone in OP intoxication models.

| Study type and protocol | Species | Overall outcomes | |
|---|---|---|---|
| | | DFP model | Soman model |
| (a) Anticonvulsant efficacy: drug given at 40, 60, or 120 min after OP exposure | Rats | • Stopped electrographic SE<br>• Stopped behavioral SE<br>• Significantly decreased seizure activity<br>• Significantly decreased duration of SE<br>• 100% survival rate | • Stopped electrographic SE<br>• Stopped behavioral SE<br>• Significantly decreased seizure activity<br>• Significantly decreased duration of SE<br>• 100% survival rate |
| (b) Acute neuroprotective efficacy: drug given at 40, 60, or 120 min after OP exposure | Rats | • Significantly reduced neuronal cell injury<br>• Significantly prevented cell death of principal neurons<br>• Significantly decreased cell death of interneurons | • Significantly reduced neuronal cell injury<br>• Significantly prevented cell death of principal neurons<br>• Significantly decreased cell death of interneurons |
| (c) Chronic neuroprotective efficacy: animals tested 3 months after DFP exposure, drug given at 40, 60, or 120 min after OP exposure | Rats | • Significant reduction in neurodegeneration of principal cells and interneurons | • Significant reduction in neurodegeneration of principal cells and interneurons. |
| (d) Chronic neuroprotective efficacy: animals tested 3 months after DFP exposure, drug given at 40 or 60 min after OP exposure | | • Significant decrease in frequency and severity of epileptic seizure development<br>• Attenuation or reduction in behavioral memory deficits | • Significant reduction in incidence of epilepsy development<br>• Attenuation or reduction in behavioral memory deficits |
| (e) Combination anticonvulsant efficacy: ± midazolam, drug given at 40 min after OP exposure | Rats | • Combination regimen was a more effective anticonvulsant than midazolam alone.<br>• Neuroprotection was much better than midazolam alone. | • Combination regimen was more effective anticonvulsant than midazolam alone.<br>• Neuroprotection was moderately better than midazolam alone. |
| (f) Combination neuroprotectant efficacy: ± midazolam, drug given at 40 min after OP exposure | Rats | • Significantly reduced neuronal cell injury<br>• Combination regimen was a more effective neuroprotectant than midazolam alone. | • Significantly reduced neuronal cell injury<br>• Combination regimen was a more effective neuroprotectant than midazolam alone. |

DFP, diisopropylfluorophosphate; OP, organophosphate; SE, status epilepticus.

and the brain increased proportionately with increasing dosage. The bioavailability of ganaxolone is >95% after IM administration with a $C_{max}$ of 0.167 h and $t_{1/2}$ of 2.4 h. We tested the efficacy of IM ganaxolone in rodent models of nerve agent exposure using a delayed post-exposure protocol in rats [30]. Ganaxolone produced a dose-dependent protection against DFP- and soman-induced seizures. In addition, it produced protection against SE even when administered 40–120 min after agent exposure (Table 1). It displayed strong neuroprotectant activity even with delayed treatment (40 min) after soman exposure. Ganaxolone therapy significantly prevented cell deaths of principal neurons and markedly decreased the loss of inhibitory interneurons. In the same setting, midazolam alone failed to protect against soman-induced SE and neuronal damage. Multiple combination regimens of ganaxolone and midazolam have been tested in the DFP and soman models. A combination regimen of ganaxolone+midazolam produced a superior efficacy for controlling SE than midazolam alone. This combination produced a greater neuroprotectant efficacy, indicating a strong synergistic protective potential of the combination regimen. These findings are highly consistent with the premise that neurosteroids produce synergistic effects with benzodiazepines on seizure protection as evident from our recent pharmacological studies [51,52].

Many analogs based on the ganaxolone structure are synthesized and tested for anticonvulsant efficacy [36,37], including several water-soluble analogs of ganaxolone (an essential feature for intravenous formulation). Ganaxolone analogs are designed to exhibit greater potency and efficacy than ganaxolone on extrasynaptic GABA-A receptor–mediated tonic inhibition (Fig. 3). The ganaxolone analogs at the C-21 position (e.g. SGE-516) displayed a stronger potentiation of GABA currents, possibly conveying a stronger antiseizure effect than ganaxolone. There is strong evidence

that ganaxolone and its analogs are preferential allosteric modulators and direct activators of extrasynaptic δGABA-A receptors, regulating network inhibition and seizures. Hence, these findings provide a mechanistic rationale for the clinical use of ganaxolone or its analogs for OP intoxication [34]. Neurosteroid therapy with ganaxolone partly meets or exceeds the expectations of a practical anticonvulsant antidote for OP intoxication in military and civilian persons. It offers many advantages over the current benzodiazepines, including its broad-spectrum efficacy, lack of tolerance on repeated use, rapid onset and intermediate duration of action, well-characterized mechanism of action, safety profile from clinical trials, and amenability for autoinjector formulations for rapid use by first responders [48–50]. Future studies will ascertain if this drug may be valuable for the treatment of refractory SE, specifically OP-induced SE.

## Conclusion and future research needs

Nerve agents have been used in Syria, Iraq and Japan and pose a threat to civilian and military personnel. Acute OP poisoning can cause convulsions, SE, paralysis, breathing problems and death. Current treatment for OP intoxication includes a specialized drug combination containing atropine sulfate, 2-PAM, and diazepam. Intramuscular midazolam (*Seizalam*), which is approved in 2018 by the FDA for treatment of seizures, will replace diazepam in the above regimen. However, benzodiazepine anticonvulsants have significant limitations. Recent mechanistic studies with diazepam and midazolam in OP intoxication models support this conclusion. Currently, there are no FDA-approved postexposure medical countermeasures available to mitigate the effects of OP intoxication [53]. Available pretreatments (pyridostigmine bromide) and post-exposure countermeasures (atropine, 2-PAM, and diazepam) do not effectively prevent or mitigate all symptoms of nerve agent intoxication. Overall, there are urgent unmet medical needs for novel and innovative antidotes to protect civilians and soldiers against neurotoxic effects of OP nerve agents.

Recently, neurosteroids have been proposed as more effective anticonvulsant antidotes than benzodiazepines for OP poisonings [34]. Dual-acting neurosteroids at extrasynaptic and synaptic GABA-A receptors appear to be more effective than benzodiazepines in controlling seizures, even when administered very late after OP exposure. Neurosteroids are able to mitigate the chronic neurological effects caused by OP neurotoxicity. Neurosteroids can produce synergistic protection in combination with midazolam, making them practical medical countermeasures for OP attacks [52]. The synthetic neurosteroid ganaxolone is currently under development as a broad-spectrum antiepileptic for genetic epilepsies and SE. This neurosteroid has great potential as a countermeasure against chemical threats. Our previous work confirmed ganaxolone's ability to cross the blood brain barrier and achieve sufficient levels to exert its protective effects after IM injection. Ganaxolone has shown significant protective effects against chemoconvulsants, OP chemicals, and nerve agents in animal models. Intramuscular administration of ganaxolone 40, 60 and 120 minutes after nerve agent exposure resulted in suppression of electrographic markers of SE and histopathological markers of neuronal damage. An anticonvulsant with the potential to protect against multiple threats would be of significant benefit in both the military and civilian efforts to protect citizens against potential threats. The FDA has a special "Animal Rule" under which compounds may be approved for use against chemical threats on the strength of animal efficacy studies, which allows the potential for an accelerated approval path versus conventional new drug applications. Ganaxolone is being considered for advanced development and FDA approval for treatment of OP poisonings. Future studies will ascertain the utility of the neurosteroid–benzodiazepine combination and its potential biological variability for treating OP seizures and SE [42,54].

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## Conflict of interest statement

Nothing declared.

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