HHS Public Access

Author manuscript

Ann N Y Acad Sci. Author manuscript; available in PMC 2017 August 01.

Published in final edited form as:

Ann N Y Acad Sci. 2016 August ; 1378(1): 25–32. doi:10.1111/nyas.13160.

Neurosteroids for the potential protection of humans against organophosphate toxicity

Doodipala Samba Reddy

Department of Neuroscience and Experimental Therapeutics, College of Medicine, Texas A&M University Health Science Center, Bryan, Texas

Abstract

This article describes the therapeutic potential of neurosteroids as anticonvulsant antidotes for chemical intoxication caused by organophosphate pesticides and nerve agents or gases like sarin and soman. Toxic manifestations following nerve agent exposure, as evident in chemical attacks in Japan and Syria, include hypersecretion, respiratory distress, tremors, convulsions leading to status epilepticus (SE), and death. Benzodiazepines, such as diazepam, are the current anticonvulsants of choice for controlling nerve agent–induced life-threatening seizures, SE, and brain injury. Benzodiazepines can control acute seizures when given early, but they are less effective for delayed treatment of SE, which is characterized by rapid desensitization of synaptic $GABA_A$ receptors, benzodiazepine resistance, and brain injury. Neurosteroid-sensitive, extrasynaptic $GABA_A$ receptors remain unaffected by such events, however. Thus, anticonvulsant neurosteroids may produce more effective protection than benzodiazepines against a broad spectrum of chemical agents, even when given late after nerve agent exposure.

Keywords

benzodiazepine; nerve agent; neurosteroid; neuroactive steroid; organophosphate

Introduction

Chemical weapons are a serious threat to civilians, as evident from the Syrian gas attack in 2013. Nerve agents and organophosphate (OP) pesticides are credible terrorist threat agents. Nerve agents (sarin, soman, tabun, cyclosarin, and VX) and OP pesticides (chlorpyrifos, parathion, and paraoxon) are extremely lethal chemicals with potent neurotoxicity. These compounds can adversely affect the human nervous system, even at low levels of exposure. Acute exposure to nerve agents or OP pesticides can result in hypersecretion, respiratory distress, tremors, persistent seizures, status epilepticus (SE), brain injury, and death.[1–6] The current treatment regimen for nerve agents (atropine, 2-PAM, and diazepam) can prevent mortality if administered early after exposure; however, these treatments do not sufficiently

Address for correspondence: D. Samba Reddy, Ph.D., R.Ph., FAAPS, FAAAS, Professor, Department of Neuroscience and Experimental Therapeutics, College of Medicine, Texas A&M University Health Science Center, 8447 State Highway 47, MREB Building 2008, Bryan, TX 77807. reddy@medicine.tamhsc.edu.

Conflict of interest

The author declares no conflicts of interest.

protect the brain from SE—prolonged seizure activity lasting 30 min or longer with significant neuronal damage and mortality. There is emerging discussion that benzodiazepines often fail to guard against persistent SE and neurodegeneration occurring at later times after nerve agent exposure.[1] Thus, there is a crucial need for new and better anticonvulsants as medical countermeasures for nerve agents. Newer antidotes include those capable of being administered as clinically effective delayed treatments for nonlethal and potentially lethal OP exposure. Effective on-scene treatment by non-medical personnel and emergency first responders provides sufficient immediate attention to allow time for transport of an exposed subject to a medical facility for further treatment. Neurosteroids are being developed to meet these criteria as broad-spectrum anticonvulsant antidotes for nerve agents and OP intoxication. This article provides a brief overview of current treatments and neurosteroid-based strategies for the protection against OP neurotoxicity in humans and animals.

Organophosphate intoxication and treatments

Nerve agents are chemical warfare agents that have long attracted the attention of terrorists for attacking civilian populations. Nerve agents (the “gases” sarin, soman, tabun, cyclosarin, and VX) directly target the nervous system and irreversibly impair neural signaling within minutes of exposure.[3–9] OP pesticides, such as diisopropyl-fluorophosphate (DFP), parathion, and paraoxon, are considered credible threat agents because they are readily obtainable and are highly neurotoxic to humans and animals when exposed by a deliberate terrorist attack or by accident or natural disaster.[10–18] Nerve agents and OP pesticides are extremely lethal and produce neurotoxicity via common mechanisms. They cause devastating damage to the brain primarily due to their irreversible inhibition of acetylcholinesterase (AChE), leading to an excessive accumulation of acetylcholine (ACh), an excitatory neurotransmitter, in the brain. Acute exposure to nerve agents or OP poisoning results in cholinergic hyperactivation and causes a set of predictable toxic signs: hypersecretion, fasciculations, tremors, convulsions, respiratory distress, and death. CNS signs following nerve agent exposure include convulsive seizures resulting in death or long-term neuronal damage. The OP intoxication is categorized into cholinergic and non-cholinergic crisis. Cholinergic crisis results as a consequence of ACh accumulation at postsynaptic sites. Specific symptoms vary according to the afflicted organ. OP intoxication also results in non-cholinergic crisis, producing profound brain damage typified by neuronal injury, neuronal death, neuroinflammation, and deleterious effects on brain structure and function. The effects of OP intoxication are persistent, and survivors may suffer chronic brain damage, including the risk of neurological and cognitive deficits for the duration of their lives.[19–22]

Antidotes for OP intoxication consist of a pretreatment with carbamates (pyridostigmine bromide) to protect AChE from inhibition by OP compounds and postexposure treatments of anticholinergics and pyridinium oximes. Current treatment includes a specialized drug regimen: (1) atropine sulfate, a muscarinic receptor antagonist; (2) 2-PAM (pralidoxime chloride), a drug that regenerates AChE activity; and (3) diazepam, a benzodiazepine anticonvulsant.[1–3,7,23] This regimen is distributed in CHEMPACKs with autoinjectors for use in case of chemical attacks or accidents. A diazepam injection (5 mg/mL) is packaged in

a 2-m: disposable autoinjector. A prefilled DuoDot*e*® autoinjector provides a single intramuscular dose of atropine (3 mg/mL) and 2-PAM (600 mg/2 mL). These antidote products are designated for administration by emergency medical services personnel. Atropine sulfate (AtropenR®) autoinjectors are available in a variety of doses (0.25, 0.5, 1, and 2 mg), specifically designed for self or caregiver administration. Midazolam is being considered as a replacement anticonvulsant for diazepam for treatment of OP intoxication.[24]

There are some limitations with the current medical regimen for OP intoxication.[1–3,25–34] Anticholinergic drugs work to counteract the effects of excess ACh by blocking cholinergic receptors. Atropine is an effective antidote in conjunction with 2-PAM or other pyridinium oximes, such as trimedoxime or obidoxime, for OP intoxication. Such drugs reactivate AChE that has undergone covalent modification by OP chemical nerve agents.[25,26] However, the use of oximes has been found to be less beneficial, perhaps even harmful, in at least two meta-analyses.[27–29] A serious limitation of these drugs is their poor central nervous system (CNS) bioavailability, owing to their permanent positive charge and lack of suitable active transporters at the blood–brain barrier. Therefore, the oximes 2-PAM, obidoxime, and HI-6 cannot directly reactivate nerve agent–inhibited AChE in the brain. As a result, there is little neurological protection from 2-PAM treatment.

Presently, there are few effective antidotes for delayed treatment (40 min or later) of lethal signs and symptoms of nerve agent poisoning. Medical treatment for OP intoxication earlier than 40 min is not practical in most instances of mass chemical exposure, owing to the expected delay of first responders. The most common postexposure treatment, atropine, is very effective at preventing lethality from OP intoxication, but it lacks the ability to prevent postexposure incapacitation, performance deficits, and permanent brain damage. While atropine is highly effective in antagonizing ACh at most peripheral muscarinic receptors and partly at central muscarinic receptors, it is ineffective at nicotinic receptors and also for intoxication mediated by such receptors in the brain and peripheral system. It is likely that nicotinic receptors are involved in OP intoxication and morbidity.[35] Nicotinic antagonists have not been used, owing to the difficulties of administering a dose of a competitive neuromuscular blocker sufficient to antagonize the effects of excessive ACh, but not so great that it paralyzes the muscles. A noncompetitive nicotinic antagonist can produce significant protection against nerve agent poisoning, as evident from sarin or tabun exposure in guinea pigs.[36] Atropine may not effectively mitigate the central muscarinic and nicotinic effects of OP intoxication, owing to its limited brain penetration at therapeutic doses.[37] Larger doses are required to get appreciable concentrations of atropine into the CNS; however, researchers seek compounds (scopolamine) that can do so more quickly and at greater concentrations. Nevertheless, OP intoxication can immediately produce generalized seizures and brain damage despite atropine administration. This notion is supported by the occurrence of chronic neurological symptoms in survivors of sarin attacks in Japan[19–22] as well as in ample animal studies that seek new anticonvulsants for nerve agents.[8,12–14,30,33,36]

Benzodiazepines have long been the first line of treatment for the control of seizures and SE, including seizures induced by OP intoxication.[38,39] Benzodiazepines act as positive allosteric modulators of synaptic $GABA_A$ receptors. The $GABA_A$ receptor mediates two types of inhibition, now characterized as synaptic (phasic) and extrasynaptic (tonic)

inhibition. Synaptic release of GABA results in the activation of low-affinity γ2-containing synaptic receptors, while high-affinity δ-containing extrasynaptic receptors are persistently activated by the ambient GABA present in the extracellular fluid. Benzodiazepines bind specifically to γ2-containing synaptic receptors and augment phasic inhibition, but do not modulate extrasynaptic δ$GABA_A$ receptor–mediated tonic inhibition. Thus, benzodiazepines do not require extrasynaptic $GABA_A$ receptors for anticonvulsant activity.[40] The benzodiazepine diazepam is currently the only U.S. Food and Drug Administration (FDA)–approved injectable anticonvulsant for the cessation of seizures caused by nerve agents and OP pesticides. However, there are many concerns with the use of diazepam for controlling nerve agent seizures. As evident from animal studies, the efficacy of diazepam decreases as the interval between OP intoxication or initiation of seizures and the drug administration increases.[33,34] Diazepam must be administered within a few minutes of OP intoxication for effective protection against seizures and SE. This timeline is often not practical in many incidents, such as emergencies and mass casualties. The development of resistance to diazepam is also a concern, because seizures gradually acquire resistance to benzodiazepines, as noted in animal studies.[33,34] Additionally, seizures often recur after termination of the initial SE by benzodiazepines. Seizures induced by cholinergic hyperactivation can thus become self-sustaining and develop time-dependent refractory SE—a serious condition associated with significant brain injury and mortality.[41] Although animal studies are largely supportive of the above limitations of benzodiazepines, apparently there is little published data from human incidents. In clinical studies, diazepam has variable pharmacokinetics and adverse effects at multiple dosages.[38,39]

The mechanisms underlying the intractability of nerve agent seizures are unclear. A variety of mechanistic premises have been proposed for the development of pharmacoresistance to SE and benzodiazepine insensitivity. Studies in animal models of SE indicate that such a phenomenon may involve internalization and downregulation of synaptic $GABA_A$ receptors.[42–46] Following nerve agent exposure, it is likely that the rate of synaptic $GABA_A$ receptor internalization increases rapidly, and the subunit composition of these receptors swiftly changes, causing benzodiazepines to ultimately lose efficacy due to lack of receptor availability.[42,43,46] A significant decrease in the surface expression of γ2-containing synaptic $GABA_A$ receptors (targets for benzodiazepines) is observed during persistent SE, while no such change is evident in δ-containing extrasynaptic $GABA_A$ receptors.[44,45] This reduced synaptic GABA inhibition is also evident in the CA1 and CA3 regions during SE,[46] indicating that anticonvulsants that exclusively target synaptic $GABA_A$ receptors may exert less efficient protection against persistent SE, such as that which occurs following OP intoxication.

Neurosteroid treatment of OP intoxication

Neurosteroids are innovative experimental countermeasures for OP intoxication. Neurosteroids are steroid compounds that can rapidly modify neuronal excitability through non-genomic mechanisms.[47–50] They function in the brain as endogenous modulators of seizure susceptibility. Although a variety of neurosteroids are present in the brain, the most examined are allopregnanolone (5α-pregnan-3α-ol-20-one), THDOC (5α-pregnan-3α,21-

diol-20-one), and androstanediol (5α-androstan-3α-ol-20-diol). Neurosteroids are highly lipophilic molecules, and therefore can easily cross the blood–brain barrier.[47,48]

Neurosteroids act rapidly to decrease neuronal excitability through direct interaction with membrane $GABA_A$ receptors in the brain (Fig. 1). Neurosteroids are positive allosteric agonists of both synaptic and extrasynaptic $GABA_A$ receptors.51–53 There are two discrete binding sites for neurosteroids: an allosteric site within the α-subunit transmembrane domain and a site of direct activation at the α–β subunit interface.[53,54] Structure–activity relationship studies at synaptic γ$GABA_A$ receptors show that the C3α-OH steroid structure is essential for the binding and the receptor-enhancing function of neurosteroids.[55,56] Apart from 5α-H stereoselectivity, the C17- or C20-ketone group is important for allosteric modulation.[57–62] Neurosteroids bind to all $GABA_A$ receptors, but δ-containing receptors at peri- and extrasynaptic sites exhibit preferential sensitivity.[63–65] The allosteric binding of neurosteroid to low-efficacy δ$GABA_A$ receptors induces a pronounced conformational change, greater channel opening, and non-desensitizing tonic inhibition.[66] Increased δGABA-A receptor expression enhances neurosteroid sensitivity through greater potentiation of tonic current.[67–70] Conversely, deficiency of δ$GABA_A$ receptor expression reduces the sensitivity to neurosteroids.[71–74] Thus, neurosteroids can produce robust inhibition in the brain by acting at both synaptic and extrasynaptic $GABA_A$ receptors that are intricately involved in the control of network hyperexcitability and seizure activity.

Several synthetic neurosteroids have been prepared and tested for their anesthetic, anxiolytic, and antiseizure effects.[75,76] The best known of these are alphaxolone, minaxolone, and ganaxolone. As therapeutic agents, neurosteroids have some advantages, in that tolerance do not appear to develop with chronic use.[77] Neurosteroids are broad-spectrum anticonvulsants and confer seizure protection in a variety of animal models. Allopregnanolone and related neurosteroids protect against seizures induced by $GABA_A$ receptor antagonists (pentylenetetrazol, picrotoxin, TBPS, bicuculline), 6-Hz electrical stimulation, pilocarpine administration, and electrical kindling stimulation.[75–85] The potencies of neurosteroids in models where they confer seizure protection vary largely in accordance with their activities as positive allosteric modulators of $GABA_A$ receptors.[60,79,80] Neurosteroids are highly active in the 6-Hz model, a better paradigm in which limbic-like seizures are induced by electrical stimulation of lower frequency and longer duration than in the maximal electroshock test.[79] Unlike benzodiazepines, anticonvulsant tolerance is not evident with chronic neurosteroid treatment. Additionally, neurosteroids can promote neuroprotection and inhibit epileptogenesis.[77,81]

Neurosteroids are viable anticonvulsants that can surpass the limitations of benzodiazepines for OP intoxication. Unlike diazepam, neurosteroids can be effective anticonvulsants when administered late after chemical exposure. These suggestions are based on the emerging molecular mechanisms of OP intoxication seizures and SE. Extrasynaptic δ-containing $GABA_A$ receptors that generate tonic inhibition supposedly do not internalize during SE;[44,45] thus, neurosteroids, which activate both extrasynaptic and synaptic receptors, could be more effective treatments for SE. There is emerging preclinical data in support of this premise. In a rodent model of SE induced by the cholinergic agonists or other convulsants, neurosteroids have been highly effective in protecting against SE, including seizure models

that are designed to simulate refractory SE.[85–88] Although neurosteroids show promise in the treatment of diverse forms of seizures, none are currently approved by the FDA for clinical use.

In the National Institutes of Health CounterACT U01 project, entitled “Neurosteroid treatment for OP intoxication,” we discovered the efficacy of neurosteroids, which enhance phasic and extrasynaptic tonic inhibition,[89] in protecting against chemical neurotoxicity (results were presented and discussed at the annual CounterACT Research Network Symposium, June 15–27, 2016 in New York, hosted by the New York Academy of Sciences). We tested a variety of natural and synthetic neurosteroids in the DFP pesticide model (a surrogate for OP nerve agents) and the nerve agent soman model using a delayed (40-min) postexposure protocol in rats. We utilized a DFP protocol that was described previously.[12] We adapted the soman protocol from the USMRICD report.[13] We found that pregnane neurosteroids produced a dose-dependent protection against DFP- and soman-induced seizures and SE, indicating their potential anticonvulsant efficacy in OP intoxication models in rats. In addition, neurosteroids have significant neuroprotectant activity even with delayed treatment after soman exposure. The benzodiazepine midazolam, which does not activate extrasynaptic δGABA$_A$ receptors,[89] is utilized as the comparative anticonvulsant. In the delayed (40-min) protocol, midazolam showed a suboptimal protection of DFP- and soman-induced SE and seizure activity returned following initial suppression. Overall, it is suggested that neurosteroids have potential efficacy in OP intoxication models, especially for delayed postexposure therapy, because of their unique mechanistic and other advantages.[90]

Conclusions

Current treatment for OP intoxication includes a specialized drug combination containing atropine, 2-PAM, and diazepam. Benzodiazepines are effective anticonvulsants for OP agents when administered within a few minutes postexposure; however, protection is limited following delayed administration, as evident from animal studies. Late-stage seizures, especially refractory SE, can cause profound brain damage. Presently, there are a few effective antidotes for delayed treatment of OP intoxication, especially for rapid and effective termination of persistent seizures and brain damage. Recently, neurosteroids have been identified as novel experimental antidotes for nerve agents. Neurosteroids are stronger anticonvulsants, even when administered very late after chemical exposure. It is suggested that neurosteroids that enhance phasic and extrasynaptic tonic inhibition produce more effective protection against persistent SE, prevent brain injury, and extend the therapeutic window when compared to benzodiazepine treatments. Certain neurosteroids have been approved by the FDA for preliminary trials, making them practical potential countermeasures in case of a chemical incident.

Acknowledgments

This work was supported by the CounterACT Program, National Institutes of Health, Office of the Director and the National Institute of Neurologic Disorders and Stroke [Grant U01 NS083460].

References

1. Dolgin E. Syrian gas attack reinforces need for better anti-sarin drugs. Nature Medicine. 2013; 19:1194–1195.
2. Okumura T, Takasu N, Ishimatsu S, Miyanoki S, Mitsuhashi A, Kumada K, Tanaka K, Hinohara S. Report on 640 victims of the Tokyo subway sarin attack. Ann Emerg Med. 1996; 28:129–135. [PubMed: 8759575]
3. Jett DA, Yeung DT. The counterACT research network: basic mechanisms and practical applications. Proc Am Thorac Soc. 2010; 7:254–256. [PubMed: 20601628]
4. Morita H, Yanagisawa N, Nakajima T, Shimizu M, Hirabayashi H, Okudera H, Nohara M, Midorikawa Y, Mimura S. Sarin poisoning in Matsumoto, Japan. Lancet. 1995; 346:290–293. [PubMed: 7630252]
5. Suzuki T, Morita H, Ono K, Maekawa K, Nagai R, Yazaki Y. Sarin poisoning in Tokyo subway. Lancet. 1995; 345:980–980.
6. Bajgar J. Complex view on poisoning with nerve agents and organophosphates. Acta Medica (Hradec Kralove). 2005; 48:3–21.
7. Bajgar J. Organophosphates/nerve agent poisoning: mechanism of action, diagnosis, prophylaxis, and treatment. Adv Clin Chem. 2004; 38:151–216. [PubMed: 15521192]
8. Shih TM, McDonough JH Jr. Organophosphorus nerve agents-induced seizures and efficacy of atropine sulfate as anticonvulsant treatment. Pharmacol Biochem Behav. 1999; 64:147–153. [PubMed: 10495009]
9. Joosen MJ, van der Schans MJ, Kuijpers WC, van Helden HP, Noort D. Timing of decontamination and treatment in case of percutaneous VX poisoning: a mini review. Chem Biol Interact. 2013; 203:149–153. [PubMed: 23085122]
10. Bouzarth WF, Himwich HE. Mechanism of seizures induced by diisopropylflurophosphate (DFP). Am J Psychiatry. 1952; 108:847–855. [PubMed: 14923898]
11. Wright LK, Liu J, Nallapaneni A, Pope CN. Behavioral sequelae following acute diisopropylfluorophosphate intoxication in rats: comparative effects of atropine and cannabinomimetics. Neurotoxicol Teratol. 2009; 32:329–335. [PubMed: 20034559]
12. Deshpande LS, Carter DS, Blair RE, Delorenzo RJ. Development of a prolonged calcium plateau in hippocampal neurons in rats surviving status epilepticus induced by the organophosphate diisopropylfluorophosphate. Toxicol Sci. 2010; 116:623–631. [PubMed: 20498005]
13. Figueiredo TH, Qashu F, Apland JP, Aroniadou-Anderjaska V, Souza AP, Braga MF. The GluK1 (GluR5) Kainate/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist LY293558 reduces soman-induced seizures and neuropathology. J Pharmacol Exp Ther. 2011; 336:303–312. [PubMed: 20962029]
14. McDonough JH Jr, Shih TM. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. Neurosci Biobehav Rev. 1997; 21:559–579. [PubMed: 9353792]
15. Jokanović M, Kosanović M. Neurotoxic effects in patients poisoned with organophosphorus pesticides. Environ Toxicol Pharmacol. 2010; 29:195–201. [PubMed: 21787602]
16. Krause K-H, van Thriel C, De Sousa PA, Leist M, Hengstler JG. Monocrotophos in Gandaman village: India school lunch deaths and need for improved toxicity testing. Arch Toxicol. 2013; 87:1877–1881. [PubMed: 23943209]
17. Savage EP, Keefe TJ, Mounce LM, Heaton RK, Lewis JA, Burcar PJ. Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch Environ Health. 1988; 43:38–45. [PubMed: 3355242]
18. Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N. Chronic neurobehavioral and central and autonomic nervous system effects of Tokyo subway sarin poisoning. J Physiol Paris. 1998; 92:317–323. [PubMed: 9789830]
19. Yanagisawa N, Morita H, Nakajima T. Sarin experiences in Japan: acute toxicity and long-term effects. J. Neurol. Sci. 2006; 249:76–85. [PubMed: 16962140]
20. Miyaki K, Nishiwaki Y, Maekawa K, Ogawa Y, Asukai N, Yoshimura K, Etoh N, Matsumoto Y, Kikuchi Y, Kumagai N, Omae K. Effects of sarin on the nervous system of subway workers seven

years after the Tokyo subway sarin attack. J Occup Health. 2005; 47:299–304. [PubMed: 16096354]

21. Murata K, Araki S, Yokoyama K, Okumura T, Ishimatsu S, Takasu N, White RF. Asymptomatic sequelae to acute sarin poisoning in the central and autonomic nervous system 6 months after the Tokyo subway attack. J Neurol. 1997; 244:601–606. [PubMed: 9402534]
22. Ohtani T, Iwanami A, Kasai K, Yamasue H, Kato T, Sasaki T, Kato N. Post-traumatic stress disorder symptoms in victims of Tokyo subway attack: a 5-year follow-up study. Psychiatry Clin Neurosci. 2004; 58:624–629. [PubMed: 15601387]
23. Eddleston M, Buckley NA, Eyer P, Dawson AH. Management of acute organophosphorus pesticide poisoning. Lancet. 2008; 371(9612):597–607. [PubMed: 17706760]
24. Reddy SD, Reddy DS. Midazolam as an anticonvulsant antidote for OP intoxication−A pharmacotherapeutic appraisal. Epilepsia. 2015; 56:813–821. [PubMed: 26032507]
25. Jokanović M. Structure-activity relationship and efficacy of pyridinium oximes in the treatment of poisoning with organophosphorus compounds: a review of recent data. Curr Top Med Chem. 2012; 12:1775–89. [PubMed: 23030612]
26. Balali-Mood M, Shariat M. Treatment of organophosphate poisoning. Experience of nerve agents and acute pesticide poisoning on the effects of oximes. J Physiol Paris. 1998; 92:375–378. [PubMed: 9789840]
27. Rahimi R, Nikfar S, Abdollahi M. Increased morbidity and mortality in acute human organophosphate-poisoned patients treated by oximes: a meta-analysis of clinical trials. Hum Exp Toxicol. 2006; 25:157–162. [PubMed: 16634335]
28. Peter JV, Moran JL, Graham P. Oxime therapy and outcomes in human organophosphate poisoning: an evaluation using meta-analytic techniques. Crit Care Med. 2006; 34:502–510. [PubMed: 16424734]
29. Banerjee I, Tripathi SK, Roy AS. Efficacy of pralidoxime in organophosphorus poisoning: revisiting the controversy in Indian setting. J Postgrad Med. 2014; 60:27–30. [PubMed: 24625936]
30. Shih TM, Duniho SM, McDonough JH. Control of nerve agent-induced seizures is critical for neuroprotection and survival. Toxicol Appl Pharmacol. 2003; 188:69–80. [PubMed: 12691725]
33. McDonough JH, McMonagle JD, Shih TM. Time-dependent reduction in the anticonvulsant effectiveness of diazepam against soman-induced seizures in guinea pigs. Drug Chem Toxicol. 2010; 33:279–83. [PubMed: 20429808]
34. Apland JP, Aroniadou-Anderjaska V, Figueiredo TH, et al. The limitations of diazepam as a treatment for nerve agent-induced seizures and neuropathology in rats; Comparison with UBP302. J Pharmacol Exp Ther. 2014; 351:359–372. [PubMed: 25157087]
35. Hassel B. Nicotinic mechanisms contribute to soman-induced symptoms and lethality. Neurotoxicology. 2006; 27:501–507. [PubMed: 16500708]
36. Turner SR, Chad JE, Price M, Timperley CM, Bird M, Green AC, Tattersall JE. Protection against nerve agent poisoning by a noncompetitive nicotinic antagonist. Toxicol Lett. 2011; 206:105–111. [PubMed: 21641979]
37. Bardin PG, van Eeden SF, Moolman JA, Foden AP, Joubert JR. Organophopshate and carbamate poisoning. Arch Intern Med. 1994; 154:1433–1441. [PubMed: 8017998]
38. Kellinghaus C, Stögbauer F. Treatment of status epilepticus in a large community hospital. Epilepsy Behav. 2012; 23:235–240. [PubMed: 22341964]
39. Singhi S, Murthy A, Singhi P, et al. Continuous midazolam versus diazepam infusion for refractory convulsive status epilepticus. J Child Neurol. 2002; 17:106–110. [PubMed: 11952069]
40. Reddy SD, Younus I, Clossen BL, Reddy DS. Antiseizure activity of midazolam in mice lacking δ-subunit extrasynaptic GABA-A receptors. J Pharmacol Exp Ther. 2015; 353:517–528. [PubMed: 25784648]
41. Wasterlain CG, Chen JW. Mechanistic and pharmacologic aspects of status epilepticus and its treatment with new antiepileptic drugs. Epilepsia. 2008; 49(Suppl 9):63–73. [PubMed: 19087119]
42. Goodkin HP, Yeh JL, Kapur J. Status epilepticus increases the intracellular accumulation of GABA-A receptors. J Neurosci. 2005; 25:5511–5520. [PubMed: 15944379]
43. Goodkin HP, Sun C, Yeh JL, Mangan PS, Kapur J. GABA-A receptor internalization during seizures. Epilepsia. 2007; 48(Suppl 5):109–113. [PubMed: 17910589]

44. Zhan RZ, Nadler JV. Enhanced tonic GABA current in normotopic and hilar ectopic dentate granule cells after pilocarpine-induced status epilepticus. J Neurophysiol. 2009; 102:670–681. [PubMed: 19474175]
45. Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. Subunit-specific trafficking of GABA-A receptors during status epilepticus. J Neurosci. 2008; 28:2527–2538. [PubMed: 18322097]
46. Sun HY, Goodkin HP. The pervasive reduction of GABA-mediated synaptic inhibition of principal neurons in the hippocampus during status epilepticus. Epilepsy Res. 2016; 119:30–33. [PubMed: 26656782]
47. Reddy DS. Neurosteroids: Endogenous role in the human brain and therapeutic potentials. Progr Brain Res. 2010; 186:113–137.
48. Mellon SH, Griffin LD. Neurosteroids: biochemistry and clinical significance. Trends Endocrinol Metab. 2002; 13:35–43. [PubMed: 11750861]
49. Reddy DS, Castaneda DC, O'Malley BW, Rogawski MA. Anticonvulsant activity of progesterone and neurosteroids in progesterone receptor knockout mice. J Pharmacol Exp Ther. 2004; 310:230–239. [PubMed: 14982969]
50. Reddy DS. Role of anticonvulsant and antiepileptogenic neurosteroids in the pathophysiology and treatment of epilepsy. Front Endocrinol. 2011; 2(38):1–11.
51. Chisari M, Eisenman LN, Krishnan K, Bandyopadhyaya AK, Wang C, Taylor A, Benz A, Covey DF, Zorumski CF, Mennerick S. The influence of neuroactive steroid lipophilicity on GABA-A receptor modulation: evidence for a low-affinity interaction. J Neurophysiol. 2009; 102:1254–1264. [PubMed: 19553485]
52. Akk G, Covey DG, Evers AS, Steinbach JH, Zorumski CF, Mennerick S. Mechanisms of neurosteroid interactions with GABA-A receptors. Pharmacol Ther. 2007; 116:35–57. [PubMed: 17524487]
53. Hosie AM, Wilkins ME, Smart TG. Neurosteroid binding sites on GABA-A receptors. Pharmacol Ther. 2007; 116:7–19. [PubMed: 17560657]
54. Hosie AM, Wilkins ME, da Silva HM, Smart TG. Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. Nature. 2006; 444(7118):486–489. [PubMed: 17108970]
55. Harrison NL, Majewska MD, Harrington JW, Barker JL. Structure-activity relationships for steroid interaction with the GABA-A receptor complex. J Pharmacol Exp Ther. 1987; 241:346–353. [PubMed: 3033209]
56. Mitchell EA, Herd MB, Gunn BG, Lambert JJ, Belelli D. Neurosteroid modulation of GABA-A receptors: molecular determinants and significance in health and disease. Neurochem Int. 2008; 52:588–595. [PubMed: 18055067]
57. Gee KW, Bolger MB, Brinton RE, Coirini H, McEwen BS. Steroid modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. J Pharmacol Exp Ther. 1988; 246:803–812. [PubMed: 2841455]
58. Kokate TG, Svensson BE, Rogawski MA. Anticonvulsant activity of neurosteroids: correlation with GABA-evoked chloride current potentiation. J Pharmacol Exp Ther. 1994; 270:1223–1229. [PubMed: 7932175]
59. Upasani RB, Yang KC, Acosta-Burruel M, Konkoy CS, McLellan JA, Woodward RM, Lan NC, Carter RB, Hawkinson JE. 3α-hydroxy-3β(phenylethnyl)-5β-pregan-20-ones: synthesis and pharmacological activity of neuroactive steroids with high affinity GABA-A receptors. J Med Chem. 1997; 40:73–84. [PubMed: 9016330]
60. Reddy DS, Jian K. The testosterone-derived neurosteroid androstanediol is a positive allosteric modulator of GABAA receptors. J Pharmacol Exp Ther. 2010; 334:1031–1041. [PubMed: 20551294]
61. Covey DF, Nathan D, Kalkbrenner M, Nilsson KR, Hu Y, Zorumsk CF, Evers AS. Enantioselectivity of pregnanolone-induced GABA-A receptor modulation and anesthesia. J Pharm Exp Ther. 2000; 293:1009–1116.
62. Qian M, Krishnan K, Kudova E, Li P, Manion BD, Taylor A, Elias G, Akk G, Evers AS, Zorumski CF, Mennerick S, Covey DF. Neurosteroid analogues. 18. Structure-activity studies of ent-steroid

potentiators of GABA-A receptors and comparison of their activities with those of alphaxolone and allopregnanolone. J Med Chem. 2014; 57:171–190. [PubMed: 24328079]

63. Belelli D, Casula A, Ling A, Lambert JJ. The influence of subunit composition on the interaction of neurosteroids with GABA-A receptors. Neuropharmacology. 2002; 43:651–661. [PubMed: 12367610]
64. Meera P, Wallner M, Otis TS. Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA-A receptors. J Neurophysiol. 2011; 106:2057–2064. [PubMed: 21795619]
65. Carver CM, Reddy DS. Neurosteroid interactions with synaptic and extrasynaptic $GABA_A$ receptors: regulation of subunit plasticity, phasic and tonic inhibition, and neuronal network excitability. Psychopharmacology. 2013; 230:151–188. [PubMed: 24071826]
66. Bianchi MT, Macdonald RL. Neurosteroids shift partial agonist activation of GABA-A receptor channels from low- to high-efficacy gating patterns. J Neurosci. 2003; 23:10934–10943. [PubMed: 14645489]
67. Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary GABA-A receptors containing the δ subunit. J Neurosci. 2002; 22:1541–1549. [PubMed: 11880484]
68. Sanna E, Mostallino MC, Luca Murru, Carta M, Talani G, Zucca S, Mura ML, Maciocco E, Biggio G. Changes in expression and function of extrasynaptic GABA-A receptors in rat hippocampus during pregnancy and after delivery. J Neurosci. 2009; 29:1755–1765. [PubMed: 19211882]
69. Wu X, Gangisetty O, Carver CM, Reddy DS. Estrous cycle regulation of extrasynaptic δ-containing GABA-A receptor-mediated tonic inhibition and limbic epileptogenesis. J Pharmacol Exp Ther. 2013; 346:146–160. [PubMed: 23667248]
70. Carver CM, Wu X, Gangisetty O, Reddy DS. Perimenstrual-like hormonal regulation of extrasynaptic δ-containing GABA-A receptors mediating tonic inhibition and neurosteroid sensitivity. J Neurosci. 2014; 34:14181–14197. [PubMed: 25339733]
71. Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi Z, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Zhiwei Li, DeLorey TM, Olsen RW, Homanics GE. Attenuated sensitivity to neuroactive steroids in GABA-A receptor delta subunit knockout mice. Proc Natl Acad Sci USA. 1999; 96:12905–12910. [PubMed: 10536021]
72. Spigelman I, Li Z, Liang J, Cagetti E, Samzadeh S, Mihalek RM, Homanics GE, Olsen RW. Reduced inhibition and sensitivity to neurosteroids in hippocampus of mice lacking the GABA-A receptor δ subunit. J Neurophysiol. 2003; 90:903–910. [PubMed: 12702713]
73. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing GABA-A receptors. Proc Natl Acad Sci USA. 2003; 100:14439–14444. [PubMed: 14623958]
74. Pandit S, Jeong JA, Jo JY, Cho HS, Kim DW, Kim JM, Ryu PD, Lee SY, Kim HW, Jeon BH, Park JB. Dual mechanisms diminishing tonic GABA-A inhibition of dentate gyrus granule cells in Noda epileptic rats. J Neurophysiol. 2013; 110:95–102. [PubMed: 23576696]
75. Belelli D, Bolger MB, Gee KW. Anticonvulsant profile of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Eur J Pharmacol. 1989; 166:325–329. [PubMed: 2792198]
76. Reddy DS, Woodward R. Ganaxolone: a prospective overview. Drugs Future. 2004; 29:227–242.
77. Reddy DS, Rogawski MA. Chronic treatment with the neuroactive steroid ganaxolone in the rat induces anticonvulsant tolerance to diazepam but not to itself. J Pharmacol Exp Ther. 2000; 295:1241–1248. [PubMed: 11082461]
78. Frye CA. The neuroactive steroid 3α,5α-THP has anti-seizure and possible neuroprotective effects in an animal model of epilepsy. Brain Res. 1995; 696:113–120. [PubMed: 8574658]
79. Kaminski RM, Livingood MR, Rogawski MA. Allopregnanolone analogs that positively modulate GABAA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. Epilepsia. 2004; 45:864–867. [PubMed: 15230714]
80. Kaminski RM, Marini H, Kim WJ, Rogawski MA. Anticonvulsant activity of androsterone and etiocholanolone. Epilepsia. 2005; 46:819–827. [PubMed: 15946323]

81. Biagini G, Baldelli E, Longo D, Pradelli L, Zini I, Rogawski MA, Avoli M. Endogenous neurosteroids modulate epileptogenesis in a model of temporal lobe epilepsy. Exp Neurol. 2006; 201:519–524. [PubMed: 16780839]

82. Reddy DS. Anticonvulsant activity of the testosterone-derived neurosteroid 3alpha-androstanediol. Neuroreport. 2004; 15:515–518. [PubMed: 15094514]

83. Reddy DS, Rogawski MA. Enhanced anticonvulsant activity of ganaxolone after neurosteroid withdrawal in a rat model of catamenial epilepsy. J Pharmacol Exp Ther. 2000; 294:909–915. [PubMed: 10945840]

84. Reddy DS, Rogawski MA. Ganaxolone suppression of behavioral and electrographic seizures in the mouse amygdala kindling model. Epilepsy Re. 2010; 89:254–260.

85. Wieland S, Belluzzi JD, Stein L, Lan NC. Comparative behavioral characterization of the neuroactive steroids 3α-OH,5α-pregnan-20-one and 3α-OH,5β-pregnan-20-one in rodents. Psychopharmacology. 1995; 118:65–71. [PubMed: 7597124]

86. Reddy DS. Gender differences in antiseizure sensitivity of neurosteroids in the pilocarpine model of status epilepticus. Epilepsia. 2009; 50(Suppl.11):126–126.

87. Briyal S, Reddy DS. Neuroactive steroid therapy of status epilepticus in epilepsy rats. Epilepsia. 2008; 49(Suppl. 7):3055–3055.

88. Rogawski MA, Loya CM, Reddy K, Zolkowska D, Lossin C. Neuroactive steroids for the treatment of status epilepticus. Epilepsia. 2013; 54(Suppl 6):93–98. [PubMed: 24001085]

89. Carver CM, Reddy DS. Neurosteroid structure-activity relationships for functional activation of extrasynaptic δ-GABA-A receptors. J Pharmacol Exp Therap. 2016; 357:188–204. [PubMed: 26857959]

90. Reddy DS. Clinical potential of neurosteroids for CNS disorders. Trends Pharmacol Sci. 2016; 37(6):1–19. [PubMed: 26521094]

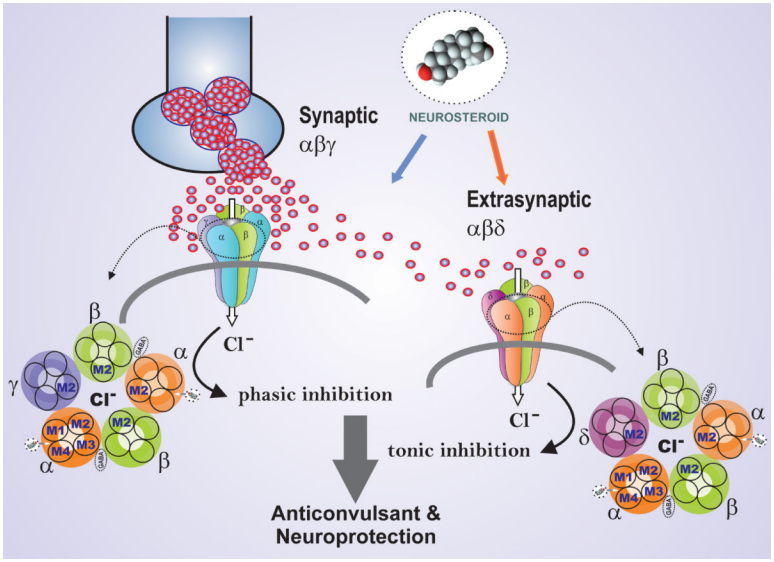

Figure 1.

Neurosteroid-based therapeutic strategy for organophosphate intoxication. Neurosteroids that enhance $GABA_A$ receptor synaptic phasic and extrasynaptic tonic inhibition in the brain have been demonstrated to be effective for controlling organophosphate chemical intoxication–induced seizures, neuronal damage, and related morbidity.