REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
2012	Open Literature	

4. TITLE AND SUBTITLE
The anticholinergic and antiglutamatergic drug caramiphen reduces seizure duration in soman-exposed rats: Synergism with the benzodiazepine diazepam

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Schultz, MK, Wright, LK, Stone, MF, Schwartz, JE, Kelley, NR, Moffett, MC, Lee, RB, Lumley, LA

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDT-N
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-P11-034

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Defense Threat Reduction Agency
8725 John J. Kingman Road STOP 6201
Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
Published in Toxicology and Applied Pharmacology, 259(3), 376-386, 2012.This research was supported by the Defense Threat Reduction Agency, Medical S&T Division.

14. ABSTRACT
See reprint.

15. SUBJECT TERMS
soman, caramiphen, diazepam, physostigmine, seizure, neuroprotection, chemical warfare nerve agents, medical chemical defense , medical countermeasures

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Lucille A. Lumley
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	11	**19b. TELEPHONE NUMBER** *(include area code)* 410-436-8377

Toxicology and Applied Pharmacology 259 (2012) 376–386

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

The anticholinergic and antiglutamatergic drug caramiphen reduces seizure duration in soman-exposed rats: Synergism with the benzodiazepine diazepam

M.K. Schultz, L.K.M. Wright, M.F. Stone, J.E. Schwartz, N.R. Kelley, M.C. Moffett, R.B. Lee, L.A. Lumley *

US Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, USA

ARTICLE INFO

Article history:
Received 9 December 2011
Revised 20 January 2012
Accepted 22 January 2012
Available online 30 January 2012

Keywords:
Soman
Caramiphen
Diazepam
Physostigmine
Seizure
Neuroprotection

ABSTRACT

Therapy of seizure activity following exposure to the nerve agent soman (GD) includes treatment with the anticonvulsant diazepam (DZP), an allosteric modulator of γ-aminobutyric acid A ($GABA_A$) receptors. However, seizure activity itself causes the endocytosis of $GABA_A$ receptors and diminishes the inhibitory effects of GABA, thereby reducing the efficacy of DZP. Treatment with an N-methyl-D-aspartic acid (NMDA) receptor antagonist prevents this reduction in GABAergic inhibition. We examined the efficacy of the NMDA receptor antagonist caramiphen edisylate (CED; 20 mg/kg, im) and DZP (10 mg/kg, sc), administered both separately and in combination, at 10, 20 or 30 min following seizure onset for attenuation of the deleterious effects associated with GD exposure (1.2 LD_{50}; 132 μg/kg, sc) in rats. Outcomes evaluated were seizure duration, neuropathology, acetylcholinesterase (AChE) activity, body weight, and temperature. We also examined the use of the reversible AChE inhibitor physostigmine (PHY; 0.2 mg/kg, im) as a therapy for GD exposure. We found that the combination of CED and DZP yielded a synergistic effect, shortening seizure durations and reducing neuropathology compared to DZP alone, when treatment was delayed 20–30 min after seizure onset. PHY reduced the number of animals that developed seizures, protected a fraction of AChE from GD inhibition, and attenuated post-exposure body weight and temperature loss independent of CED and/or DZP treatment. We conclude that: 1) CED and DZP treatment offers considerable protection against the effects of GD and 2) PHY is a potential therapeutic option following GD exposure, albeit with a limited window of opportunity.

Published by Elsevier Inc.

Introduction

Soman (GD) is an organophosphorus (OP) compound that inhibits the cholinesterase (ChE) family of enzymes including acetylcholinesterase (AChE), butyrylcholinesterase (BChE) (Junge and Krisch, 1975). The toxicity of GD is due to inhibition of AChE, the enzyme responsible for hydrolyzing the neurotransmitter acetylcholine (ACh) (McDonough and Shih, 1993). Excess ACh at synapses and neuromuscular junctions results in the prolonged stimulation of both muscarinic and nicotinic receptors leading to miosis, hypersecretions, fasciculations, respiratory distress, cardiac dysfunction, and seizures that can rapidly progress to self-sustained seizures (*status epilepticus*, SE) and result in extensive neuropathology as seen in rats (de Araujo Furtado et al., 2009, 2010) and guinea pigs (McDonough and Shih, 1997).

The current US Army-fielded treatment strategy against nerve agent-induced toxicity includes the antimuscarinic atropine, the oxime 2-pralidoxime (2-PAM), and the benzodiazepine diazepam (reviewed in Cannard, 2006; Taylor, 2001). Pretreatment with the reversible ChE inhibitor pyridostigmine or physostigmine is also used in the United States and the United Kingdom, respectively, to prevent the nerve agent from binding to a fraction of the enzyme (Eckert et al., 2007; Gordon et al., 1978; Shih et al., 1991; Wetherell et al., 2006, 2007). While these treatments protect against nerve agent-induced lethality, complete protection is not provided in terms of epileptiform activity, neuropathology, and cognitive deficits (de Araujo Furtado et al., 2009, 2010; Langston et al., 2011; McDonough and Shih, 1997; Moffett et al., 2011; Raveh et al., 2003; Shih et al., 1991, 2003).

A review by McDonough and Shih (1997) postulated a triphasic model of seizure progression following nerve agent exposure. Phase 1 is characterized by cholinergic hyperactivity and lasts about 5 min after seizure onset. Phase 2 is a transitional phase characterized by both cholinergic and glutamatergic hyperactivity and lasts another 5 to 40 min. Phase 3 is predominantly characterized by glutamatergic hyperactivity, and it is the activation of N-methyl-D-aspartic acid (NMDA) receptors and the subsequent accumulation of intracellular calcium (Ca^{2+}) in this phase that is associated with nerve agent-induced neuropathology. In the later phases, glutamatergic activation sustains the seizure even when the cholinergic system has been antagonized. Drugs with both anticholinergic and antiglutamatergic properties have been shown to be beneficial adjunct treatments for nerve agent exposure (reviewed in Weissman and Raveh, 2008).

* Corresponding author at: US Army Medical Research Institute of Chemical Defense 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, USA. Fax: +1 410 436 8377.
E-mail address: lucille.a.lange@us.army.mil (L.A. Lumley).

0041-008X/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.taap.2012.01.017

Intracellular Ca^{2+} accumulation following the prolonged stimulation of NMDA receptors during seizure activity activates Ca^{2+}-sensitive protein kinases (Churn and DeLorenzo, 1998), which modulate the trafficking of both NMDA (Lan et al., 2001) and γ-aminobutyric acid A ($GABA_A$) receptors (Jovanovic et al., 2004). $GABA_A$ receptor-mediated inhibition is diminished during SE (Kapur and Coulter, 1995), which may reduce the effectiveness of anticonvulsants such as diazepam that act on this receptor. Similarly, epileptic activity has been shown to cause the endocytosis of $GABA_A$ receptors (Blair et al., 2004; Naylor et al., 2005; Wasterlain and Chen, 2008). The administration of NMDA receptor antagonists such as ketamine or MK-801, however, prevents the seizure-induced reduction of GABAergic inhibition in experimentally induced SE (Kapur and Lothman, 1990; Wasterlain and Chen, 2008). It remains to be determined whether similar effects occur following nerve agent-induced seizures.

When glutamatergic antagonists are used alone (i.e., without anticonvulsant or anticholinergic treatments), complete protection from OP toxicity is not achieved (Lallement et al., 1999; Myhrer et al., 2008; Shih et al., 1991). However, protection is improved when antiglutamatergic treatments are combined with anticholinergics (Myhrer et al., 2008; Shih et al., 1991; Sparenborg et al., 1990). The combination of the NMDA receptor antagonist ketamine (50 mg/kg) and diazepam (20 mg/kg) has been shown to terminate pilocarpine-induced SE when the individual administration of these same doses was ineffective (Martin and Kapur, 2007). Our laboratory recently observed that the combination of ketamine and diazepam reduced seizure duration, cognitive impairment, and neuropathology in rats exposed to GD (Lumley et al., 2009). Taken together, these studies demonstrate the effectiveness of NMDA receptor antagonism as an adjunct therapy to benzodiazepine in the treatment of nerve agent exposure.

Caramiphen

Caramiphen, an anticholinergic that acts as both a muscarinic (Hudkins and DeHaven-Hudkins, 1991) and nicotinic antagonist (Gao et al., 1998), has been shown to have anticonvulsant effects in rats (Apland and Braitman, 1990; Diana et al., 1993; Sparenborg et al., 1990; Szekely et al., 1994; Tortella et al., 1988). In addition, caramiphen binds to the zinc binding site on the NMDA receptor ion channel (Raveh et al., 1999) and attenuates NMDA-evoked currents (Figueiredo et al., 2011; Fletcher et al., 1995). Though it antagonizes the NMDA receptor, CED is unable to terminate NMDA-induced epileptiform activity, which indicates that the anticonvulsant function of CED may be attributed to its anticholinergic properties (Apland and Braitman, 1990). Recently, Figueiredo et al. (2011) demonstrated that caramiphen facilitates GABA-evoked currents at 100 and 300 μM concentrations, but reduces currents at 1 mM, which suggests the possibility of a high affinity binding site that potentiates currents through the $GABA_A$ channel and a low affinity site that has the opposite effect. Additionally, caramiphen has been shown to antagonize voltage-gated Ca^{2+} channels (Church and Fletcher, 1995; Fletcher et al., 1995).

In a rat model of nerve agent exposure, caramiphen was more effective than the antimuscarinic scopolamine as an adjunct treatment to oxime (TMB4) and atropine therapy for sarin exposure in terms of reduced signs of cholinergic toxicity, reduced brain damage, and the reversal of spatial learning impairments in the Morris water maze (Raveh et al., 2008). Caramiphen was also shown to prevent the GD-induced downregulation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor site involved in learning and memory (Raveh et al., 2002), which likely contributed to the cognitive preservation in rats treated with caramiphen either prior to or following exposure to nerve agent (Raveh et al., 2002, 2008).

Caramiphen pretreatment of GD exposure by microinfusion into area tempestas (located deep in the piriform cortex) of rats was shown to delay the onset of epileptiform activity and convulsions longer than pretreatment with other drugs with similar pharmacological profiles (benactyzine, biperiden, and trihexyphenidyl) (Myhrer et al., 2008). In a second experiment in this study, drugs with only anticholinergic activity (atropine and scopolamine) or only antiglutamatergic activity (ketamine and MK-801) were used as pretreatments for GD exposure. Only the anticholinergic drugs were able to antagonize GD-induced seizures, supporting the suggestion that the anticonvulsant property of caramiphen is a result of anticholinergic rather than antiglutamatergic activity.

Physostigmine

Unlike pyridostigmine, the carbamate physostigmine crosses the blood brain barrier (Gordon et al., 1978) and thereby protects a fraction of AChE in both the peripheral and central nervous systems from being inhibited by nerve agent (Wetherell et al., 2007). A combination of physostigmine, HI-6 (an oxime that has been shown to be more effective against GD toxicity than 2-PAM Kassa and Fusek, 2002), and scopolamine protected against the lethal and incapacitating effects of the nerve agents GD, sarin, cyclosarin, VX, and tabun when administered 1 min after 5.0 LD_{50} in guinea pigs (Wetherell et al., 2006, 2007). However, a potential drawback of post-exposure treatment with carbamates is that the additive effect of AChE inhibition from both the nerve agent and the carbamate can potentiate the cholinergic crisis. Recently, it has been shown that physostigmine given 1 min after GD exposure (in combination with obidoxime and atropine) reduced the occurrence and duration of seizures in guinea pigs at 0.1 and 0.3 mg/kg; however, a higher dose (0.8 mg/kg) increased mortality (Joosen et al., 2011).

In the current study, we evaluated combination therapies with caramiphen, diazepam, and physostigmine as adjunct treatments of atropine and HI-6, for alleviation of the deleterious effects of GD exposure in rats. We used outcomes of seizure duration, neuropathology, AChE activity in whole blood, body weight, and body temperature to determine the most effective treatment combination. We hypothesized that the NMDA receptor antagonism of caramiphen would yield a synergistic effect in combination with the allosteric $GABA_A$ modulation of diazepam and would terminate GD-induced seizures earlier than treatment with diazepam or caramiphen alone. The expected result was that the anticholinergic property of caramiphen would antagonize seizures in the early phase of seizure generation and that the NMDA receptor antagonist property of caramiphen would potentiate the anticonvulsant effect of diazepam when the seizure progressed to the point where it was refractory to anticholinergic treatment. Based on recent research involving physostigmine, we hypothesized that physostigmine would attenuate the toxic effects of GD when administered 1 min after exposure.

Methods

Subjects. Male Sprague–Dawley rats (250–300 g) were individually housed and maintained on a reverse light–dark cycle (lights on 2100–0900) with food and water available ad libitum. The rats were surgically implanted with telemetry transmitters (F40-EET, Data Sciences International [DSI], Inc., St. Paul, MN, USA) for the continuous monitoring and collection of electroencephalographic (EEG) activity, body temperature, and general motor activity. Rats were weighed daily, and treatment groups were counterbalanced according to pre-exposure weight.

Chemicals. GD (pinacolyl methylphosphonofluoridate) was obtained from the US Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD, USA). Atropine sulfate (ATR) and physostigmine (PHY) were purchased from Sigma Aldrich (St. Louis, MO, USA). HI-6 dimethanesulfonate salt was prepared by Starkes Associates (Buffalo, NY, USA) under contract to the Walter Reed Army Institute of Research (Silver Spring, MD, USA). Caramiphen edisylate (CED) was a generous gift from Dr. James Apland at USAMRICD and was originally synthesized at Sigma Aldrich. Nuclear magnetic resonance analyses conducted by

Dr. Benedict Capacio's laboratory demonstrated that the sample of CED was >97% pure. Other chemicals were purchased as follows: 1) diazepam (DZP; United States Pharmacopeia, USP) from Hospira Inc. (Lake Forrest, IL, USA), 2) buprenorphine hydrochloride from Reckitt Benckiser Pharmaceuticals Inc. (Richmond, VA, USA), 3) bacitracin from Perrigo (Allegan, MI, USA), 4) Vetbond™ from 3 M Animal Care Products (St. Paul, MN, USA), and 5) isoflurane (USP) from Minrad Inc. (Bethleham, PA, USA). Chemicals used in transcardial perfusion (saline and 4% paraformaldehyde in 0.1 M phosphate buffer) as well as 20% sucrose were purchased from FD Neurotechnologies (Catonsville, MD, USA).

Surgery. For the implantation of telemetry devices, rats were administered isoflurane (3% induction, 1.5–2% maintenance) and placed in a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Cortical stainless steel screw electrodes were implanted on the skull 2 mm bilaterally to the midline and 1.6 mm anterior and 4 mm posterior to bregma. Stainless steel wires from the F40-EET transmitters were implanted subcutaneously, wrapped around the electrodes, and secured in place using dental acrylic. The incision sites were sutured, treated with topical bacitracin, and sealed with Vetbond™. Rats were administered buprenorphine (buprenex solution; 1:1 dilution in sterile water; 0.07 ml, sc) immediately after removal from anesthesia. The rats were given one week to recover prior to GD exposure.

Telemetry equipment. The home cage was placed on a DSI physiotel receiver model RPC-1 in the colony room for EEG acquisition. Data were digitized at 250 Hz, 60 Hz notch filter, 0.1 Hz hi-pass filter, 1 kHz low-pass filter and recorded using Dataquest ART 4.1 (Acquisition software; Data Systems International — DSI, St. Paul, MN, USA). Body temperature was also recorded.

Exposure. After a week of recovery from surgery, the rats were exposed to 1.2 LD_{50} GD (132 μg/kg, sc; in saline, 0.5 ml/kg) followed 1 min later by administration of HI-6 (93.6 mg/kg, im) and ATR (2 mg/kg, im) in the same injection (sterile water, 0.5 ml/kg) (Fig. 1). This dose of GD was selected to maximize survival while still allowing neuropathology to occur (Moffett et al., 2011). PHY (0.2 mg/kg, im) was administered in the same injection as HI-6 and ATR 1 min after GD exposure. CED (20 mg/kg, im; in sterile water, 0.5 ml/kg, based on Raveh et al. (2008)) and/or DZP (10 mg/kg, sc; 2 ml/kg) were administered 10, 20 or 30 min after seizure onset. Rats were given a wet mash of food, water and sugar for 3 days following exposure. Experimental groups are described in Table 1. Rats that did not seize were excluded from statistical analyses unless otherwise noted. One GD-exposed rat died prior to treatment with diazepam, and a second died within 24 h, and were omitted from analysis, with the exception of analysis of PHY effects on preventing seizure onset.

Body weight. Rats were weighed daily between 0800 and 0830. Any rat that lost more than 10 g from the previous day's weight was treated with 3.0 ml saline (0.9% saline, sc). The change in body weight following GD exposure was converted to a percentage of baseline body weight.

Electroencephalograph (EEG) scoring. EEG data were collected continuously for 3 days prior to and 3 days following GD exposure. Electrographic seizure activity was determined as rhythmic high-amplitude spikes that lasted at least 10 s (D'Ambrosio et al., 2009; de Araujo Furtado et al., 2009), and seizures were considered terminated when the EEG no longer displayed this rhythmic high-amplitude spiking. The time points for initial seizure onset and termination were recorded, and seizure duration was calculated as the difference between onset and termination. For statistical analyses, seizures lasting longer than 24 h after onset were given a maximal duration of 1440 min.

Body temperature. Body temperature was recorded continuously throughout the experiment as average temperature per minute. Recordings were normalized to the time of exposure and reduced to 1-h moving averages. As the transmitter was implanted subcutaneously the temperature values were not a measure of core body temperature. However, our laboratory has observed similar temperature values between subcutaneous transmitter implants and those implanted within the abdominal cavity (unpublished data).

Neuropathology assessment. Rats were deeply anesthetized (sodium pentobarbital; 75 mg/kg, ip) and euthanized by exsanguination 72 h after exposure (i.e., post-exposure day [PED] 3). Whole blood was collected via cardiac puncture for the WRAIR ChE assay. Rats were perfused via the ascending aorta with saline followed by 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde. The brains were then post-fixed in the same fixative for 6 h at 4 °C. After cryoprotection in 0.1 M phosphate buffer containing 20% sucrose for 72 h at 4 °C, brains were rapidly frozen and stored at −75 °C until processed. Brains were sectioned (50 μm, coronal) and processed by FD Neurotechnologies, Inc. using a proprietary silver stain (FD NeuroSilver™) and Fluoro-Jade B for the assessment of neuropathological damage, as well as cresyl violet for the identification of brain structures.

For the silver stain, each brain region of interest (piriform cortex, thalamus, amygdala, hippocampus, and fiber tracts) at coronal section bregma −3.00 mm was qualitatively scored for neuronal degeneration using a rank scale of 0–4 by an observer blind to treatment groups (de Araujo Furtado et al., 2009, 2010; McDonough and Shih, 1997; Moffett et al., 2011; Shih et al., 2003). For Fluoro-Jade B, fluorescing profiles indicating degenerating neurons were quantified per region, and then divided by the area of the region to obtain a profile density score. Brain regions were determined using *The Rat Brain in Stereotaxic*

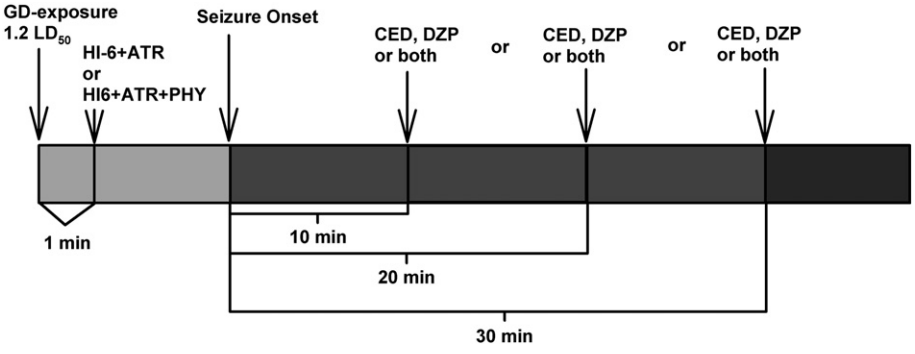

Fig. 1. Exposure outline. Rats were exposed to GD and then treated 1 min later with ATR/HI6 or ATR/HI6/PHY. At three different times after seizure onset (10, 20 or 30 min), rats were treated with CED, DZP or a combination of both drugs.

Table 1
Groups. Rats were assigned to groups based on treatment (CED and/or DZP), presence or absence of PHY, and treatment time (10, 20 or 30 min) after seizure onset.

	10 min	20 min	30 min
DZP	n = 6	n = 5	n = 8
DZP/PHY	n = 6	n = 7	n = 5
CED	n = 8	n = 9	n = 5
CED/PHY	n = 8	n = 7	n = 5
CED/DZP	n = 8	n = 9	n = 8
CED/DZP/PHY	n = 7	n = 8	n = 7

Coordinates (Paxinos and Watson, 2005). These brain regions were selected for scoring as we have previously seen neuronal degeneration in these regions following GD exposure in our rat model (Moffett et al., 2011).

WRAIR whole blood cholinesterase assay. The method for the WRAIR ChE assay has been previously described (U.S. Patent No. 6,746,850, Feaster et al., 2004; Gordon et al., 2005; Haigh et al., 2008). Briefly, the concentration of AChE was calculated based on the rate of hydrolysis for three thiocholine substrates (acetyl-, butyryl-, and propionyl-thiocholine) in whole blood samples collected 3 days after GD exposure. Cholinesterase data previously analyzed from un-exposed historical controls were used for comparison to experimental GD-exposed groups.

Statistical analysis. Statistical analyses were conducted using PASW Statistics 17 (SPSS Inc., an IBM company, Chicago, IL, USA), and statistical significance was set at $p<0.05$. Seizure duration within the first 24 h was estimated and compared among treatment groups using a Kaplan–Meier analysis. A Log Rank (Mantel–Cox) analysis was performed to compare each treatment group to the DZP group at each treatment time. Presence or absence of seizures in terms of PHY treatment was analyzed using a Fisher's exact test. Percent of body weight change from baseline following exposure was analyzed using a four-factor analysis of variance (ANOVA) with one repeated measure; the four factors were treatment group (CED, DZP, and CED/DZP), treatment time, presence or absence of PHY, and observation time (repeated measure). The Greenhouse-Geisser correction was used to correct for violations of sphericity. Body temperature was also analyzed using a similar four-factor ANOVA with one repeated measure. A linear regression analysis was used to compare temperature recovery between treatment groups. AChE in whole blood was analyzed using a three-factor ANOVA with treatment group, treatment time, and presence or absence of PHY as the three factors. A three-factor (treatment group, treatment time, and presence or absence of PHY) multivariate ANOVA (MANOVA) was used to compare treatment groups and assess neuropathology in selected brain regions. Post hoc analyses were conducted using a Dunnett's test to compare treatment groups to control conditions within each factor.

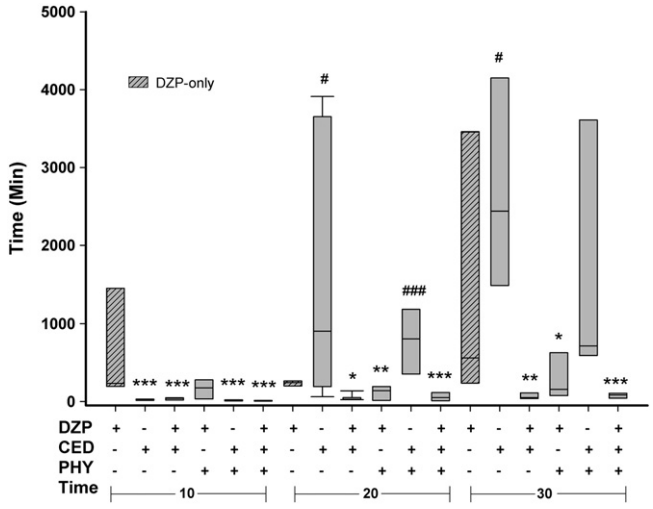

Fig. 2. Seizure duration. Seizure duration (box and whisker plot; box represents 25th–75th percentile, the line within the box marks the median, whiskers represent 10th and 90th percentiles) following GD exposure grouped by PHY or non-PHY treatment (1 min after GD exposure along with HI-6 and ATR) and by DZP, CED, or CED/DZP treatment (10, 20 or 30 min after GD exposure). Comparisons are made within each time point to the DZP group (hatched box). CED, with or without DZP, at 10 min decreased seizure duration relative to DZP, regardless of PHY treatment. CED at 20 and 30 min and CED/PHY at 20 min in the absence of DZP increased seizure duration relative to DZP. CED/PHY at 30 min was not significantly different from DZP. CED/DZP decreased seizure duration relative to DZP at all time points regardless of PHY treatment. (*$p<0.05$, **$p<0.01$, ***$p<0.001$ < DZP at respective time point; #$p<0.05$, ###$p<0.001$ > DZP only at respective time point).

Table 2
Seizure occurrence in the presence or absence of PHY treatment.

	Seizure	No seizure	Total
NonPHY	67 (95.7%)	3	70
PHY	63 (78.7%)**	17	80

** $p<0.01$.

Results

Seizure duration

As depicted in Fig. 2, at the 10-min treatment time, the following groups displayed shorter seizure durations compared to the DZP: CED/DZP, CED/DZP/PHY, CED/PHY, and CED. The seizure duration for the DZP/PHY group did not significantly differ from the DZP group. At the 20-min treatment time, the following groups had shorter seizure durations compared to the DZP: CED/DZP, CED/DZP/PHY, and DZP/PHY. The following groups had significantly greater seizure durations than the DZP: CED/PHY and CED. At the 30-minute treatment time, the following groups had shorter seizure durations compared to the DZP: CED/DZP, CED/DZP/PHY, and DZP/PHY. The seizure duration of the CED/PHY group did not differ significantly from that of the DZP group. The CED group had greater seizure duration compared to the DZP group. Statistics are displayed in Suppl. Table. 1.

Of the 80 animals that received PHY, 63 (78.7%) experienced seizure versus 67 (95.7%) of the 70 animals that did not receive PHY (Table 2). This difference in seizure incidence between the non-PHY and PHY groups was significant using a Fisher's exact test ($p<0.01$).

AChE in whole blood

Rats treated with PHY had 29.9% more AChE activity in whole blood at 72 h after GD exposure than rats not treated with PHY ($F(1,109) = 9.075$, $p<0.01$). No other significant effects were observed (data not shown). The mean AChE activity for PHY-treated rats was 55.3% of historical controls, compared to 43.7% for non-PHY-treated rats.

Body temperature

At the time of exposure, the mean body temperature was 36.3 ± 1.2 °C. Main effects of the repeated observation (hour), treatment, PHY, and treatment time are displayed in Suppl. Table 2A. In sum, there were significant main effects of hour, treatment, PHY and significant interactions between multiple factors (treatment × time; treatment × hour X time; PHY × hour). Suppl. Table 2B–D displays statistics for the analysis when stratified by treatment time (10, 20 and 30 min, respectively). All three time points show a transient temperature drop. There was no effect of treatment at 10 min (Fig. 3A; Suppl. Table 2B). Rats treated with CED at 20 min had significantly lower

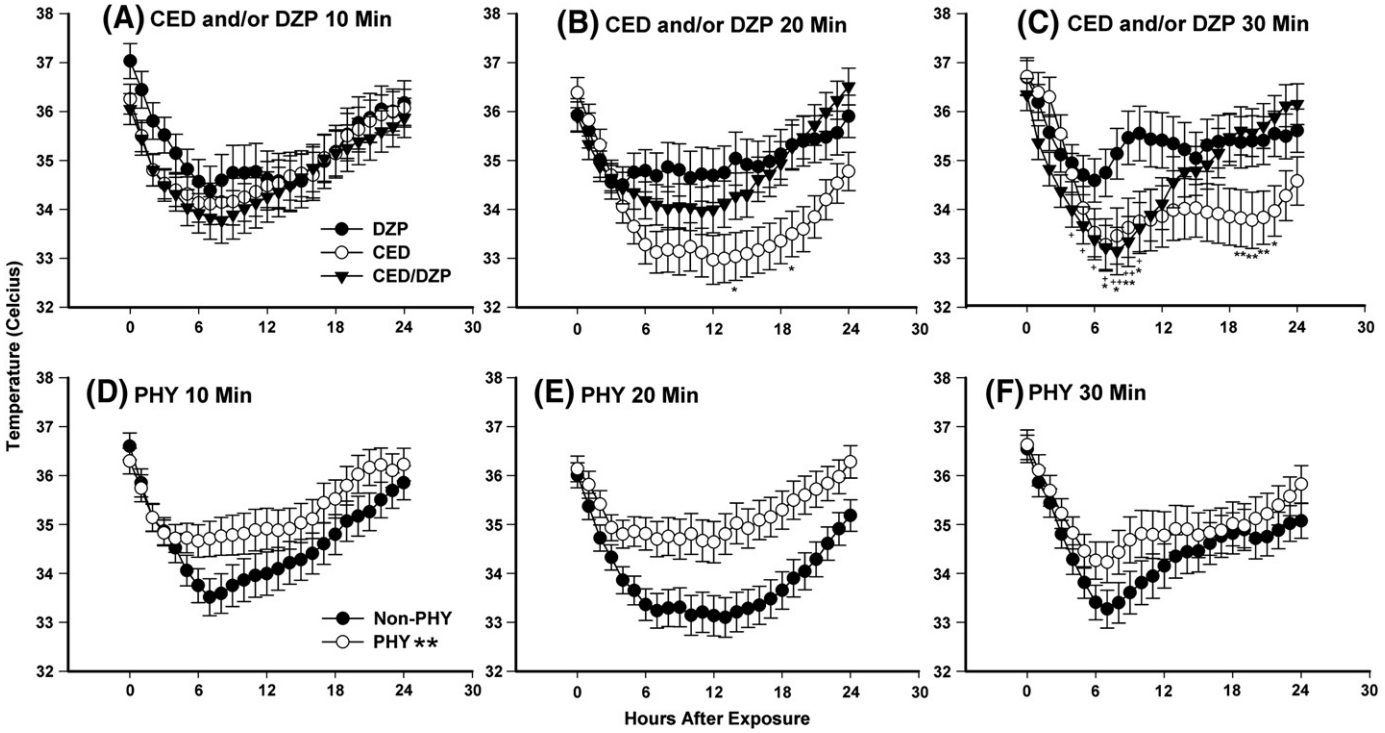

Fig. 3. Body temperature. Body temperature (mean ± SEM) following GD exposure grouped by treatment time (10 [A, D], 20 [B, E] or 30 [C, F] min), CED and/or DZP treatment (A–C) and PHY treatment (D–F). (Note: PHY treatment was given 1 min after GD.) PHY attenuated temperature loss following GD-exposure. CED and CED/DZP treatment resulted in increased temperature loss following GD exposure when administered at 30 min. (*p<0.05 CED ≠ DZP C *p<0.05 **p<0.01 CED ≠ DZP; +p<0.05, ++p<0.01 CED/CED ≠ DZP).

temperature than DZP rats for a brief period 19 h following GD-exposure (Fig. 3B; Suppl. Table 2C). At 30 min, CED and CED/DZP treated rats had transient periods of lower temperature than DZP rats (Fig. 3C; Suppl. Table 2D). PHY treated rats had higher temperature than non-PHY treated rats from 4 to 24 h following GD-exposure (Figs. 3D–F; Suppl. Table 2E).

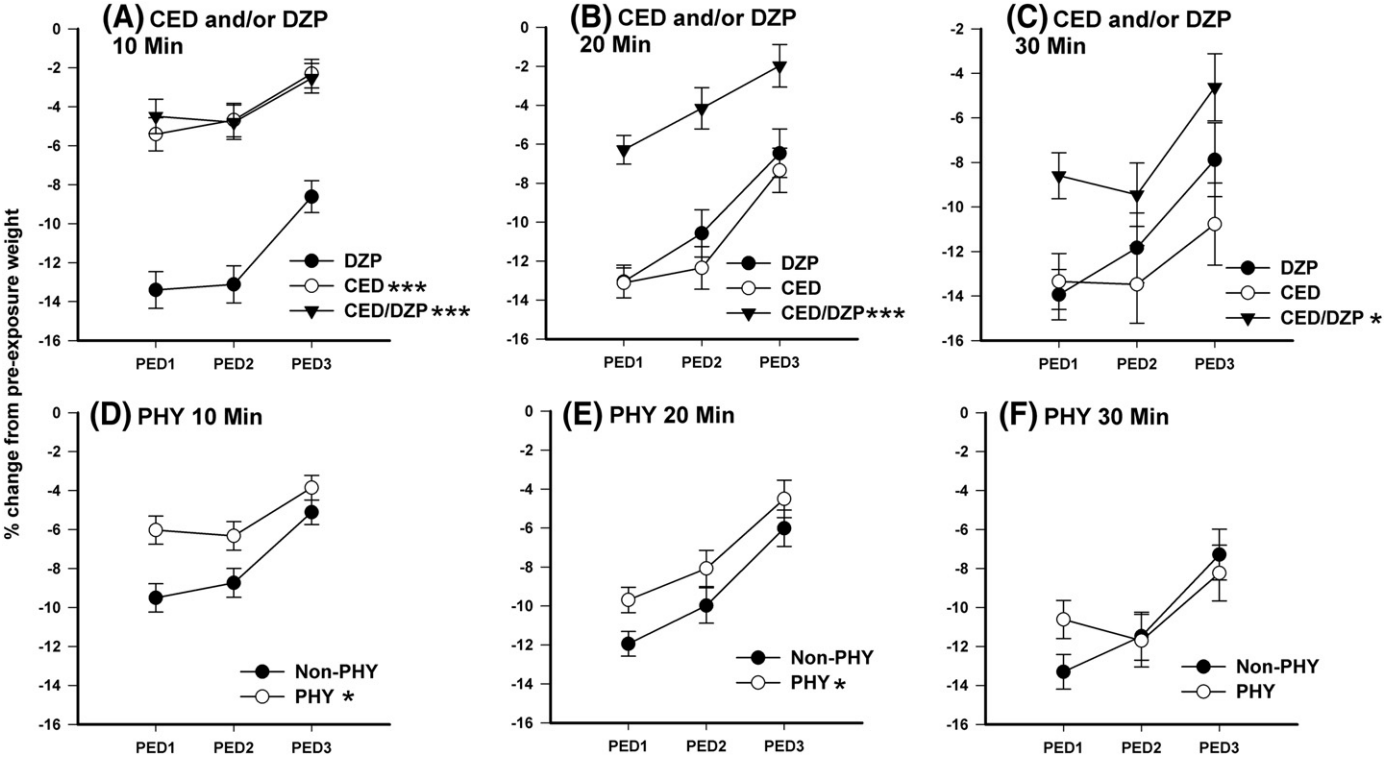

Fig. 4. Body weight loss. Change in body weight (mean ± SEM) following GD exposure grouped by treatment time (10 [A, D], 20 [B, E] or 30 [C, F] min), CED and/or DZP treatment (A–C) and PHY treatment (D–F). PED is the abbreviation for post-exposure day. CED/DZP treatment attenuated body weight loss at 10, 20 and 30 min. PHY attenuated body weight loss at 10 and 20 min. CED attenuated body weight loss at 10 min.

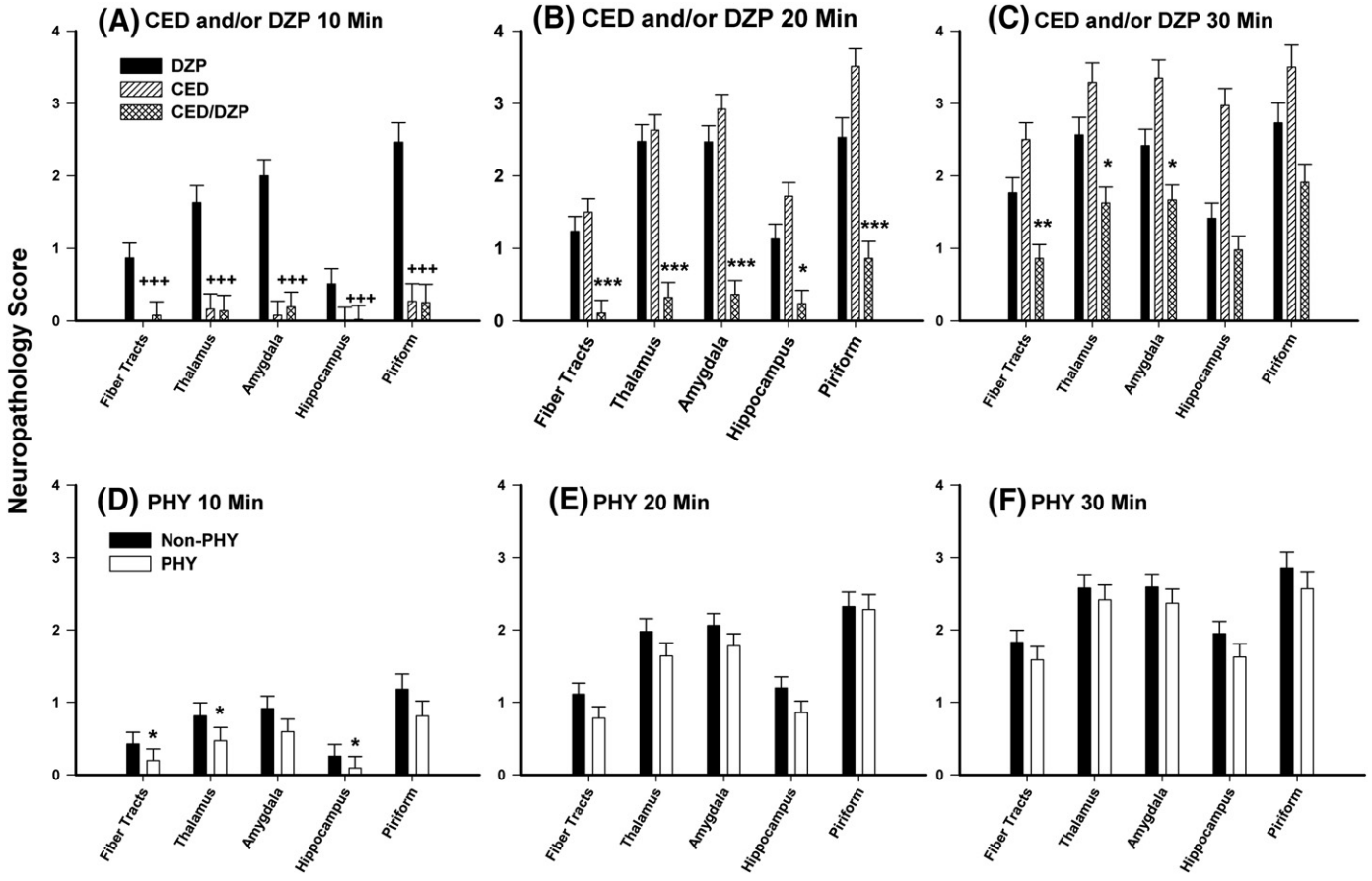

Fig. 5. Neuropathology graphs (silver). Neuropathology score within each region (mean + SEM) following GD exposure grouped by treatment time (10 min [A, D], 20 min [B, E] or 30 min [C, F]), CED and/or DZP treatment (A–C) and PHY treatment (D–F). CED/DZP treatment resulted in lower neuropathology compared to DZP at all time points. CED resulted in lower neuropathology when administered at 10 min only. There was no overall effect of PHY. Treatments administered at 10 min resulted in lower neuropathology than did treatments at 20 or 30 min. In the fiber tracts, thalamus and hippocampus, treatment at 20 min resulted in lower neuropathology than at 30 min. (+++ both CED and CED/DZP less than DZP, $p<0.001$).

Body weight

Statistics for body weight is presented in Suppl. Table 3. There were significant effects of repeated day, treatment, PHY, and treatment time on body weight, as well as interactions between factors (treatment × treatment time, day × PHY, and day × treatment × PHY; further investigation into these interactions are displayed in Suppl. Table 3B–D). In general, rats lost weight following GD-exposure. CED and CED/DZP reduced the amount of weight lost when treated at 10 min in comparison to DZP only rats (Fig. 4A); however, when delayed 20 and 30 min, attenuation of weight loss was only seen in rats treated with CED/DZP (Figs. 4B–C). PHY treatment had marginal success at attenuating weight loss following GD-exposure on day 1, but not when DZP and/or CED administration was delayed 30 min (Figs. 4D–F; Suppl. Table 3B–D).

Neuropathology assessments

For the silver stain (Figs. 5–6; Suppl. Table 4), there was significant effect of treatment, treatment time, and a significant interaction between treatment and treatment time on neuropathology. There was no overall effect of PHY and no interactions between PHY and time or treatment. At 10 min, there was a significant effect of CED and/or DZP treatment. CED and CED/DZP treatment resulted in lower neuropathology compared to DZP in all regions measured. At 20 min, there was a significant effect of CED and/or DZP treatment. CED/DZP treatment resulted in significantly lower neuropathology compared to DZP in all regions scored. At 30 min, there was a significant effect of CED and/or DZP treatment. CED/DZP treatment resulted in lower neuropathology in the fiber tracts, thalamus and amygdala.

A MANOVA was conducted within each treatment group to compare the effect of treatment time (Suppl. Table 4D). For each treatment, the earlier treatment time of 10 min was more protective than delayed treatment (Fig. 5). Within the DZP group, there was a significant effect of time. Treatment with DZP at 10 min resulted in lower neuropathology in fiber tracts and hippocampus when compared to DZP at 30 min. DZP at 20 min did not differ from DZP at 30 min. Within the CED group, there was a significant overall effect of time. CED treatment at 10 min resulted in lower neuropathology in all regions in comparison to 30 min. CED treatment at 20 min resulted in lower neuropathology in fiber tracts, thalamus and hippocampus in comparison to 30 min. Within the CED/DZP treatment, there was a significant effect of time. CED/DZP treatment at 10 min or 20 min resulted in lower neuropathology in all regions in comparison to 30 min.

A stepwise linear regression analysis between seizure duration and damage to all sub-regions revealed that damage to the hippocampus had a predictive relationship with seizure duration ($R^2=0.463$, $F(1,124)=106.122$, $p<0.001$; Hippocampus $\beta=0.681$, $p<0.001$, data not shown). Damage to other regions within this analysis did not yield significant predictive relationships with seizure duration. This suggests that the hippocampus may be more sensitive to longer seizures than other regions in terms of neuropathology determined via silver stain.

For Fluoro-Jade B stain (Figs. 7–8; Suppl. Table 5), there was a significant effect of treatment, time, and an interaction between treatment and time. There was no overall effect of PHY and no interactions

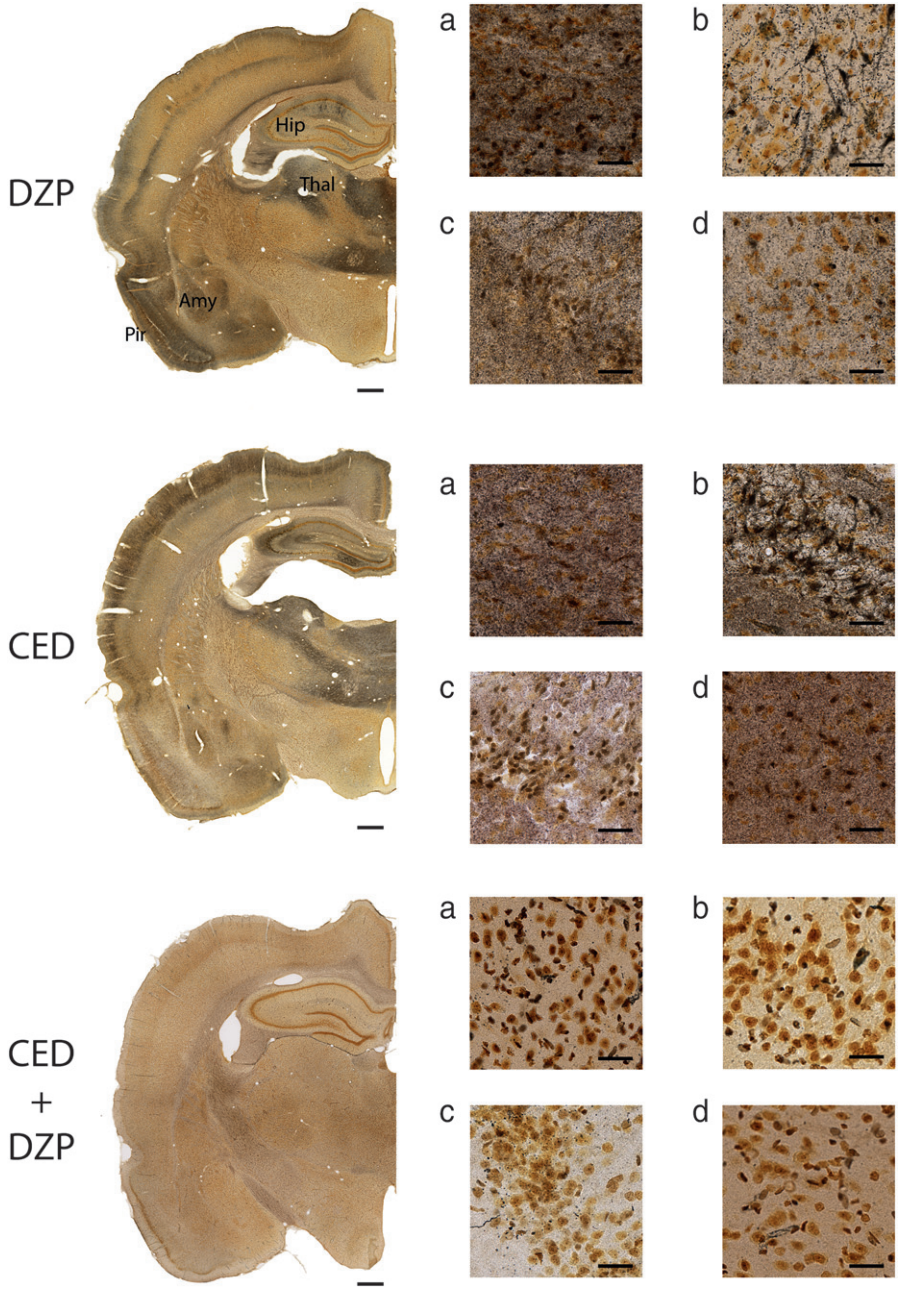

Fig. 6. Silver neuropathology images. Representative images of silver-stained histological preparations from rats treated with DZP, CED or CED/DZP at 30 min (non-PHY) organized in rows. Left: half brain images, scale bar = 500 μm. Right: enlarged images of specific nuclei (in parentheses) within a larger region scored, scale bar = 40 μm. A: Thalamus (reiunnes). B: Hippocampus (dentate gyrus). C: Piriform cortex (layer 2). D: Amygdala (basolateral).

between PHY and treatment or time (Fig. 7). At 10 min, both CED and CED/DZP resulted in lower neuropathology than DZP in all regions. At 20 min the CED/DZP group, but not the CED group, had lower neuropathology than the DZP group in the thalamus, amygdala, and piriform cortex. At 30 min the CED/DZP group, but not the CED group, had lower neuropathology than DZP in the amygdala.

A MANOVA was conducted within each treatment group to compare the effect of treatment time (Suppl. Table 5D). For DZP, there were no differences in profile density between different treatment time points. Treatment with CED at 10 min resulted in lower neuropathology in all regions compared to the 30 min. Treatment with CED at 20 min resulted in lower neuropathology in the thalamus and amygdala in comparison to the 30 min group. Treatment with CED/DZP at 10 or 20 min resulted in lower neuropathology in the thalamus, amygdala and piriform cortex in comparison to the 30 min group. These results support the findings using silver stain in that treatment with DZP is less time sensitive than CED or CED/DZP.

A stepwise linear regression analysis between seizure duration and profile density in all subregions scored indicated that profile density in the hippocampus and amygdala had a significant predictive relationship with seizure duration ($R^2 = 0.397$, $F(1,124) = 40.878$, $p < 0.001$; amygdala $\beta = 0.435$, $t = 5.47$, $p < 0.001$; hippocampus $\beta = 0.292$, $t = 3.665$, $p < 0.001$). This data suggests that both the hippocampus and amygdala may be more sensitive to longer seizures than other regions in terms of neuropathology as determined via Fluoro-Jade-B staining (data not shown).

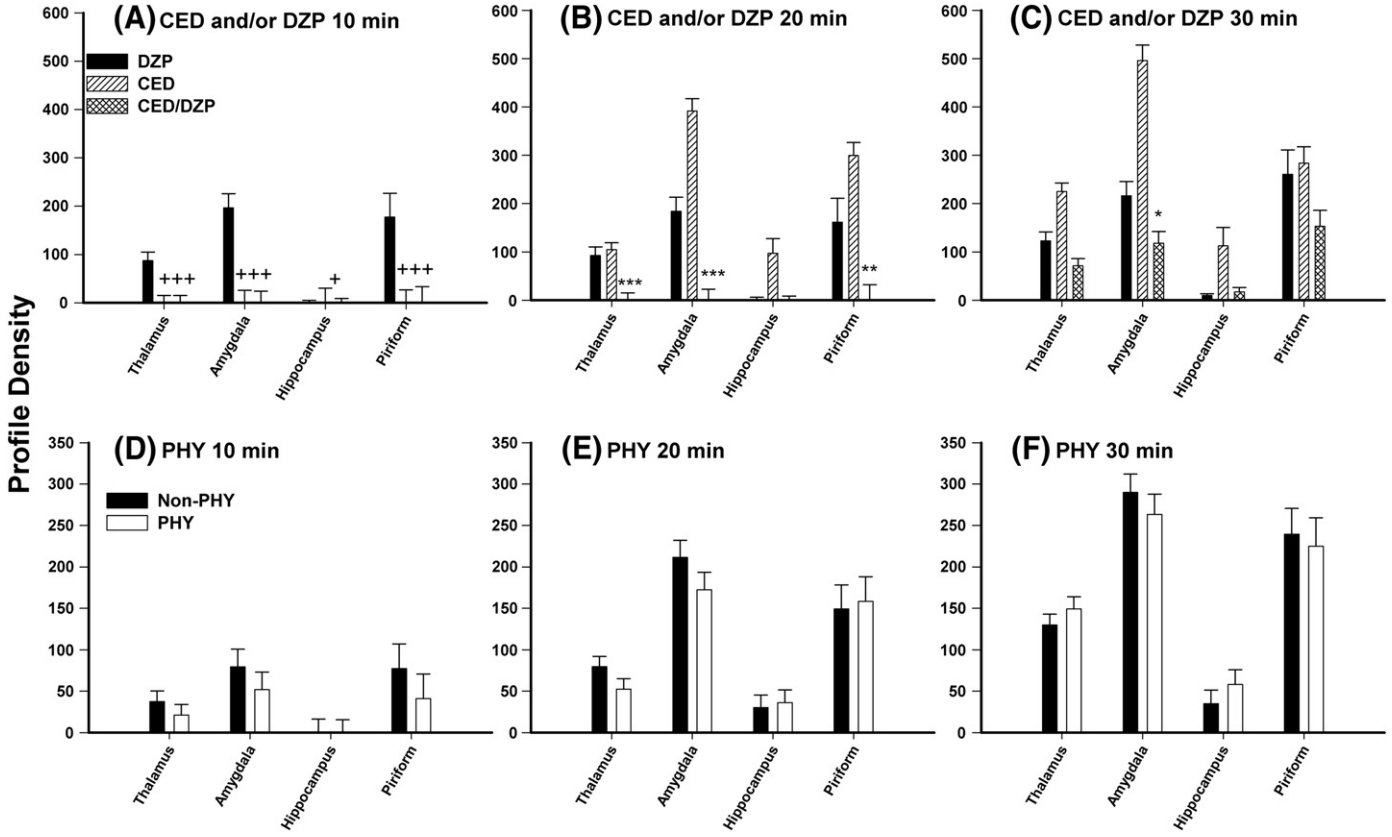

Fig. 7. Neuropathology graphs (Fluoro-Jade B). Neuropathology score within each region (mean + SEM) following GD exposure grouped by treatment time (10 min [A, D], 20 min [B, E] or 30 min [C, F]), CED and/or DZP treatment (A–C) and PHY treatment (D–F). CED/DZP treatment resulted in lower neuropathology compared to DZP at all time points. CED resulted in lower neuropathology when administered at 10 min only. There was no overall effect of PHY. Treatments administered at 10 min resulted in lower neuropathology than did treatments at 20 or 30 min. In the fiber tracts, thalamus and hippocampus, treatment at 20 min resulted in lower neuropathology than at 30 min. (+++ both CED and CED/DZP less than DZP, $p<0.001$).

Discussion

The standard treatment regimen for nerve agent exposure includes an anticholinergic drug to counteract the acute cholinergic syndrome, an oxime to reactivate AChE, and an anticonvulsant to treat seizures and prevent neuropathology. Unfortunately, complete protection is not provided by these treatments. In fact, our laboratory recently showed that contextual and auditory fear conditioning is impaired in GD-exposed rats despite being administered the standard treatment regimen (Moffett et al., 2011). Moreover, moderate to severe neuropathology was observed in the amygdala, piriform cortex, and thalamus of these rats. Additional treatments to reduce the seizure duration, neuropathology and/or functional impairments that follow nerve agent exposure are needed. One class of potential adjunct therapies to current treatments includes drugs with both anticholinergic and antiglutamatergic properties, such as CED. Drugs with this pharmacological profile have the potential to 1) inhibit the propagation of epileptiform activity through antagonism of cholinergic receptors, 2) mitigate the excitotoxicity produced by acceleration of NMDA receptor stimulation and subsequent accumulation of intracellular Ca^{2+} (Weissman and Raveh, 2008) and 3) prevent the reduction of GABAergic currents through NMDA receptor inhibition and increase the efficacy of anticonvulsant treatments. The present study was conducted to evaluate the efficacy of CED, with or without DZP, as an adjunct therapeutic against GD-induced seizures and neuropathology. We hypothesized that the co-administration of CED and DZP would terminate GD-induced seizures faster than DZP alone and thereby reduce/prevent neuropathology. The effectiveness of PHY as a therapeutic was also evaluated.

GD-exposed rats treated with DZP continued to have seizures for more than 3 h, which is consistent with previous studies in our laboratory (Langston et al., 2011; Moffett et al., 2011), regardless of when the treatment was administered. Body temperature in DZP-treated rats was reduced over the course of the first 8 h following exposure irrespective of treatment time. Meeter and Wolthuis (1968) observed a 4–6 °C drop in body temperature with GD-exposed rats; however, their rats did not receive any therapy. ATR has been shown to reduce nerve agent-induced hypothermia (Clement, 1993; Meeter and Wolthuis, 1968), which suggests that this effect is mediated by muscarinic receptors. Interestingly, DZP has been shown to dose-dependently decrease body temperature (Elliot and White, 2001) and to reduce stress-induced hyperthermia (Vinkers et al., 2009). DZP-treated rats also lost ~14% of their body weight within the first 24 h following exposure irrespective of treatment time.

GD-exposed rats treated with CED at 10 min had shorter seizure durations, less body weight loss, and reduced neuropathology compared to DZP-treated rats. Early body weight loss is an indicator of seizure severity and, by corollary extension, neuropathology following GD exposure in rats (Churchill et al., 1985). Neuropathology, specifically in the amygdala, piriform cortex, and thalamus, was observed in these rats, but the severity was reduced by earlier treatment with CED with or without DZP. Unfortunately, CED by itself was not an effective therapeutic at the later treatment times when GD-induced seizures are no longer solely controlled by cholinergic mechanisms. GD-exposed rats treated with CED at 20 or 30 min had seizures that lasted longer than those of DZP-treated rats and, in many cases, continued until the end of the EEG recording (72 h). In addition, there was no difference in terms of body weight or neuropathology between rats that received CED treatment alone or DZP-treatment alone at these treatment times. At 30 min, CED-treated rats experienced a greater drop in body temperature than did DZP-treated rats. Recently, a higher dose of CED

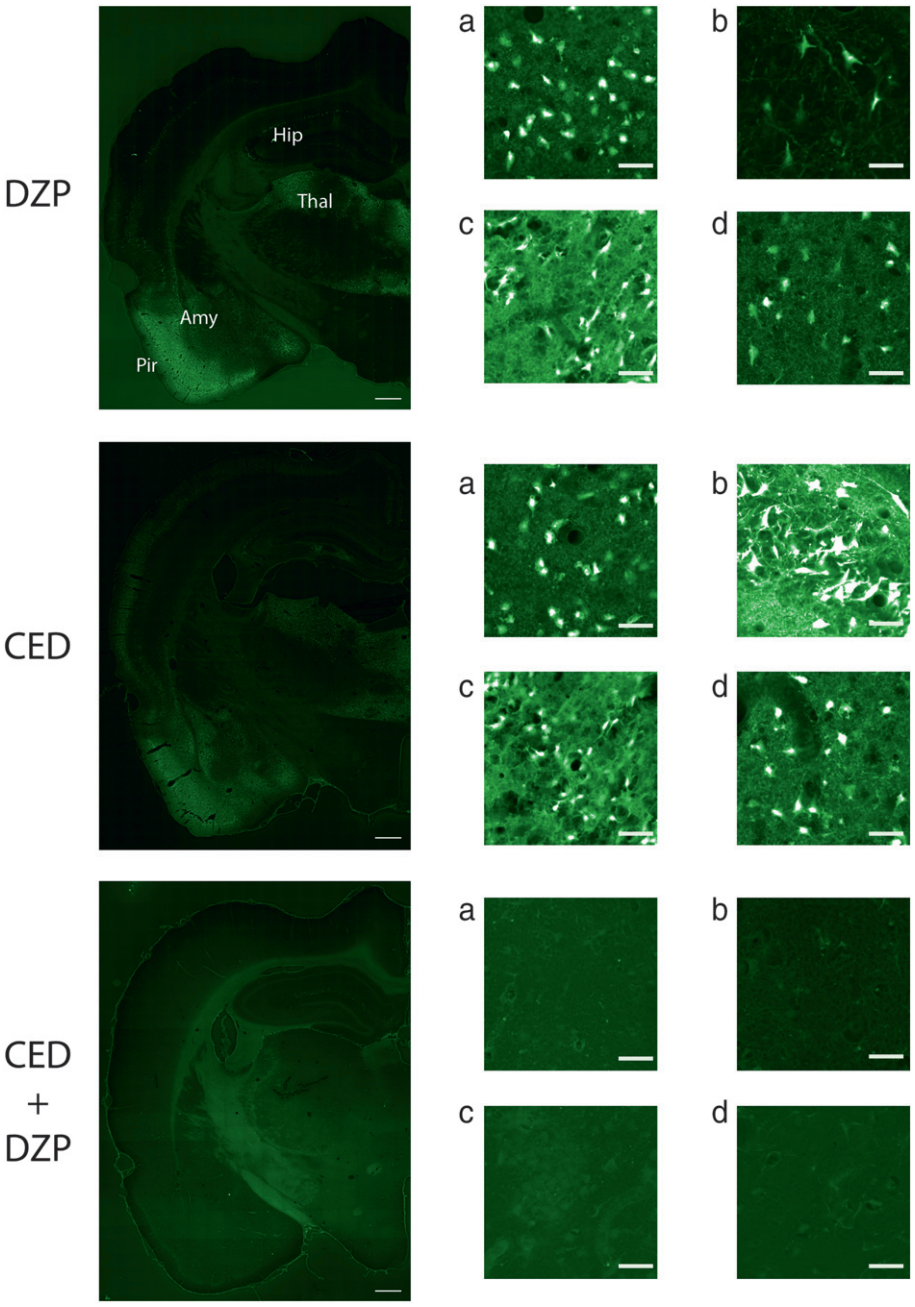

Fig. 8. Fluoro-Jade B neuropathology images. Representative images of Fluoro-Jade B-stained histological preparations from rats treated with DZP, CED or CED/DZP at 30 min (non-PHY) organized in rows. Left: half brain images, scale bar = 500 μm. Right: enlarged images of specific nuclei (in parentheses) within a larger region scored, scale bar = 40 μm. A: Thalamus (reiunnes). B: Hippocampus (dentate gyrus). C: Piriform cortex (layer 2). D: Amygdala (basolateral).

(100 mg/kg) was shown to reduce the behavioral signs of seizure associated with GD exposure, in the absence of DZP or another anticonvulsant treatment, when administered 30 or 60 min after seizure onset (Figueiredo et al., 2011). Seizures were abolished within 1–2 h or 4–4.5 h, respectively, and neuropathology was also reduced in these rats. Thus, it seems that a higher dose of CED than the one used in this study in the absence of diazepam would be required to achieve a superior anticonvulsant effect compared to DZP treatment. When co-administered with levetiracetam (Keppra®), an antiepileptic drug suspected of reducing glutamate release (Lynch et al., 2004), 40 min after seizure onset, CED (20 mg/kg ip) completely terminated 1.6 LD_{50} GD-induced epileptiform activity in the absence of benzodiazepine treatment (Myhrer et al., 2011).

GD-exposed rats treated with CED/DZP had shorter seizure durations and reduced neuropathology compared to DZP-treated rats regardless of treatment time. CED/DZP-treated rats also had less body weight loss than DZP-treated rats. Although the initial drop in body temperature was similar at treatment time 30 min, CED/DZP-treated rats recovered at a faster rate from GD-induced mild hypothermia than did CED-treated rats. Thus, the combination of CED and DZP proved to be the most effective therapeutic strategy against GD-induced toxicity.

A possible mechanism for this synergistic action between CED and DZP would be the prevention of seizure-induced reduction of GABAergic inhibition through NMDA receptor antagonism. The influx and accumulation of intracellular Ca^{2+} through NMDA receptors during epileptiform activity may be responsible for the alteration of GABAergic

signaling (Goodkin et al., 2005; Kapur and Coulter, 1995; Kapur and Lothman, 1990; Martin and Kapur, 2007; Naylor et al., 2005; Wasterlain and Chen, 2008) and thus the reduced efficacy of benzodiazepine treatment. In addition, SE in rats has been shown to result in the mobilization of spare NMDA receptors to the cell surface (Wasterlain et al., 2002). It's possible that attenuation of NMDA receptor activation by CED reduced the NMDA receptor-dependent alteration of $GABA_A$ receptor trafficking in addition to blocking NMDA receptor-dependent neurotoxicity, thus preventing the reduction of benzodiazepine anticonvulsant efficacy. Recently, GD has been shown to dose-dependently cause $GABA_A$ receptor endocytosis as well as to reduce GABAergic inhibitory post synaptic currents (IPSC) (Wang et al., 2011). To provide an effective treatment for nerve agent exposure, treatment strategies must be developed to mitigate this reduction in GABAergic signaling, thereby restoring efficacy of benzodiazepine treatment. The results presented in this paper show that CED was able to increase the anticonvulsant efficacy of DZP and reduce neuropathology in the rat brain.

Post-exposure treatment with PHY reduced the percentage of rats developing seizures. Although PHY had no effect on seizure duration or neuropathology in animals that did develop seizures, PHY-treated rats did not experience as severe a drop in body temperate or lose as much body weight as non-PHY-treated rats. PHY-treated rats also had more AChE in whole blood at 72 h following exposure than did non-PHY-treated rats. Thus, we have shown that PHY treatment (under the conditions used herein) does not potentiate GD-induced seizure activity. However, it has recently been shown that a higher dose of PHY (0.8 mg/kg) can increase mortality following GD exposure (Joosen et al., 2011), suggesting that the safety margin for PHY treatment is very small.

In conclusion, we determined that the combination of CED, DZP, and PHY along with ATR and HI-6 provided additional protection against GD exposure above that provided by just ATR, HI-6, and DZP. The anticonvulsant efficacy of CED was evident at the earliest treatment time, and the synergistic effect between CED and DZP allowed for shorter seizure durations and reduced neuropathology across all treatment times. PHY prevented seizures in a subset of GD-exposed rats and attenuated the hypothermia and body weight loss associated with GD exposure. In future studies, we intend to further examine the treatment combination of CED and DZP on the prevention of long-term cognitive consequences related to GD exposure.

Supplementary materials related to this article can be found online at doi:10.1016/j.taap.2012.01.017.

Conflict of interest statement

The authors declare that there are no conflicts of interest related to this study.

Disclaimers

The views expressed in this article are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89–544), as amended.

Abbreviations

GD	soman
DZP	diazepam
GABA	γ-aminobutyric acid
CED	caramiphen edisylate
AChE	acetylcholinesterase
ACh	acetylcholine
PHY	physostigmine
OP	organophosphorus
BuChE	butyrylcholinesterase
ChE	cholinesterase
SE	status epilepticus
ATR	atropine sulfate
2-PAM	2-pralidoxime
NMDA	N-methyl-D-aspartic acid
AMPA	α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid
EEG	electroencephalograph
PED	post-exposure day
IPSC	inhibitory post-synaptic currents

Acknowledgments

This research was supported by the Defense Threat Reduction Agency-Joint Science and Technology Office, Medical S&T Division and Physical Science Division (PI: Dr. Lucille Lumley). Mark Schultz and Dr. Linnzi Wright were supported by appointments to the Student/Postdoctoral Research Participation Program at the U.S. Army Medical Research Institute of Chemical Defense administered by the Oak Ridge Institute for Science and Education through an interagency appointment between the U.S. Department of Energy and USAMRMC.

The authors would like to thank Dr. James Apland, Dr. Gary Rockwood, Dr. Taiza Figueiredo and Cindy Kronman for institutional review of this manuscript. In addition, the authors acknowledge Dr. Apland for his generous gift of CED, Dr. Sunil Soni for nuclear magnetic resonance analysis of CED and Wuya Lumeh, Allison Nelson and Harlan Schafer for technical assistance.

References

Apland, J.P., Braitman, D.J., 1990. Effects of non-opioid antitussives on epileptiform activity and NMDA responses in hippocampal and olfactory cortex slices. Brain Res. 529, 277–285.

Blair, R.E., Sombati, S., Lawrence, D.C., McCay, B.D., DeLorenzo, R.J., 2004. Epileptogenesis causes acute and chronic increases in GABAA receptor endocytosis that contributes to the induction and maintenance of seizures in the hippocampal culture model of acquired epilepsy. J. Pharmacol. Exp. Ther. 310, 871–880.

Cannard, K., 2006. The acute treatment of nerve agent exposure. J. Neurol. Sci. 249, 86–94.

Church, J., Fletcher, E.J., 1995. Blockade by sigma site ligands of high voltage-activated Ca2 + channels in rat and mouse cultured hippocampal pyramidal neurones. Br. J. Pharmacol. 116, 2801–2810.

Churchill, L., Pazdernik, T.L., Jackson, J.L., Nelson, S.R., Samson, F.E., McDonough Jr., J.H., McLeod Jr., C.G., 1985. Soman-induced brain lesions demonstrated by muscarinic receptor autoradiography. Neurotoxicology 6, 81–90.

Churn, S.B., DeLorenzo, R.J., 1998. Modulation of GABAergic receptor binding by activation of calcium and calmodulin-dependent kinase II membrane phosphorylation. Brain Res. 809, 68–76.

Clement, J.G., 1993. Pharmacological nature of soman-induced hypothermia in mice. Pharmacol. Biochem. Behav. 44, 689–702.

D'Ambrosio, R., Hakimian, S., Stewart, T., Verley, D.R., Fender, J.S., Eastman, C.L., Sheerin, A.H., Gupta, P., Diaz-Arrastia, R., Ojemann, J., Miller, J.W., 2009. Functional definition of seizure provides new insight into post-traumatic epileptogenesis. Brain 132, 2805–2821.

de Araujo Furtado, M., Zheng, A., Sedigh-Sarvestani, M., Lumley, L., Lichtenstein, S., Yourick, D., 2009. Analyzing large data sets acquired through telemetry from rats exposed to organophosphorous compounds: an EEG study. J. Neurosci. Methods 184, 176–183.

de Araujo Furtado, M., Lumley, L.A., Robison, C., Tong, L.C., Lichtenstein, S., Yourick, D.L., 2010. Spontaneous recurrent seizures after status epilepticus induced by soman in Sprague-Dawley rats. Epilepsia 51, 1503–1510.

Diana, G., Scotti de Carolis, A., Popoli, P., Pezzola, A., Sagratella, S., 1993. Non-opioid antitussives potentiate some behavioural and EEG effects of N-methyl-D-aspartate channel blockers. Life Sci. 52, 1547–1557.

Eckert, S., Eyer, P., Worek, F., 2007. Reversible inhibition of acetylcholinesterase by carbamates or huperzine A increases residual activity of the enzyme upon soman challenge. Toxicology 233, 180–186.

Elliot, E.E., White, J.M., 2001. The acute effects of zolpidem compared to diazepam and lorazepam using radiotelemetry. Neuropharmacology 40, 717–721.

Feaster, S.R., Gordon, R.K., Doctor, B.P., 2004. Assay for detecting, measuring and monitoring the activities and concentrations of proteins and methods of use thereof Patent (US 6,746,850 B2), June 8, 2004.

Figueiredo, T.H., Aroniadou-Anderjaska, V., Qashu, F., Apland, J.P., Pidoplichko, V., Stevens, D., Ferrara, T.M., Braga, M.F.M., 2011. Neuroprotective efficacy of caramiphen against soman and mechanisms of action. Br. J. Pharmacol. doi:10.1111/j.1476-5381.2011.01427.x

Fletcher, E.J., Church, J., Abdel-Hamid, K., MacDonald, J.F., 1995. Blockade by sigma site ligands of N-methyl-D-aspartate-evoked responses in rat and mouse cultured hippocampal pyramidal neurones. Br. J. Pharmacol. 116, 2791–2800.
Gao, Z.G., Liu, B.Y., Cui, W.Y., Li, L.J., Fan, Q.H., Liu, C.G., 1998. Anti-nicotinic properties of anticholinergic antiparkinson drugs. J. Pharm. Pharmacol. 50, 1299–1305.
Goodkin, H.P., Yeh, J.L., Kapur, J., 2005. Status epilepticus increases the intracellular accumulation of GABAA receptors. J. Neurosci. 25, 5511–5520.
Gordon, J.J., Leadbeater, L., Maidment, M.P., 1978. The protection of animals against organophosphate poisoning by pretreatment with a carbamate. Toxicol. Appl. Pharmacol. 43, 207–216.
Gordon, R.K., Haigh, J.R., Garcia, G.E., Feaster, S.R., Riel, M.A., Lenz, D.E., Aisen, P.S., Doctor, B.P., 2005. Oral administration of pyridostigmine bromide and huperzine A protects human whole blood cholinesterases from ex vivo exposure to soman. Chem. Biol. Interact. 157–158, 239–246.
Haigh, J.R., Lefkowitz, L.J., Capacio, B.R., Doctor, B.P., Gordon, R.K., 2008. Advantages of the WRAIR whole blood cholinesterase assay: comparative analysis to the micro-Ellman, test-mate ChE, and Michel (DeltapH) assays. Chem. Biol. Interact. 175, 417–420.
Hudkins, R.L., DeHaven-Hudkins, D.L., 1991. M1 muscarinic antagonists interact with sigma recognition sites. Life Sci. 49, 1229–1235.
Joosen, M.J., Smit, A.B., van Helden, H.P., 2011. Treatment efficacy in a soman-poisoned guinea pig model: added value of physostigmine? Arch. Toxicol. 85, 227–237.
Jovanovic, J.N., Thomas, P., Kittler, J.T., Smart, T.G., Moss, S.J., 2004. Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating GABA(A) receptor phosphorylation, activity, and cell-surface stability. J. Neurosci. 24, 522–530.
Junge, W., Krisch, K., 1975. The carboxylesterases/amidases of mammalian liver and their possible significance. CRC Crit. Rev. Toxicol. 3, 371–435.
Kapur, J., Coulter, D.A., 1995. Experimental status epilepticus alters gamma-aminobutyric acid type A receptor function in CA1 pyramidal neurons. Ann. Neurol. 38, 893–900.
Kapur, J., Lothman, E.W., 1990. NMDA receptor activation mediates the loss of GABAergic inhibition induced by recurrent seizures. Epilepsy Res. 5, 103–111.
Kassa, J., Fusek, J., 2002. The influence of oxime selection on the efficacy of antidotal treatment of soman-poisoned rats. Acta Medica (Hradec Kralove) 45, 19–27.
Lallement, G., Baubichon, D., Clarencon, D., Galonnier, M., Peoc'h, M., Carpentier, P., 1999. Review of the value of gacyclidine (GK-11) as adjuvant medication to conventional treatments of organophosphate poisoning: primate experiments mimicking various scenarios of military or terrorist attack by soman. Neurotoxicology 20, 675–684.
Lan, J.Y., Skeberdis, V.A., Jover, T., Grooms, S.Y., Lin, Y., Araneda, R.C., Zheng, X., Bennett, M.V., Zukin, R.S., 2001. Protein kinase C modulates NMDA receptor trafficking and gating. Nat. Neurosci. 4, 382–390.
Langston, J.L., Wright, L.K.M., Connis, N.C., Lumley, L.A., 2011. Characterizing the behavioral effects of nerve agent-induced seizure activity in rats: increased startle reactivity and preservative behavior. Pharmacol. Biochem. Behav. doi:10.1016/j.pbb.2011.09.011
Lumley, L.A., Furtado, M.D., Moffett, M.C., Schultz, M.K., Schwartz, J.E., Stone, M.F., Maida, D.M., Robison, C.L., 2009. Ketamine and diazepam combination therapy reduces seizures, seizure associated neuropathology and functional impairment induced by soman exposure in rats. Program No. 638.14/P13. 2009 Neuroscience Meeting Planner. Society for Neuroscience, Chicago, IL. Online.
Lynch, B.A., Lambeng, N., Nocka, K., Kensel-Hammes, P., Bajjalieh, S.M., Matagne, A., Fuks, B., 2004. The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proc. Natl. Acad. Sci. U. S. A. 101, 9861–9866.
Martin, B.S., Kapur, J., 2007. A combination of ketamine and diazepam synergistically controls refractory status epilepticus induced by cholinergic stimulation. Epilepsia 49, 248–255.
McDonough, J.H., Shih, T.M., 1993. Pharmacological modulation of soman-induced seizures. Neurosci. Biobehav. Rev. 17, 203–215.
McDonough, J.H., Shih, T.M., 1997. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. Neurosci. Biobehav. Rev. 21, 559–579.
Meeter, E., Wolthuis, O.L., 1968. The effects of cholinesterase inhibitors on the body temperature of the rat. Eur. J. Pharmacol. 4, 18–24.
Moffett, M.C., Schultz, M.K., Schwartz, J.E., Stone, M.F., Lumley, L.A., 2011. Impaired auditory and contextual fear conditioning in soman-exposed rats. Pharmacol. Biochem. Behav. 98, 120–129.
Myhrer, T., Enger, S., Aas, P., 2008. Anticonvulsant efficacy of drugs with cholinergic and/or glutamatergic antagonism microinfused into area tempestas of rats exposed to soman. Neurochem. Res. 33, 348–354.
Myhrer, T., Enger, S., Jonassen, M., Aas, P., 2011. Enhanced efficacy of anticonvulsants when combined with levetiracetam in soman-exposed rats. Neurotoxicology doi:10.1016/j.neuro.2011.04.008.
Naylor, D.E., Liu, H., Wasterlain, C.G., 2005. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. J. Neurosci. 25, 7724–7733.
Paxinos, G., Watson, C., 2005. The Rat Brain in Stereotaxic Coordinates. Elsevier Academic Press, San Diego.
Raveh, L., Chapman, S., Cohen, G., Alkalay, D., Gilat, E., Rabinovitz, I., Weissman, B.A., 1999. The involvement of the NMDA receptor complex in the protective effect of anticholinergic drugs against soman poisoning. Neurotoxicology 20, 551–559.
Raveh, L., Weissman, B.A., Cohen, G., Alkalay, D., Rabinovitz, I., Sonego, H., Brandeis, R., 2002. Caramiphen and scopolamine prevent soman-induced brain damage and cognitive dysfunction. Neurotoxicology 23, 7–17.
Raveh, L., Brandeis, R., Gilat, E., Cohen, G., Alkalay, D., Rabinovitz, I., Sonego, H., Weissman, B.A., 2003. Anticholinergic and antiglutamatergic agents protect against soman-induced brain damage and cognitive dysfunction. Toxicol. Sci. 75, 108–116.
Raveh, L., Rabinovitz, I., Gilat, E., Egoz, I., Kapon, J., Stavitsky, Z., Weissman, B.A., Brandeis, R., 2008. Efficacy of antidotal treatment against sarin poisoning: the superiority of benactyzine and caramiphen. Toxicol. Appl. Pharmacol. 227, 155–162.
Shih, T.M., Koviak, T.A., Capacio, B.R., 1991. Anticonvulsants for poisoning by the organophosphorus compound soman: pharmacological mechanisms. Neurosci. Biobehav. Rev. 15, 349–362.
Shih, T.M., Duniho, S.M., McDonough, J.H., 2003. Control of nerve agent-induced seizures is critical for neuroprotection and survival. Toxicol. Appl. Pharmacol. 188, 69–80.
Sparenborg, S., Brennecke, L.H., Braitman, D.J., 1990. Prevention of soman neurotoxicity by non-opioid antitussives. Neurotoxicology 11, 509–520.
Szekely, J.I., Sharpe, L.G., Katz, J.L., 1994. Effects of caramiphen and phencyclidine alone and in combination on behavior in the rat. Pharmacol. Biochem. Behav. 47, 709–713.
Taylor, P., 2001. Anticholinesterase agents. In: Hardman, J.G., Limbird, L.E., Gilman, A.G. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, pp. 175–191.
Tortella, F.C., Witkin, J.M., Musacchio, J.M., 1988. Caramiphen: a non-opioid antitussive with potent anticonvulsant properties in rats. Eur. J. Pharmacol. 155, 69–75.
Vinkers, C.H., Klanker, M., Groenink, L., Korte, S.M., Cook, J.M., Van Linn, M.L., Hopkins, S.C., Olivier, B., 2009. Dissociating anxiolytic and sedative effects of GABAAergic drugs using temperature and locomotor responses to acute stress. Psychopharmacology (Berl) 204, 299–311.
Wang, Y., Liu, L., Weiss, T., Stewart, C., Mikler, J., 2011. Effect of acute soman exposure on GABA(A) receptors in rat hippocampal slices and cultured hippocampal neurons. Neurotox. Res. 20, 334–350.
Wasterlain, C.G., Chen, J.W., 2008. Mechanistic and pharmacologic aspects of status epilepticus and its treatment with new antiepileptic drugs. Epilepsia 49, 63–73.
Wasterlain, C., Liu, H., Mazarati, A., Baldwin, R., 2002. NMDA receptor trafficking during the transition from single seizures to status epilepticus. Ann. Neurol. 52, S16.
Weissman, B.A., Raveh, L., 2008. Therapy against organophosphate poisoning: the importance of anticholinergic drugs with antiglutamatergic properties. Toxicol. Appl. Pharmacol. 232, 351–358.
Wetherell, J., Price, M., Mumford, H., 2006. A novel approach for medical countermeasures to nerve agent poisoning in the guinea-pig. Neurotoxicology 27, 485–491.
Wetherell, J., Price, M., Mumford, H., Armstrong, S., Scott, L., 2007. Development of next generation medical countermeasures to nerve agent poisoning. Toxicology 233, 120–127.