Pretreatment of Guinea Pigs with Galantamine Prevents Immediate and Delayed Effects of Soman on Inhibitory Synaptic Transmission in the Hippocampus

Elena A. Alexandrova, Yasco Aracava, Edna F. R. Pereira, and Edson X. Albuquerque

Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland

Received March 2, 2010; accepted June 15, 2010

ABSTRACT

Galantamine has emerged as a potential antidote to prevent the acute toxicity of organophosphorus (OP) compounds. Changes in inhibitory GABAergic activity in different brain regions can contribute to both induction and maintenance of seizures in subjects exposed to the OP nerve agent soman. Here, we tested the hypothesis that galantamine can prevent immediate and delayed effects of soman on hippocampal inhibitory synaptic transmission. Spontaneous inhibitory postsynaptic currents (IPSCs) were recorded from CA1 pyramidal neurons in hippocampal slices obtained at 1 h, 24 h, or 6 to 9 days after the injection of guinea pigs with saline (0.5 ml/kg i.m.), 1 x LD50 soman (26.3 μg/kg s.c.), galantamine (8 mg/kg i.m.), or galantamine at 30 min before soman. Soman-challenged animals that were not pretreated showed mild, moderate, or severe signs of acute intoxication. At 1 h after the soman injection, the mean IPSC amplitude recorded from slices of mildly intoxicated animals and the mean IPSC frequency recorded from slices of severely intoxicated animals were larger and lower, respectively, than those recorded from slices of control animals. Regardless of the severity of the acute toxicity, at 24 h after the soman challenge the mean IPSC frequency was lower than that recorded from slices of control animals. At 6 to 9 days after the challenge, the IPSC frequency had returned to control levels, whereas the mean IPSC amplitude became larger than control. Pretreatment with galantamine prevented soman-induced changes in IPSCs. Counteracting the effects of soman on inhibitory transmission can be an important determinant of the antitodal effectiveness of galantamine.

INTRODUCTION

Organophosphorus (OP) nerve agents, including soman, sarin, and VX, are among the most lethal chemical warfare agents. They are chemically related to, although far more toxic than, OP pesticides used in agriculture and households worldwide. Although OPs interact with numerous molecular targets (Albuquerque et al., 1985; Huff et al., 1994; Duysen et al., 2001), acute OP intoxication results primarily from the irreversible inhibition of acetylcholinesterase (AChE) that leads to acetylcholine (ACh) accumulation and, consequently, overstimulation of cholinergic receptors in the peripheral and central nervous systems (Newmark, 2007).

Changes in the activity of the excitatory glutamatergic and the inhibitory GABAergic systems in brain regions enriched with cholinergic inputs, including the hippocampus, seem to contribute to the maintenance of OP-induced seizures (Shih and McDonough, 1997; Myhrer, 2007). In hippocampal microdialysates, levels of the inhibitory neurotransmitter GABA have been shown to decrease in guinea pigs at 1 to 2 h after exposure to soman (Fosbraey et al., 1997). On the other hand, levels of GABA have been found to be significantly increased in hippocampal tissue obtained from rats at 80 min after the onset of soman-induced seizures (Shih and McDonough, 1997). Neurotransmitter levels detected in microdialysates reflect both synaptic release and nonspecific overflow from synaptic and nonsynaptic (metabolic) sources. Likewise, tissue levels of neurotransmitters reflect total (metabolic and synaptic) contents of the transmitter. Thus, very little is known regarding the immediate and protracted effects of an acute in vivo exposure to soman on GABAergic synaptic transmission in the hippocampus.

ABBREVIATIONS: OP, organophosphorus; ACh, acetylcholine; AChE, acetylcholinesterase; IPSC, inhibitory postsynaptic current; IACUC, Institutional Animal Care and Use Committee; ANOVA, analysis of variance; nAChR, nicotinic acetylcholine receptor; mAChR, muscarinic acetylcholine receptor; ACSF, artificial cerebrospinal fluid.

This work was supported in part by the National Institutes of Health National Institute of Neurological Disorders and Stroke CounterACT Program [Grant U01-NS059344]; and the National Institutes of Health National Institute of Environmental Health Sciences [Grant 5T32ES007263]. Part of this work has been presented previously: Alexandrova E, Aracava Y, Pereira EFR, and Albuquerque EX (2008) Galantamine counteracts changes in inhibitory synaptic transmission in the CA1 region of the guinea pig hippocampus; the 2008 Annual Meeting of the Society for Neuroscience; 2008 Nov 15–19, Washington, DC; Society for Neuroscience, Washington, DC.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.110.167700.
Galantamine prevents the acute toxicity of OP nerve agents and pesticides in guinea pigs, the best nonprimate model to predict the effectiveness of OP antidotes in humans (Albuquerque et al., 2005, 2006; Pereira et al., 2008). Galantamine, a drug approved for treatment of mild to moderate Alzheimer’s disease, has a dual mode of action; it is a reversible, competitive AChE inhibitor and a positive allosteric modulator of nAChRs (Maelicke and Albuquerque, 2000; Pereira et al., 2002). However, the actions of galantamine that contribute to its effectiveness as a therapeutic countermeasure against OP poisoning remain poorly understood.

The present study was designed to test the hypothesis that an acute exposure of guinea pigs to soman has immediate and delayed effects on GABAergic transmission in the CA1 field of the hippocampus that are preventable by pretreatment of the animals with galantamine. To test this hypothesis, the whole-cell patch-clamp technique was used to record spontaneous inhibitory postsynaptic currents (IPSCs) from CA1 pyramidal neurons in hippocampal slices obtained at 1 h, 24 h, and 6 to 9 days after a single exposure guinea pigs to of soman and/or galantamine.

Evidence is provided herein that GABAergic transmission impinging onto CA1 pyramidal neurons changes significantly with time after a subcutaneous injection of guinea pigs with soman (1×LD50). At 1 h after the exposure, an increase in the IPSC amplitudes was observed in the hippocampi of mildly intoxicated animals, and a decrease in the frequency of IPSCs was seen in the hippocampi of severely intoxicated animals. Regardless of the severity of the acute toxicity exhibited by the animals, the IPSC frequency was lower and the IPSC amplitudes were larger than control at 24 h and 6 to 9 days after the exposure, respectively. Although a single intramuscular injection of galantamine (8 mg/kg) had no long-term effects on the IPSCs in the CA1 region, pretreatment of the guinea pigs with galantamine prevented the effects of soman. Galantamine’s ability to prevent the effects of soman on GABAergic transmission may contribute to its effectiveness as an antidotal therapy against soman poisoning.

Materials and Methods

Animal Care and Treatments. Female Hartley guinea pigs [Crl/HABr; Charles River Laboratories, Inc., Wilmington, MA] were housed in groups of four in stainless-steel cages (60 × 60 × 25 cm) in a climate-controlled animal care facility with constant temperature of 21 ± 0.5°C and a 12-h light/dark cycle. Animals were 30 to 33 days old upon arrival and were acclimated for at least 48 h before any treatment. The study complied with the regulations and standards of the Animal Welfare Act and adhered to the principles of the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

Guinea pigs were divided into four treatment groups. Group I (n = 30) received an injection of saline (0.9% NaCl; 0.5 ml/kg i.m.). Group II (n = 49) received an injection of 26.3 μg/kg soman (1×LD50 s.c.). Group III (n = 19) was treated with galantamine (8 mg/kg i.m.) 30 min before the challenge with 26.3 μg/kg s.c. soman. This treatment regimen was selected on the basis of our earlier demonstration that all guinea pigs survive with no signs of acute toxicity when they are treated with galantamine (8 mg/kg i.m.) and 30 min later exposed to 1×LD50 soman (Aracava et al., 2009; Gullapalli et al., 2010). Group IV (n = 30) received only galantamine (8 mg/kg i.m.).

Experiments were conducted at 1 h, 24 h, and 6 to 9 days after the injections. Animals were monitored every 15 min during the first 2 h after the injections, hourly during the next 6 h, and daily subsequently. Acute reactions to treatments were scored according to a modified Racine scale (Aracava et al., 2009) as described under Results. The experimenter who performed the electrophysiological experiments and analyzed the data was blind to the severity of the acute toxicity. Whenever animals developed life-threatening signs of intoxication, including gasping and unremitting motor convulsions, they were euthanized according to the protocol approved by the Institutional Animal Care and Use Committee (IACUC).

Preparation of Hippocampal Slices. Following the IACUC guidelines, guinea pigs were euthanized by asphyxiation in a CO2 atmosphere followed by decapitation. Their brains were removed, and the hippocampi were dissected out in cold artificial cerebrospinal fluid (ACSF). The middle third section of each hippocampus was used for slicing. Using a vibratome (Leica VT1000S; Leica Microsystems Inc., Deerfield, IL), transverse slices (300-μm thick) were cut in ice-cold ACSF bubbled with 95% O2/5% CO2. They were maintained for at least 1 h at room temperature in a humidified environment on the surface of ACSF that was continuously bubbled with 95% O2/5% CO2. ACSF was composed of 125 mM NaCl, 25 mM NaHCO3, 2.5 mM KCl, 2 mM CaCl2, 1.6 mM MgCl2, and 11 mM Na-glucose.

Electrophysiological Recordings. Recordings were obtained at room temperature (20–22°C) from the soma of CA1 pyramidal neurons by using the conventional whole-cell patch-clamp technique and an LM-EPIC amplifier (List Electronics, Darmstadt, Germany). Hippocampal slices were transferred to the recording chamber and perfused with oxygenated ACSF at 2 ml/min. In the chamber, they were kept submerged in ACSF and held in place by two nylon fibers. Patch pipettes were pulled from borosilicate glass capillaries (i.d. 1.2 mm; World Precision Instruments Inc., Sarasota, FL) with a P-97 Flaming-Brown puller (Sutter Instrument Company, Novato, CA) and had resistances of 3.5 to 6.5 MΩ when filled with the internal solution. The internal solution was composed of 130 mM Cs-methane sulfonylate, 10 mM CsCl, 2 mM MgCl2, 10 mM EGTA, 10 mM HEPES, and 5 mM lidocaine N-ethyl bromide. The pH was adjusted to 7.3 with CsOH. Biocytin (final concentration 0.3–0.5%) was added to the pipette solution to label the neurons from which recordings were obtained.

Neurons were visualized by infrared-assisted video microscopy. Currents were filtered at 3 kHz, digitized at 10 kHz, and recorded with pClamp9.2 software (Molecular Devices, Sunnyvale, CA). The series resistance estimated from the amplitude of the initial capacitive transient in response to a 5-mV pulse was 8 to 24 MΩ. It was not compensated and was monitored during each experiment. Experiments were terminated if the series resistance changed by more than 15%. Spontaneous IPSCs were recorded for 5 min at the reversal potential for glutamatergic currents (i.e., 0 mV). The GABAa, receptor antagonist bicuculline (10 μM) was applied at the end of the experiments to confirm that the synaptic events were indeed GABAergic in nature.

Data Analysis and Statistics. The Mini Analysis 6.0.3 software (Synapsoft Inc., Decatur, GA) was used to analyze the frequency and amplitude of synaptic events. Amplitudes, rise times (10–90%), decay-time constants (rd), and area (pA×ms) of single events were also measured as described below. The threshold amplitude for detecting IPSCs was set at twice the baseline noise (root mean square), and the IPSCs detected by the software were visually inspected to minimize errors. Events that did not show a typical synaptic wave form were rejected manually.

For kinetic analysis, only single events with a sharp rising phase and an exponential decay were chosen during visual inspection of the recordings. Double- and multiple-peak currents were excluded. Rise times and rd were determined during the analysis of the averaged chosen single events aligned at half rise time in each cell. More than 90% of the synaptic events were fitted with a single exponential decay. Charge transfer of IPSCs was taken as the area under the curve of the currents. To minimize potential sampling bias, a maximum of two cells per animal was studied. Data are expressed as...
mean ± S.E.M. of results obtained from various animals, and statistical significance was analyzed by using ANOVA or the Mann–Whitney test.

**Drugs.** Stock solution of soman (1.88–1.9 mg/ml) was obtained from the U.S. Army Edgewood Chemical Biological Center via an agreement with the U.S. Army Medical Research Institute of Chemical Defense. Soman (methylphosphonofluoridic acid 1,2,2-trimethylpropyl ester) was stored, handled, and disposed of according to the regulations set forth by the U.S. Army Medical Research Institute of Chemical Defense. Galantamine HBr was generously provided by Dr. Alfred Maelicke (Galantos Pharma, Mainz, Germany).

**Results**

**Acute Effects and Lethality of 1×LD₅₀ Soman Are Prevented by Treatment of the Guinea Pigs with Galantamine.** The severity of the acute toxicity presented by guinea pigs challenged with 1×LD₅₀ soman was qualitatively defined according to a modified Racine scale as described in Aracava et al. (2009). Animals were considered mildly (stages 0 and 1), moderately (stages 2 and 3), and severely intoxicated (stages 4 and 5) (see Table 1). In animals that reached stages 1 to 3, signs of acute intoxication were not life-threatening and subsided within a few hours after the soman challenge. According to the IACUC-approved protocol, animals had to be euthanized as soon as signs of intoxication became life-threatening. Thus, only a few animals that reached stages 4 and 5 remained alive after 24 h of exposure to soman.

Of 90 soman-injected guinea pigs, 49 were used in the present electrophysiological studies (see Table 1). Of those, 38 animals did not go beyond stage 3; 8, 13, and 17 animals were euthanized at 1 h, 24 h and 6 to 9 days after the challenge, respectively. Seven of the other 11 animals reached stage 4 within 45 to 60 min and were euthanized at 1 h after challenge. The remaining four reached stage 4 within a few hours. Two of these animals were euthanized and used at 24 h, because they still displayed signs of toxicity at that time. The other two guinea pigs were euthanized and used between days 6 and 9 after the challenge. As soon as the animals were euthanized, their brains were removed and their hippocampi were dissected for slicing as described under Materials and Methods.

At 24 h after the injection of soman, the surviving guinea pigs had lost 10 to 15% of their initial weight. At 6 to 9 days, they were gaining weight, albeit at a rate slower than control pigs had lost 10 to 15% of their initial weight. At 6 to 9 days, their hippocampi were dissected for slicing as described in Aracava et al. (2009). According to the IACUC-approved protocol, animals had to be euthanized whenever signs of intoxication became life-threatening. The far-right column shows the number of animals that were not used in this electrophysiological study because they had to be euthanized earlier than the experimental time points.

**TABLE 1**

Classification of the severity of soman-induced toxicity

The severity of the acute toxicity presented by guinea pigs challenged with 1×LD₅₀ soman was classified as described in Aracava et al. (2009). According to the IACUC-approved protocol, animals had to be euthanized whenever signs of intoxication became life-threatening. The far-right column shows the number of animals that were not used in this electrophysiological study because they had to be euthanized earlier than the experimental time points.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Progression of Behavioral Changes</th>
<th>Classification of Toxicity</th>
<th>Euthanized and Used in Experiments</th>
<th>Euthanized, Not Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No abnormal gross behavior</td>
<td>Mild</td>
<td>3  8  11</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Facial twitches, pawing at whiskers and mouth, chewing</td>
<td>Mild</td>
<td>3  8  11</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Head tremor and/or nodding, short periods of immobility</td>
<td>Moderate</td>
<td>5  5  6</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Forelimb clonus</td>
<td>Moderate</td>
<td>5  5  6</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Rearing with no loss of balance, strong grinding, gnashing or bruxism</td>
<td>Severe</td>
<td>7  2  2</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>Rearing with loss of balance, frank convulsions</td>
<td>Severe</td>
<td>7  2  2</td>
<td>39</td>
</tr>
</tbody>
</table>

As in previous studies (Alkondon et al., 2009; Aracava et al., 2009; Gullapalli et al., 2010), guinea pigs treated with galantamine (8 mg/kg i.m.) alone or challenged with soman 30 min after the treatment with galantamine survived with no apparent signs of acute toxicity and gained weight at the same rate as control animals (data not shown).

**Characteristics of IPSCs Recorded from CA1 Pyramidal Neurons in Guinea Pig Hippocampal Slices.** Spontaneous postsynaptic currents recorded from CA1 pyramidal neurons at the reversal potential of glutamatergic events (0 mV) were outward events. These events were blocked by the GABAᵦ receptor antagonist bicuculline (10 µM) (Fig. 1A). In addition, in the presence of the glutamate receptor antagonists 6-cyano-7-nitroquinolinone-2,3-dione (10 µM) and 2-amino-5-phosphonopentoic acid (50 µM), the synaptic currents reversed between −30 and −60 mV (Fig. 1B). Based on the Nernst equation, the null potential for Cl⁻ under the present experimental condition is approximately −52 mV. Thus, outward currents recorded at 0 mV are primarily IPSCs mediated by GABAᵦ receptors.

The frequency (expressed as number of IPSCs/recording time), amplitude, τd, and rise time of IPSCs recorded from slices obtained at 1 h were not significantly different from those recorded at 24 h or 6 to 9 days after the saline injection (Table 2). Therefore, results from all control animals were pooled together for subsequent comparison with results obtained from soman-exposed animals.

**Immediate and Delayed Effects of the Acute Exposure of Guinea Pigs to Soman on IPSCs.** The mean frequency and amplitude of IPSCs recorded from hippocampal slices taken from guinea pigs at 24 h or 6 to 9 days after soman challenge did not vary with the animals’ score of acute toxicity (Fig. 2A). Therefore, for subsequent comparison with control values, data from all animals were pooled together for each time point. Data obtained at 1 h after soman could not be pooled, because the mean IPSC amplitudes were significantly higher in mildly intoxicated (scores 0–1) than in moderately intoxicated (scores 2–3) or severely intoxicated (scores 4–5) animals (Fig. 2B).

At 1 h after soman, the IPSC amplitudes recorded from slices of mildly intoxicated guinea pigs were significantly larger than control (Fig. 3A). In those slices, the IPSC frequency remained unchanged (Fig. 3B). On the other hand, in slices obtained at 1 h after soman from severely intoxicated animals the IPSC frequency was significantly lower than...
control (Fig. 3B); there was no concomitant change in the IPSC amplitude (Fig. 3A).

At 24 h after soman challenge, the frequency of IPSCs was significantly lower than control (Fig. 4A). The cumulative distribution of interevent intervals was also shifted toward longer intervals in comparison with control (Fig. 4B). The reduction of IPSC frequency was transient, because it was no longer detected in slices taken at 6 to 9 days after soman challenge (Fig. 4A).

The mean amplitude of IPSCs recorded at 24 h after soman was comparable with control. However, at 6 to 9 days after soman, it was significantly larger than control (Fig. 4C); the histogram and cumulative distribution of IPSC amplitudes were also skewed toward larger amplitudes (Fig. 4D). The charge transfer through individual IPSCs recorded from control animals was 410 ± 41 pA·ms and increased to 585 ± 74 pA·ms at 6 to 9 days after soman challenge (p < 0.05 according to the unpaired Student’s t test). This increase was caused by the increase in IPSC amplitudes, because the exposure to soman had no significant effect on the rundown events. At 1 h, 24 h, and 6 to 9 days after soman, the IPSCs were 26.3 ± 1.5, 26.1 ± 1.3, and 24.3 ± 0.7 ms, and their rise times were 2.05 ± 0.33, 1.82 ± 0.13, and 1.88 ± 0.15 ms, respectively. These results are comparable with those obtained from control animals (see Table 1).

Effects of an Acute Treatment with Galantamine on IPSCs Impinging onto CA1 Pyramidal Neurons in Naïve and Soman-Injected Guinea Pigs. Pretreatment with galantamine (8 mg/kg i.m.) prevented the effects of soman...
on the frequency and amplitudes of IPSCs (Fig. 5). In slices from galantamine-treated, soman-challenged guinea pigs, the frequency and amplitude of IPSCs were comparable with control (Fig. 5).

In guinea pigs treated intramuscularly with 8 mg/kg galantamine, brain concentrations of the drug peak at 30 min after the injection and decay with a half-life of approximately 70 min (Albuquerque et al., 2006). Pharmacologically relevant concentrations of galantamine remain present in brain tissue up to 3 h after treatment. Thus, during the first 3 h after the injection of galantamine, timing to obtain the hippocampal slices is an important determinant of the remaining tissue concentrations of the drug. For meaningful comparison of results, hippocampal slices were obtained at 1.5 h after treatment with galantamine alone, because they were also obtained at 1.5 h after the galantamine treatment of soman-challenged animals.

At 1.5 h, 24 h, or 6 to 9 days after the injection of galantamine, the frequency of IPSCs was not significantly different from control (Fig. 6A). However, at 1.5 h after galantamine the IPSC amplitudes were larger than control (Fig. 6B); the histogram distribution and cumulative plot of IPSC amplitudes were also shifted toward larger amplitudes (Fig. 6C). The effect of galantamine on the IPSC amplitudes was transient and could not be detected at 24 h or 6 to 9 days after the treatment (Fig. 6B).

**Fig. 4.** Changes in IPSC frequency and amplitude recorded from CA1 pyramidal neurons at 24 h or 6 to 9 days after injection of guinea pigs with 1×LD₅₀ soman. A, mean frequency of IPSCs recorded from neurons in slices taken at 24 h after soman was significantly lower than control. ***, p < 0.01** according to ANOVA followed by Dunnett post hoc comparison with control. B, cumulative probability plot of inter-event intervals recorded at 24 h after soman was displaced to the right in comparison with control, p < 0.05 according to the Mann–Whitney test. C, mean amplitudes of IPSCs recorded from neurons in slices obtained at 6 to 9 days after soman were significantly larger than control. ***, p < 0.01** according to ANOVA followed by Dunnett post hoc comparison with control. D, compared with control, the histogram and cumulative probability plot of IPSC amplitudes recorded at 6 to 9 days after soman were skewed toward larger amplitudes. **p < 0.05** according to the Mann–Whitney test. Data are presented as mean and S.E.M. of results obtained from 30 (saline), 21 (24 h after soman), and 22 neurons (6–9 days after soman).

**Fig. 5.** Pretreatment of the guinea pigs with galantamine prevented soman-induced changes in the frequency and amplitude of IPSCs. Animals were treated with galantamine (8 mg/kg i.m.) and 30 min later they were challenged with 1×LD₅₀ soman (28.3 μg/kg s.c.). Hippocampal slices were obtained from animals at 1 h, 24 h, or 6 to 9 days after the soman challenge. Graphs show frequency (A) and amplitudes (B) of IPSCs in galantamine-treated, soman-challenged animals were comparable with control levels. Graph and error bars represent mean and S.E.M., respectively, of results obtained from the following number of neurons: 30 (saline), 12 (1 h after soman), 19 (24 h after soman), and 10 (6–9 days after soman).

**Fig. 6.** Effects of the acute treatment of guinea pigs with galantamine on IPSC frequency and amplitudes. A and B, graphs of mean IPSC frequency (A) and amplitudes (B) recorded from CA1 pyramidal neurons in slices obtained at 1.5 h, 24 h, or 6 to 9 days after the injection of guinea pigs with galantamine (8 mg/kg i.m.). Treatment with galantamine caused a significant transient increase in the IPSC amplitudes. ***, p < 0.05** according to ANOVA followed by Dunnett post hoc comparison with control. C, the histogram and cumulative probability plot of IPSC amplitudes recorded at 1.5 h after the injection of galantamine were displaced toward larger amplitudes in comparison with control, p < 0.05 according to the Mann–Whitney test. Data are presented as mean and S.E.M. of results obtained from the following number of neurons: 30 (saline), 17 (galantamine, 1.5 h), 12 (galantamine, 24 h), and 11 (galantamine, 6–9 days).
The rise time of IPSCs recorded at 1.5 h, 24 h, and 6 to 9 days after the galantamine treatment were 1.97 ± 0.29, 2.17 ± 0.46, and 1.78 ± 0.23 ms, respectively. The 1/ds of these events were 22.9 ± 1.3, 22.3 ± 1.9, and 25.4 ± 1.8 ms, respectively. These parameters were not significantly different from control (see Table 1).

Discussion

The present study demonstrates that an acute exposure of guinea pigs to soman has distinct immediate and delayed effects on GABAAergic synaptic transmission impinging onto CA1 pyramidal neurons. The ability of galantamine to prevent these effects can be an important determinant of its antidotal effectiveness.

Immediate and Delayed Effects of an Acute Soman Challenge on GABAAergic Transmission: Potential Mechanisms and Toxicological Relevance. In severely intoxicated guinea pigs, the IPSC frequency recorded from CA1 pyramidal neurons at 1 h after soman challenge was significantly lower than control. Because the IPSC amplitudes remained unchanged, the decreased IPSC frequency may have resulted from reduced firing of GABAAergic interneurons synapsing onto the pyramidal neurons. The acute reduction of inhibitory inputs to CA1 pyramidal neurons can have a dual causal–consequential relationship with the convulsions the animals presented. Disinhibition of the pyramidal neurons can lead to development of seizures (Trevelyan, 2009), which can trigger loss of CA1 interneurons (Nadler, 1981; Ben-Ari, 1985) and further reduce inhibitory inputs to pyramidal cells. In fact, CA1 interneurons are highly vulnerable to seizure-induced death (Nadler, 1981; Ben-Ari, 1985), and neurodegeneration is always present in the hippocampus of animals severely intoxicated with soman (Filliat et al., 1999; Myhrer et al., 2005; Albuquerque et al., 2006).

The hippocampus receives cholinergic innervation from the basal forebrain nuclei (Woolf, 1991). Muscarinic receptors, particularly M1, are present in the somatodendritic region and/or along the axons of most CA1 interneurons (Behrends and ten Bruggencate, 1993; Cobb and Davies, 2005). Different types of CA1 interneurons also express somatodendritic α7 and α4β2 nAChRs and receive excitatory innervation from glutamatergic neurons that express α3β4 nAChRs (Albuquerque et al., 2009; Alkondon et al., 2009). Activation of these mACHRs and nAChRs, which are known to contribute to the acute toxicity of soman (Hassel, 2006), increases neuronal firing. Thus, the reduced frequency of IPSCs in pyramidal neurons of soman-exposed guinea pigs could be explained by reduced activity/expression of mACHRs and/or nAChRs on interneurons that synapse onto the pyramidal cells. A long-lasting reduction of mACHr expression has been observed in the hippocampus of rats exposed to a single convulsive dose of soman (Churchill et al., 1990). Likewise, a sustained reduction of α3β4 nAChR-dependent excitation of CA1 stratum radiatum interneurons has been seen in the hippocampus of guinea pigs severely intoxicated with soman (Alkondon et al., 2009).

In mildly intoxicated guinea pigs, the IPSC amplitudes recorded at 1 h after soman challenge were significantly larger than control. Because the frequency of events was not altered, the increased IPSC amplitude could have resulted from a presynaptic action that increases GABA release. Activation of M2 mAChRs on presynaptic terminals of CA1 interneurons (Hájos et al., 1998) decreases quantal GABA release (Fukudome et al., 2004). Thus, desensitization of these receptors by excess ACh can facilitate synaptic release of GABA and increase the amplitude without affecting the frequency of IPSCs. Previous studies have proposed that animals will not present convulsions when exposed to soman if brain AChE inhibition does not reach at least 65% (Tonduli et al., 1999). Therefore, the degree of AChE inhibition in the hippocampus of mildly intoxicated guinea pigs may have been too low to cause sufficient build-up of ACh to over stimulate mACHRs and/or nAChRs. However, a small, yet persistent, rise in the ACh concentration can be sufficient to desensitize some of these receptor subtypes (Alkondon et al., 2000).

AChE-unrelated mechanisms can also contribute to the increased synaptic release of GABA onto CA1 pyramidal neurons. For example, soman inhibits adenosine A1 receptors with high affinity (Lau et al., 1991). These receptors are present in axon terminals of CA1 interneurons and their activation decreases GABA release (Jeong et al., 2003). Thus, soman-induced inhibition of A1 receptors would increase the amplitude without affecting the frequency of IPSCs. One cannot rule out the possibility that an increase in the number of post synaptic GABAB receptors contributes to the increased IPSC amplitudes.

Regardless of the severity of the signs of acute intoxication presented by the guinea pigs, the frequency of IPSCs recorded at 24 h after soman challenge was significantly lower than control. Because AChE activity remains significantly inhibited by 24 h after an exposure of guinea pigs to 1×LD50 soman (Lintern et al., 1998), the low frequency of IPSCs could be explained by ACh-induced desensitization of mACHRs and nAChRs on CA1 interneurons. Long-lasting loss of mACHR function caused by desensitization by excess ACh has been observed on the papillary sphincter muscle of rats exposed to low levels of soman (Dabisch et al., 2007). In addition, the decreased α7 nAChR activity recorded from CA1 stratum oriens interneurons at 24 h after an acute exposure of guinea pigs to 1×LD50 soman has been attributed to receptor desensitization by accumulated ACh (Alkondon et al., 2009).

Although the frequency of IPSCs returned to control levels by 6 to 9 days after soman challenge, their amplitudes became larger than control. Because a large portion of AChE activity is recovered by 7 days after soman exposure (Lintern et al., 1998), compensatory mechanisms may have overcome the decreased inhibitory input to the pyramidal neurons. Drugs that increase GABAB receptor activity increase the expression of GABAB receptors (Raol et al., 2005), which exert a presynaptic autoinhibitory effect on GABA release (Brown and Sihra, 2008). Soman-induced suppression of GABAAergic transmission in the CA1 field of the guinea pig hippocampus can lead to a reduction of presynaptic GABAB receptors and, consequently, disinhibit the synaptic release of GABA.

Soman-induced immediate and delayed changes in inhibitory inputs to CA1 pyramidal neurons can disrupt the hippocampal network oscillations that are essential for cognitive processing (Buzsáki, 2001), and, thereby, account for the
delayed cognitive dysfunctions triggered by this nerve agent (Wolthus and Vanversch, 1984; Myhrer et al., 2005).

**Effectiveness of Galantamine as a Medical Countermeasure against Soman Intoxication.** At 1.5 h after the treatment of guinea pigs with galantamine, the IPSC amplitudes were significantly larger than control. No concurrent change in the frequency of events was noted. At this time, brain concentrations of galantamine are within the range that favors its nicotinic allosteric potentiating action, in addition to a small degree of AChE inhibition (Albuquerque et al., 2006). Acting primarily as a nicotinic allosteric potentiating ligand, galantamine increases the frequency of ACh-induced IPSCs in rat hippocampal slices and human cerebral cortical slices in vitro (Santos et al., 2003). Such an effect could not have been detected in the slices taken from galantamine-treated guinea pigs, because the slices were maintained in galantamine-free ACSF and the nicotinic allosteric potentiating action of the drug is promptly reversible upon washout. The transient increase in IPSC amplitude in galantamine-treated guinea pigs, which was similar to that seen at 1 h in guinea pigs mildly intoxicated with soman, could be explained by the transient accumulation of ACh leading to M2 mAChR desensitization/internalization, which can last long after agonist removal (Krudewig et al., 2000).

Pretreatment of guinea pigs with galantamine prevented soman-induced immediate and delayed changes on inhibitory synaptic inputs to CA1 pyramidal neurons. A number of actions of galantamine could explain its effectiveness. First, reversible inhibition of brain AChE by galantamine may be sufficient to protect a significant pool of the enzyme from soman-induced irreversible inhibition, and, thereby, prevent excessive build-up of ACh concentrations. Second, the neuroprotective action of galantamine can prevent soman-induced neuronal loss, which does not seem to be exclusively caused by the seizures induced by the nerve agent (Filliat et al., 1999). Third, as a nicotinic allosteric potentiating ligand, galantamine can increase the activity of nAChR-associated signaling mechanisms (Albuquerque et al., 2009) to prevent changes in gene expression that may contribute to the delayed effects of soman on synaptic transmission.

In conclusion, pretreatment of guinea pigs with galantamine effectively counteracts the immediate and delayed effects of a lethal dose of soman on inhibitory synaptic transmission impinging onto CA1 pyramidal neurons. Considering that inhibitory inputs to CA1 pyramidal neurons control hippocampal rhythms that are essential for cognitive processing (Buzsáki, 2001), galantamine may be an effective medical countermeasure to prevent delayed cognitive dysfunctions triggered by an exposure to soman.

**Acknowledgments**

We thank Mabel A. Zelle and Miriam Akkerman for expert technical assistance.

**References**


Address correspondence to: Dr. Edson X. Albuquerque, Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, 655 W. Baltimore St., Baltimore, MD 21201. E-mail: ealbuque@umaryland.edu