Low Concentrations of Pyridostigmine Prevent Soman-Induced Inhibition of GABAergic Transmission in the Central Nervous System: Involvement of Muscarinic Receptors

MÁRITON D. SANTOS, EDNA F. R. PEREIRA, YASCO ARACAVA, NEWTON G. CASTRO, WILLIAM P. FAWCETT, WILLIAM R. RANDALL, and EDSON X. ALBUQUERQUE

Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine (M.D.S., E.F.R.P., Y.A., W.P.F., W.R.R., E.X.A.), Baltimore, Maryland; Departamento de Farmacologia Básica e Clínica, Instituto de Ciências Biomédicas (Y.A., N.G.C., E.X.A.), and Instituto de Biofísica Carlos Chagas Filho (M.D.S.), Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Received August 16, 2002; accepted September 11, 2002

ABSTRACT

This study was designed to investigate the effects of the cholinesterase inhibitors soman and pyridostigmine bromide (PB) on synaptic transmission in the CA1 field of rat hippocampal slices. Soman (1–100 nM, 10–15 min) decreased the amplitude of GABAergic postsynaptic currents (IPSCs) evoked by stimulation of Schaffer collaterals and recorded from CA1 pyramidal neurons. It also decreased the amplitude and frequency of spontaneous IPSCs recorded from pyramidal neurons. Whereas the maximal effect of soman on evoked GABAergic transmission was observed at 10 nM, full cholinesterase inhibition was induced by 1 nM soman. After 10–15-min exposure of hippocampal slices to 100 nM PB, GABAergic transmission was facilitated and cholinesterase activity was not significantly affected. At nanomolar concentrations, soman and PB have no direct effect on GABA_A receptors. The effects of soman and PB on GABAergic transmission were inhibited by the m2 receptor antagonist 11-[[2-diethylamino-O-methyl]-1-piperidinyl] acetyl]-5,11-dihydro-6H-pyridol[2,3-b][1,4]benzodiazepine-6-one (1 nM) and the m3 receptor antagonist 4-diphenylacetoxycarbonyl-N-methyl-piperidine (100 nM), respectively, and by the nonselective muscarinic receptor antagonist atropine (1 μM). Thus, changes in GABAergic transmission are likely to result from direct interactions of soman and PB with m2 and m3 receptors, respectively, located on GABAergic fibers/neurons synapsing onto the neurons under study. Although the effects of 1 nM soman and 100 nM PB were diametrically opposed, they only canceled one another when PB was applied to the neurons before soman. Therefore, PB, acting via m3 receptors, can effectively counteract effects arising from the interactions of soman with m2 receptors in the brain.

The organophosphates sarin, soman, VX, and tabun are the most powerful agents of chemical mass destruction (for review, see Reutter, 1999). These compounds, commonly referred to as nerve agents, are extremely toxic and their toxicity has long been attributed to their ability to block cholinesterases irreversibly (Taylor, 1996). The therapeutic strategy used to prevent and/or counteract the toxic effects of nerve agents has been designed primarily to recover cholinesterase activity and thereby reduce manifestation of over-stimulation of nicotinic and muscarinic receptors by excessive accumulation of acetylcholine (ACh) at cholinergic synapses. However, persistence of neurological sequella despite the use of this cholinesterase-based therapy emphasizes the significance of nerve agents’ effects on a wider range of targets (for review, see Moore, 1998). Thus, the actions and effects of nerve agents and their antidotes in the central nervous system (CNS) continue to be investigated extensively.

Reportedly, doses of soman that are close to or higher than its LD50 cause long-lasting epileptic seizure activity that is associated with severe brain damage in rats (McLeod et al.,...
Pyridostigmine and Soman on GABAergic Transmission

Materials and Methods

Rat Hippocampal Slices. Hippocampal slices of 250-μm thickness were obtained from 15- to 25-day-old Sprague-Dawley rats according to the procedure described previously (Alkondon et al., 1999). The slices were kept in a holding chamber containing artificial cerebrospinal fluid (ACSF) bubbled with 95% O₂ and 5% CO₂ at room temperature. Each slice, as needed, was transferred to a recording chamber (capacity of 2 ml) and held submerged by two nylon fibers. The recording chamber was continuously perfused with bubbled ACSF, which had the following composition: 125 mM NaCl, 25 mM NaHCO₃, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 2 mM CaCl₂, 1 mM MgCl₂, and 25 mM glucose (osmolality ~340 mOsm).

Electrophysiological Recordings. By means of the whole-cell mode of the patch-clamp technique, spontaneous or field stimulation-evoked postsynaptic currents (PSCs) were recorded from neurons of the CA1 pyramidal layer of rat hippocampal slices. Test solutions were applied to the slices through a set of coplanar-parallel glass tubes (400-μm i.d.) glued together and assembled on a motor-driven system (Newport Corporation, Irvine, CA) controlled by microcomputer. The tubes were placed at a distance of approximately 100 to 150 μm from the slice, and the gravity-driven flow rate was adjusted to 1.0 ml/min. Each tube was connected to a different reservoir filled with test solution. Evoked PSCs were recorded after application of a supramaximal 20- to 60-μs electrical stimulus to afferent fibers via a bipolar electrode made of thin platinum wires (50–100 μm in diameter). The stimulus was delivered by an isolated stimulator unit (Digitimer Ltd., Garden City, England) connected to a digital-to-analog interface (TL-1 DMA; Axon Instruments, Union City, CA). The platinum electrode was positioned at the Schaffer collaterals. Possible changes in series resistance were detected by applying, online, a 5-mV hyperpolarizing pulse before the test pulse.

Electrophysiological signals were recorded by means of an Axopatch 200A (Axon Instruments), filtered at 2 kHz, and either stored on VCR tapes or directly sampled by a microcomputer using the pClamp6 software (Axon Instruments). Low-resistance (2–5 MΩ) electrodes were pulled from borosilicate capillary glass (World Precision Instruments, New Haven, CT) and filled with internal solution. The composition of the internal solution used for voltage-clamp recordings from neurons in the CA1 pyramidal layer was as follows: 80 mM CsCl, 80 mM CsF, 22.5 mM EGTA, 10 mM HEPES, and 5 mM QX-314 (pH adjusted to 7.3 with CsOH; 340 mMOSM). All experiments were performed at room temperature (20–22°C).

Data Analysis. Peak amplitude, 10 to 90% rise time, and decay-time constant of field stimulation-evoked PSCs were determined using the pClamp6 software. Spontaneously occurring currents were analyzed using the Continuous Data Recording software (Dempster, 1989). All the analyses were made on fixed 3-min recordings. Unless otherwise stated, data are presented as mean ± S.E.M. The Student’s t test was used for pairwise comparison of results obtained in a test group and its respective control. In addition, one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test was used to compare results of repeated measures and multiple groups.

Cholinesterase Assay. Cholinesterase activity was measured by a two-phase radiotopic assay (Johnson and Russell, 1975) using 0.788 nM [3H]ACh iodide, which was sufficient to produce 100,000 cpm when totally hydrolyzed. The reaction (100 μl) was terminated by addition of 100 μl of a termination mixture (1 M monochloroacetic acid, 2 M NaCl, and 0.5 M NaOH) to which 4 ml of scintillation mixture (10% isoamyl alcohol, 0.5% diphenylxazole, and 0.02% dimethylphenylxazolybenzene in toluene) was added. The hydrolyzed, acidicified [3H]acetate partitioned into the organic phase and was subsequently counted. All samples were assayed in the presence of the butyrylcholinesterase inhibitor tetraisopropylpyrophosphoramide (10⁻⁴ M). Protein assays were performed using a micro BCA kit (Pierce Chemical, Rockford, IL) according to the manufacturer instructions. Two sets of experiments were carried out. In one set,

1984; Lallement et al., 1997; McDonough and Shih, 1997). At much lower doses, soman induces behavioral alterations that are not accompanied by gross histopathological changes in the brain of rats (Sirkka et al., 1990; Moore, 1998; Baille et al., 2001). The epileptic activity induced by high doses of soman in laboratory animals can be prevented to a great extent by pretreatment with atropine (a muscarinic receptor antagonist), pralidoxime (an oxime that reactivates cholinesterases by removing the phosphate group bound to the esteratic site of cholinesterases), and benzodiazepines and/or reversible cholinesterase inhibitors that readily cross the blood-brain barrier such as huperzine (Lennox et al., 1992; Lallement et al., 1997; Carpentier et al., 2000).

Pretreatment of rats with pyridostigmine bromide (PB), a cholinesterase inhibitor normally included in the antidotal therapy for intoxication by nerve agents, does not prevent seizure activity induced by high doses of soman in rats (Lallement et al., 1997). However, it does improve the sensorimotor performance of rats exposed to low doses of sarin and the cognitive impairment induced by an acute, subtoxic treatment of rats with the organophosphate diisopropylfluorophosphate (Stone et al., 2000; Abou-Donia et al., 2002). There have been no studies directed at investigating whether PB can prevent and/or reverse neurological alterations associated with exposure to low levels of soman.

Although the quaternary ammonium structure of PB prevents its easy access to the brain, low levels of PB have been detected by means of radioimmunoassay in the brain of rats treated with a single dose of 1 mg/kg PB intramuscularly (Miller and Verma, 1989), and CNS-related effects have been observed in laboratory animals and humans treated with therapeutic doses of PB. These effects include enhanced CNS arousal in humans (Borland et al., 1985), increased startle response in Wistar Kyoto rats (Servatius et al., 1998), and sensorimotor alterations that are accompanied by increases in acetycholinesterase activity and binding of the selective type 2 muscarinic receptor ligand AFDX 384 in specific areas of the brain of Sprague-Dawley rats (Abou-Donia et al., 2002).

The present study was designed to determine the effects of soman and low concentrations of PB on neurotransmission in the hippocampus and to investigate possible interactions between PB and soman at the cellular level. To this end, the whole-cell mode of the patch-clamp technique was used to record spontaneous and field stimulation-evoked postsynaptic currents from neurons in the CA1 pyramidal layer of rat hippocampal slices before, during, and after their exposure to soman and/or PB. The results presented herein demonstrate that GABAergic transmission in the CA1 field of the hippocampus is inhibited by soman (1–100 nM) and potentiated by PB (100 nM) and that preexposure of hippocampal slices to 100 nM PB can effectively counteract the effects of 1 nM soman on GABAergic transmission. Evidence is also provided that the effects of soman and PB on GABAergic transmission are not related to cholinesterase inhibition. Instead, they are the result of the direct interactions of soman and PB with m2 and m3 receptors, respectively, located on GABAergic neurons/fibers synapsing onto the neurons under study. It is, therefore, concluded that, acting primarily via m3 receptors, PB can prevent some of the toxic effects arising from the interactions of soman with m2 receptors in the CNS.
hippocampal slices were first perfused for 15 min with ACSF containing no drug, 1 nM soman, or 100 nM PB and subsequently washed three times with drug-free ACSF for 1.5 min. Each slice was transferred to a microfuge tube containing 50 μl of extraction buffer (pH 7.4; 1 M NaCl, 0.019 M NaH_2PO_4-H_2O, 0.081 M NaHPO_4-7H_2O, and 1% Triton X-100) and snap frozen. Cells within the slices were lysed by five rounds of freeze-thaw, lasting approximately 5 min, and homogenates were centrifuged at 14,000 rpm at 4°C. Cholinesterase activity measured in the supernatant was normalized to the protein contents of the pellets. In the other set of experiments, hippocampal slices were first extracted as described above and cholinesterase activity was measured in the supernatants after 0-, 15-, 30-, and 60-min exposure to 100 nM PB.

**Drugs and Biological Hazards.** Soman (1,2,2-trimethylpropyl methylphosphonofluoridate) was obtained from the U.S. Army Medical Research and Development Command. Atropine sulfate, PB, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 2-amino-5-phosphonovaleric acid (APV), picrotoxin, and 4-diphenylacetox-N-methylpiperidine (4-DAMP) methiodide were purchased from Sigma-Aldrich (St. Louis, MO). 11-[[2-Diethylamino-6H]-pyridyl][2,3-b][1,4]benzodiazepine-6-one (AFDX-116) was purchased from Tocris Cookson (St. Louis, MO). Methyllycaconitine (MLA) citrate was a gift from Prof. M. H. Benn (Department of Chemistry, University of Calgary, Calgary, AB, Canada). Dihydro-β-erythroidine (DH β E) hydrobromide was a gift from Merek (Rahway, NJ). A 250 mM stock solution of picrotoxin was made in dimethyl sulfoxide, and dilutions were made in the ACSF. NaOH was used to dissolve CNQX and APV (the 10 mM stock solution of CNQX had 12.5 mM NaOH and the 50 mM stock solution of APV had 0.5 M of NaOH). ^1^Hlabeled choline (specific activity 55 μCi/ml) was obtained from PerkinElmer Life Sciences (Boston, MA).

Safe handling of organophosphates was assured according to U.S. Army Medical Research and Development Command’s recommendations. The compounds were stored at ~80°C and diluted daily in an organophosphate vapor-proof hood. All organophosphate- and/or tetrodotoxin-containing solutions were inactivated with 5% sodium hypochlorite. Latex gloves and proper goggles were used throughout the experiment.

**Results**

**Characteristics of EPSCs and IPSCs Evoked by Field Stimulation of Schaffer Collaterals and Recorded from Neurons in the CA1 Pyramidal Layer of Rat Hippocampal Slices.** Application of a supramaximal stimulus to Schaffer collaterals in the stratum radiatum in the CA1 hippocampal field via a bipolar platinum electrode generated PSCs that could be recorded from the CA1 pyramidal neurons. The finding that these currents were blocked after perfusion of the slices with ACSF containing the GABA type A receptor antagonist picrotoxin (100 μM) and the glutamate receptor antagonists CNQX (20 μM) and APV (50 μM) indicated that they were mediated by GABA or glutamate released from field-stimulated fibers onto the neurons under study. Using these receptor antagonists, IPSCs and EPSCs were pharmacologically isolated. Evoked IPSCs were recorded from neurons in slices that were continuously perfused with ACSF containing 20 μM CNQX and 50 μM APV. On the other hand, evoked EPSCs were recorded from neurons in hippocampal slices continuously perfused with ACSF containing 100 μM picrotoxin. Under control conditions, evoked IPSCs or EPSCs recorded from a single neuron had variable amplitudes. However, up to 20 min, field stimulation-evoked IPSCs or EPSCs did not show any significant rundown (Fig. 1). In most experiments, recordings under control conditions lasted 3 to 5 min, after which time a given drug was applied to the hippocampal slice via the multibarrel perfusion system. In this system, solutions were exchanged within 20 to 25 s, as determined by the 4-aminopyrindine-induced enhancement of the amplitudes of evoked PSCs.

**Effects of Soman on IPSCs and EPSCs Evoked by Field Stimulation of Schaffer Collaterals and Recorded from CA1 Neurons in Hippocampal Slices.** Perfusion of hippocampal slices with ACSF containing soman (0.1–100 nM) resulted in a significant reduction of the amplitudes of evoked IPSCs recorded from neurons in the CA1 pyramidal layer (Fig. 2A). The effect was concentration-dependent; it was statistically significant at 1 nM and reached its maximum at 10 nM (Fig. 2B). Inhibition of evoked IPSCs became apparent at 1 min after beginning of the perfusion of the slices with soman-containing ACSF. In addition, the effect of soman on evoked EPSCs was not fully reversed even after 10 min of washing the preparations with soman-free ACSF (Fig. 2A). The kinetics of evoked IPSCs was not significantly altered by 1 to 100 nM soman. The rise times of evoked IPSCs recorded under control conditions and in the presence of 100 nM soman were 4.54 ± 0.63 and 4.86 ± 0.23 ms, respectively (n = 3 neurons, each from a different slice). Likewise, the decay-time constants of the currents recorded in the absence and in the presence of 100 nM soman were 71.7 ± 1.43 and 66.86 ± 7.66 ms, respectively (n = 3 neurons, each from a different slice).

Perfusion of hippocampal slices with ACSF containing soman up to 50 nM resulted in no changes in the amplitudes (Fig. 3, A and B) or kinetics of evoked EPSCs. The rise times of evoked EPSCs recorded from hippocampal neurons before and during their exposure to 50 nM soman were 5.47 ± 0.56 and 5.74 ± 0.40 ms, respectively (n = 4 neurons, each from a different slice). Similarly, the decay-time constants of evoked EPSCs recorded from hippocampal neurons in the absence and in the presence of 50 nM soman were 107.46 ± 4.70 and 114.66 ± 8.06 ms, respectively (n = 4 neurons, each from a different slice).
Effects of Soman on Spontaneously Occurring IPSCs and Miniature IPSCs and EPSCs Recorded from Neurons in the CA1 Pyramidal Layer of Hippocampal Slices. In the absence of the Na$^+$-channel blocker tetrodotoxin and in the continuous presence of the glutamate receptor antagonists CNQX (20 μM) and APV (50 μM), spontaneously occurring IPSCs were recorded from neurons in the pyramidal layer of the CA1 field of hippocampal slices. When the Schaffer collaterals were not stimulated, 0.1 and 1 nM soman caused no significant changes in the frequency, amplitude, or kinetics of the spontaneously occurring IPSCs (Fig. 4A). Only at 100 nM did soman cause significant reduction of the peak amplitude and frequency of these events (Fig. 4A). In contrast, soman at 1 or 10 nM caused a significant reduction in the amplitudes of spontaneous IPSCs recorded from CA1 pyramidal neurons between two subsequent field stimuli applied to Schaffer collaterals (Fig. 4B). The frequency of spontaneous IPSCs recorded under the latter experimental condition was also significantly decreased by 10 nM soman (Fig. 4B).

A 10-min exposure of hippocampal slices to 50 nM soman did not result in any significant change in the amplitude, kinetics, or frequency of miniature IPSCs recorded from CA1 neurons that were continuously perfused with ACSF containing 300 nM tetrodotoxin in addition to the glutamate receptor antagonists CNQX and APV (Table 1). A previous study had shown that at high concentrations (2 μM) soman also has no effect on miniature IPSCs (Chebabo et al., 1999). These results demonstrate that soman (up to 2 μM) does not alter the activity of postsynaptic GABA$_A$ receptors and suggest that soman-induced block of GABAergic transmission is the result of a presynaptic mechanism of action.

In addition to being unable to alter evoked glutamatergic transmission, soman also had no significant effect on tetrodotoxin-insensitive glutamatergic transmission. At 50 nM, soman did not alter the amplitude, kinetics, or frequency of miniature EPSCs recorded from CA1 pyramidal neurons in hippocampal slices that were continuously perfused with ACSF containing 300 nM tetrodotoxin in addition to picrotoxin (Table 1).

Effects of the Nonselective Muscarinic Receptor Antagonist Atropine and of Nicotinic Receptor Antagonists on Soman-Induced Inhibition of Evoked IPSCs in Hippocampal Slices. In an attempt to elucidate the mechanisms underlying the effect of soman on evoked IPSCs, the following facts were taken into account: 1) nicotinic and muscarinic receptors are known to modulate GABA release from
Soman has no significant effect on miniature IPSCs and EPSCs recorded from CA1 pyramidal neurons in rat hippocampal slices. The values of average rise time, decay-time constant, and peak amplitude and the frequency of events recorded from a given neuron for 3 min in the presence of 50 nM soman are represented as percentage of the values determined for events recorded from that neuron for 3 min under control conditions. Data are presented as mean ± S.E.M. of results obtained from four to six neurons, each from a different slice.

<table>
<thead>
<tr>
<th>Miniature IPSCs</th>
<th>Miniature EPSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>% control</td>
<td></td>
</tr>
<tr>
<td>Rise time</td>
<td>105.8 ± 8.87</td>
</tr>
<tr>
<td>Decay-time constant</td>
<td>98.5 ± 7.07</td>
</tr>
<tr>
<td>Peak amplitude</td>
<td>102.5 ± 7.3</td>
</tr>
<tr>
<td>Frequency</td>
<td>113.8 ± 10.3</td>
</tr>
</tbody>
</table>

* In the absence of soman, the rise time of miniature IPSCs and EPSCs was 6.0 ± 1.08 and 3.42 ± 0.98 ms, respectively.

** In the absence of soman, the decay-time constant of miniature IPSCs and EPSCs was 47.0 ± 7.14 and 20.4 ± 5.74 ms, respectively.

Effects of PB on IPSCs and EPSCs Evoked by Field Stimulation-Evoked IPSCs in the CA1 Layer of Hippocampal Slices. In the continuous presence of CNQX and APV, perfusion of hippocampal slices with ACSF containing PB increased the amplitude of IPSCs evoked by field stimulation of the Schaffer collaterals and recorded from CA1 pyramidal neurons (Fig. 6, A and C). This effect, which was apparent at 100 nM and became negligible at 1 µM PB (Fig. 6C), was completely reversed after 10 min of washing the preparations with PB-free ACSF (Fig. 6A). In contrast, perfusion of hippocampal slices with ACSF containing PB (30 nM–1 µM) resulted in no change in the amplitude of evoked EPSCs recorded from CA1 pyramid neurons in the continuous presence of 100 µM picrotoxin (Fig. 6, B and C).

Effects of Atropine on PB-Induced Potentiation of Field Stimulation-Evoked IPSCs in the CA1 Layer of Hippocampal Slices. As aforementioned, exposure of hippocampal slices to 100 nM PB resulted in the enhancement of the amplitudes of IPSCs evoked by field stimulation of the Schaffer collaterals and recorded from neurons in the CA1 pyramidal layer of hippocampal slices. The effect reached its maximum at 30 to 60 s after beginning of the perfusion of the slices with PB-containing ACSF, remained constant for as long as the drug was present, i.e., 5 to 8 min (Fig. 7, A and B), and was blocked by subsequent exposure of the slices to ACSF containing 1 nM atropine (Fig. 7, A and C).

In the presence of 100 nM atropine, not only was PB devoid of any effect on evoked IPSCs but also the amplitude of evoked IPSCs became smaller than those of events recorded under control conditions (Figs. 2B and 5B). Taken together, these results suggest that the effect of soman on GABAergic transmission is mediated by its interaction with muscarinic receptors present on GABAergic axons/terminals synapsing onto the neurons from which recordings were obtained.

**TABLE 1**

<table>
<thead>
<tr>
<th>Miniature IPSCs</th>
<th>Miniature EPSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>% control</td>
<td></td>
</tr>
<tr>
<td>Rise time</td>
<td>105.8 ± 8.87</td>
</tr>
<tr>
<td>Decay-time constant</td>
<td>98.5 ± 7.07</td>
</tr>
<tr>
<td>Peak amplitude</td>
<td>102.5 ± 7.3</td>
</tr>
<tr>
<td>Frequency</td>
<td>113.8 ± 10.3</td>
</tr>
</tbody>
</table>

* In the absence of soman, the rise time of miniature IPSCs and EPSCs was 6.0 ± 1.08 and 3.42 ± 0.98 ms, respectively.

** In the absence of soman, the decay-time constant of miniature IPSCs and EPSCs was 47.0 ± 7.14 and 20.4 ± 5.74 ms, respectively.

happicampal neurons (Pitler and Alger, 1994; Alkondon et al., 1999); 2) organophosphates, via cholinesterase inhibition, can indirectly alter the activity of muscarinic and nicotinic receptors; and 3) organophosphates can interact directly with different subtypes of nicotinic and muscarinic receptors (Albuquerque et al., 1988; Lockhart et al., 2001).

Exposure of hippocampal slices to the nonselective muscarinic receptor antagonist atropine (1 nM) had no significant effect on the amplitude of evoked IPSCs. However, it prevented soman from reducing the amplitudes of evoked IPSCs recorded from neurons in the CA1 field of hippocampal slices (Fig. 5A). Perfusion of hippocampal slices with ACSF containing 50 nM MLA, a selective α7 nAChR antagonist, and DHβE, a nicotinic antagonist that at the concentration tested (10 µM) would have blocked by 40 and 90%, respectively, the activity of α7 and α4β2 nAChRs, caused no significant alteration in the amplitudes of evoked IPSCs. However, in the continuous presence of the nAChR antagonists, soman was still capable of reducing the amplitude of the evoked IPSCs (Fig. 5B). In the presence of the nAChR antagonists, the magnitude of soman-induced reduction of the IPSC amplitudes was about the same as that observed under control conditions (Figs. 2B and 5B). Taken together, these results suggest that the effect of soman on GABAergic transmission is mediated by its interaction with muscarinic receptors present on GABAergic axons/terminals synapsing onto the neurons from which recordings were obtained.

Effects of PB on IPSCs and EPSCs Evoked by Field Stimulation-Evoked IPSCs in the CA1 Layer of Hippocampal Slices. In the continuous presence of CNQX and APV, perfusion of hippocampal slices with ACSF containing PB increased the amplitude of IPSCs evoked by field stimulation of the Schaffer collaterals and recorded from CA1 pyramidal neurons (Fig. 6, A and C). This effect, which was apparent at 100 nM and became negligible at 1 µM PB (Fig. 6C), was completely reversed after 10 min of washing the preparations with PB-free ACSF (Fig. 6A). In contrast, perfusion of hippocampal slices with ACSF containing PB (30 nM–1 µM) resulted in no change in the amplitude of evoked EPSCs recorded from CA1 pyramid neurons in the continuous presence of 100 µM picrotoxin (Fig. 6, B and C).

Effects of Atropine on PB-Induced Potentiation of Field Stimulation-Evoked IPSCs in the CA1 Layer of Hippocampal Slices. As aforementioned, exposure of hippocampal slices to 100 nM PB resulted in the enhancement of the amplitudes of IPSCs evoked by field stimulation of the Schaffer collaterals and recorded from neurons in the CA1 pyramidal layer of hippocampal slices. The effect reached its maximum at 30 to 60 s after beginning of the perfusion of the slices with PB-containing ACSF, remained constant for as long as the drug was present, i.e., 5 to 8 min (Fig. 7, A and B), and was blocked by subsequent exposure of the slices to ACSF containing 1 nM atropine (Fig. 7, A and C).

In the presence of 100 nM atropine, not only was PB devoid of any effect on evoked IPSCs but also the amplitude of evoked IPSCs became smaller than those of events recorded under control conditions (Figs. 2B and 5B). Taken together, these results suggest that the effect of soman on GABAergic transmission is mediated by its interaction with muscarinic receptors present on GABAergic axons/terminals synapsing onto the neurons from which recordings were obtained.
with 50 nM MLA plus 10 nM CA1 pyramidal neurons in hippocampal slices that were exposed first to induced inhibition of evoked IPSCs. Evoked IPSCs were recorded from CA1 pyramidal neurons in hippocampal slices that were exposed first to 1 nM atropine for 5 to 8 min and subsequently to the admixture of 1 nM atropine plus 1 nM soman for an additional 10 min. At the end of the experiments, the preparations were washed for 10 min with drug-free ACSF. The amplitudes of events evoked at a frequency of 0.2 Hz for 3 min were averaged. The averaged amplitudes of events recorded in the presence of atropine or atropine plus soman and in the washing phase are expressed as percentage of the averaged amplitudes of events recorded at the same frequency for 3 min before exposure of the neurons to any drug. B, quantitative analysis of the effect of MLA and DHβE on evoked IPSCs and on soman-induced inhibition of evoked IPSCs. Evoked IPSCs were recorded from CA1 pyramidal neurons in hippocampal slices that were exposed first to 50 nM MLA plus 10 μM DHβE for 5 to 8 min and subsequently to the admixture of the antagonists and 1 nM soman for an additional 10 min. At the end of the experiments, the preparations were washed for 10 min with drug-free ACSF. The amplitudes of events evoked at a frequency of 0.2 Hz for 3 min were averaged. The averaged amplitudes of events recorded in the presence of drugs and in the washing phase are expressed as percentage of the averaged amplitudes of events recorded at the same frequency for 3 min under control conditions. In the graphs, each column and error bar represent mean and S.E.M., respectively, of results obtained from four neurons. According to the unpaired Student’s t test, results are significantly different from control with * p < 0.05.

than those of events recorded before exposure of the slices to PB and atropine (each at 100 nM) (Fig. 7, B and D). An earlier report had demonstrated that at concentrations ≥100 nM atropine alone can reduce the amplitude of evoked IPSCs (Chehabo et al., 1999).

Effects of PB on Spontaneously Occurring IPSCs Recorded from CA1 Pyramidal Neurons in Rat Hippocampal Slices: Blockade by Atropine. In the continuous presence of the glutamate receptor antagonists CNQX and APV, spontaneously occurring IPSCs were recorded from CA1 pyramidal neurons in slices where the Schaffer collaterals had not been subjected to field stimulation. At 1 and 30 nM, PB had no apparent effect on the amplitude or frequency of spontaneously occurring IPSCs (Fig. 8A). At 100 nM, PB caused a small, albeit significant, enhancement in the frequency and amplitude of these events. These effects became negligible as concentration of PB was increased to 1 μM (Fig. 8A). PB (100 nM)-induced increase in the frequency and amplitudes of spontaneously occurring IPSCs started at 30 s after beginning of perfusion of the hippocampal slices with PB-containing ACSF and was fully reversed after 10 min of washing the slices with PB-free ACSF. In addition, PB at all concentrations tested caused no significant change on the decay-time constants of spontaneously occurring IPSCs (data not shown).

Field stimulation of the Schaffer collaterals did not alter significantly the magnitude of the effects of 100 nM PB on spontaneously occurring IPSCs. In the presence of 100 nM PB, the amplitude and frequency of spontaneously occurring IPSCs recorded between subsequent field stimuli applied to the Schaffer collaterals were, respectively, 18.8 ± 5.3 and

Fig. 5. Effects of the muscarinic receptor antagonist atropine and the nAChR antagonists MLA and DHβE on soman-induced inhibition of evoked IPSCs in rat hippocampal slices. A, quantitative analysis of the effect of atropine on evoked IPSCs and on soman-induced inhibition of evoked IPSCs. Evoked IPSCs were recorded from CA1 pyramidal neurons in hippocampal slices that were exposed first to 1 nM atropine for 5 to 8 min and subsequently to the admixture of 1 nM atropine plus 1 nM soman for an additional 10 min. At the end of the experiments, the preparations were washed for 10 min with drug-free ACSF. The amplitudes of events evoked at a frequency of 0.2 Hz for 3 min were averaged. The averaged amplitudes of events recorded in the presence of atropine or atropine plus soman and in the washing phase are expressed as percentage of the averaged amplitudes of events recorded at the same frequency for 3 min before exposure of the neurons to any drug. B, quantitative analysis of the effect of MLA and DHβE on evoked IPSCs and on soman-induced inhibition of evoked IPSCs. Evoked IPSCs were recorded from CA1 pyramidal neurons in hippocampal slices that were exposed first to 50 nM MLA plus 10 μM DHβE for 5 to 8 min and subsequently to the admixture of the antagonists and 1 nM soman for an additional 10 min. At the end of the experiments, the preparations were washed for 10 min with drug-free ACSF. The amplitudes of events evoked at a frequency of 0.2 Hz for 3 min were averaged. The averaged amplitudes of events recorded in the presence of drugs and in the washing phase are expressed as percentage of the averaged amplitudes of events recorded at the same frequency for 3 min under control conditions. In the graphs, each column and error bar represent mean and S.E.M., respectively, of results obtained from four neurons. All experiments were carried out in the presence of CNQX and APV. Holding potential, −60 mV. According to the unpaired Student’s t test, results are significantly different from control with * p < 0.05.

Fig. 6. PB increases the amplitude of IPSCs and does alter the amplitude of EPSCs evoked by field stimulation of the Schaffer collaterals and recorded from CA1 pyramidal neurons in rat hippocampal slices. A and B, sample recordings of evoked IPSCs (A) and EPSCs (B) obtained from two neurons under control conditions, in the presence of 100 nM PB and after 10 min of washing of the preparations with PB-free ACSF. The neurons were exposed to PB for 10 min. Evoked IPSCs were recorded in the presence of 20 μM CNQX and 50 μM APV, and evoked EPSCs were recorded in the presence of 100 μM picrotoxin. Holding potential, −60 mV. C, graph of PB concentrations versus the amplitudes of evoked IPSCs or EPSCs normalized to control conditions. The amplitudes of EPSCs or IPSCs evoked at a frequency of 0.2 Hz for 3 min were averaged. The averaged amplitudes of events recorded in the presence of PB are expressed as percentage of the averaged amplitudes of events recorded at the same frequency for 3 min under control conditions. Each concentration was tested on a neuron that had not been previously exposed to PB. Each point and error bar represent mean and S.E.M., respectively, of results obtained from four neurons. According to the unpaired Student’s t test, results are different from control with * p < 0.05.
24.7 ± 11.3% larger than those recorded under control conditions (n = 3 neurons, each from a different slice). These results were not statistically different from those obtained from slices in which the Schaffer collaterals had not been field stimulated. In these preparations, 100 nM PB increased by 20.0 ± 5.0 and 6.5 ± 1.0%, respectively, the frequency and amplitude of spontaneously occurring IPSCs (Fig. 8A).

Perfusion of the hippocampal slices with ACSF containing 1 nM atropine in addition to 100 nM PB resulted in inhibition of the effects of the carbamate on spontaneously occurring IPSCs (Fig. 8B). Atropine-induced inhibition of the effects of PB on frequency and amplitude of IPSCs could not be reversed, even after 10 min of washing of the preparations with atropine-free ACSF (Fig. 8B). Furthermore, at 1 nM atropine itself had no effect on spontaneously occurring IPSCs as evidenced by the finding that after an additional 10 min of washing of the hippocampal slices with drug-free ACSF, the frequency and amplitude of these events were similar to those recorded before exposure of the preparations to any drug (Fig. 8B).

**Effects of the Muscarinic Receptor Antagonists 4-DAMP and AFDX-116 on Soman-Induced Inhibition and PB-Induced Potentiation of GABAergic Transmission in Rat Hippocampal Slices.** Numerous reports have indicated that PB and soman can interact directly with m2 and m3 receptors (Silveira et al., 1990; McBride et al., 1991; Ramnarine et al., 1996; Shibata et al., 1998; Lockhart et al., 2001). Thus, the m2- and m3-prefering antagonists AFDX-116 and 4-DAMP, respectively, were used in an attempt to identify the muscarinic receptor subtype underlying the effects of PB and soman on GABAergic transmission.

Perfusion of hippocampal slices with ACSF containing 100 nM 4-DAMP caused a small, albeit significant reduction in the amplitude of IPSCs evoked by field stimulation of the Schaffer collaterals and recorded from CA1 pyramidal neurons. A and B, amplitudes of IPSCs evoked at 0.2 Hz and recorded from CA1 pyramidal neurons under different experimental conditions. In A, recordings were obtained from a neuron in the following sequence: 1) under control condition, 2) in the presence of 100 nM PB, 3) in the presence of 100 nM PB and 1 nM atropine, and 4) during the washout with drug-free ACSF. The experimental results shown in B were obtained from another neuron subjected to the same treatment as that described in A, except that the concentration of atropine was 100 nM. C and D, quantitative analyses of the effects of 1 nM (C) and 100 nM (D) atropine on PB-induced potentiation of evoked IPSCs. The amplitudes of IPSCs evoked at a frequency of 0.2 Hz for 3 min were averaged. The averaged amplitudes of events recorded from a given neuron in the presence of PB or atropine plus PB and during the washing phase are expressed as percentage of the averaged amplitudes of events recorded at the same frequency for 3 min under control conditions. Each column and error bar represent mean and S.E.M., respectively, of results obtained from five neurons. All experiments were carried out in the presence of the glutamate receptor antagonists 20 μM CNQX and 50 μM APV. Holding potential, −60 mV. According to the ANOVA test, peak amplitudes of IPSCs recorded in the presence of PB before exposure of the neurons to 1 nM (C) or 100 nM atropine (D) are significantly different from control with *, p < 0.01. In addition, peak amplitudes of IPSCs recorded in the presence of 100 nM PB plus 100 nM atropine and after washout of both drugs are significantly different from control with *, p < 0.05. Finally, amplitudes of events recorded during exposure of neurons to PB plus atropine and after removal of both drugs were significantly different from those recorded in the presence of PB alone with **, p < 0.01.
AFDX-116 became significant at 100 nM; the amplitudes of IPSCs recorded in the presence of 100 nM AFDX-116 were 82.1 ± 3.1% of those recorded under control conditions (n = 3 neurons, each from a different slice; p < 0.01 according to the ANOVA test). Considering that activation of m2 receptors presynaptically located in GABAergic fibers/neurons inhibits GABAergic transmission in the hippocampus (Pitler and Alger, 1992), the potentiating effect of 1 nM AFDX-116 on GABAergic transmission can be the result of the blockade of tonically active m2 receptors in GABAergic fibers synapsing onto the neurons under study. The inhibition of transmission observed at higher concentrations of AFDX-116 may be accounted for by nonsynaptic blockade of other muscarinic receptor subtypes, including m3 receptors, counteracting and prevailing over the blockade of tonically active m2 receptors. Soman was unable to inhibit GABAergic transmission between Schaffer collaterals and CA1 pyramidal neurons in the presence of 1 nM AFDX-116 (data not shown) and 10 nM AFDX-116 (Fig. 9A). On the other hand, PB-induced increase in the amplitudes of evoked IPSCs could be detected in the continuous presence of 1 nM AFDX-116 (Fig. 9B).
Interactive Effects of PB and Soman on Evoked IPSCs Recorded from CA1 Neurons in the Pyramidal Layer of Rat Hippocampal Slices. The results presented herein demonstrate that whereas PB facilitates, soman inhibits GABAergic transmission between the Schaffer collaterals and CA1 neurons in the pyramidal layer of rat hippocampal slices. Reduction of the GABAergic tone in the hippocampus can underlie the proconvulsant effects of soman in animals and humans. Numerous studies have indicated that the effectiveness of PB as part of the antidotal regimen against poisoning by organophosphates is only evident when PB is administered before exposure to the toxicant. Thus, GABAergic transmission was analyzed in hippocampal slices that were exposed first to PB and subsequently to soman and in slices that were exposed first to soman and subsequently to PB. All the results described below were obtained from hippocampal slices in which the Schaffer collaterals had been subjected to field stimulation.

A 10-min exposure of hippocampal slices to 100 nM PB resulted in a 20% enhancement of the amplitudes of evoked IPSCs. Upon subsequent perfusion of the slices with ACSF containing both 100 nM PB and 1 nM soman, the amplitudes of evoked IPSCs were similar to those of evoked IPSCs recorded under control conditions (Fig. 10A). In slices preexposed to 100 nM PB, the inhibitory effect of increasing concentrations of soman eventually outweighed the potentiating effect of PB on GABAergic transmission. Nevertheless, the reduction of the amplitudes of evoked IPSCs (to below control levels) was smaller than that observed in slices that had not been preexposed to PB (Fig. 10B). However, when slices were first perfused with ACSF containing 1 nM soman and a 20% reduction in the amplitudes of evoked IPSCs recorded from CA1 neurons in response to field stimulation of the Schaffer collaterals was observed, subsequent exposure of the preparations to the admixture of 100 nM PB and 1 nM soman caused no further changes in GABAergic transmission (Fig. 10C).

Soman-induced reduction of the peak amplitude and frequency of spontaneously occurring IPSCs below control levels was also significantly smaller in slices that were preexposed for 10 min to 100 nM PB than in slices that had not been preexposed to PB (Fig. 11A). In slices that were preexposed for 10 min with a PB concentration that had no effect on spontaneous IPSCs, i.e., 300 nM, the magnitude of the reduction of the amplitude and frequency of these events by 10 nM soman was comparable to that observed in preparations that had not been preexposed to 300 nM PB (Figs. 4B and 11B).

Cholinesterase Activity in Hippocampal Slices Exposed to Soman or PB. A radiometric assay was used to determine the extent to which cholinesterase activity was affected after exposure of hippocampal slices to 1 nM soman or 100 nM PB. Cholinesterase activity in intact slices that had been exposed for 15 min to 1 nM soman was approximately 99.5% lower than that measured in untreated slices (Table 2). On the other hand, cholinesterase activity in intact slices that had been exposed to 100 nM PB was similar to that measured in untreated slices (Table 2). When added to homogenates of hippocampal slices, 100 nM PB caused a time-dependent inhibition of cholinesterase. The cholinesterase activity in homogenates that were incubated with 100 nM PB for 15, 30, and 60 min was 68.5 ± 3.2, 56.3 ± 3.1, and 40.9 ± 1.8%, respectively, of that measured in control homogenates (mean ± S.D., n = 3 slices). The lack of inhibition of cholinesterase in intact hippocampal slices exposed for 15 min to PB is likely due to the difficult penetration of PB into the slices.

---

**Fig. 10.** Preexposure of the hippocampal slices to 100 nM PB masks the inhibitory effect of soman on evoked IPSCs. A, quantitative analyses of the effects of soman on evoked IPSCs recorded in the continuous presence of 100 or 300 nM PB. The IPSCs were recorded from hippocampal slices that were exposed first to 100 nM PB, subsequently to 1, 3, or 10 nM soman plus 100 nM PB, and finally to PB alone. In another set of experiments, IPSCs were recorded from neurons that were exposed first to 300 nM PB and subsequently to 300 nM PB plus 3 or 10 nM soman. Each perfusion lasted 5 to 8 min. The amplitudes of IPSCs evoked at a frequency of 0.2 Hz for 3 min were averaged. The averaged amplitudes of events recorded from a given neuron in the presence of the test compounds are expressed as percentage of the averaged amplitudes of events recorded at the same frequency for 3 min under control conditions. Each column and error bar represent mean and S.E.M., respectively, of results obtained from three neurons. B, comparison of the effects of 1 and 10 nM soman on evoked IPSCs recorded from slices that were not preexposed to 100 nM PB and from slices that had been preexposed for 5 to 8 min to 100 nM PB. Data were extracted from Figs. 2B and 10A to allow mutual comparison. C, inhibitory effect of soman on evoked IPSCs was unaltered when hippocampal slices were first exposed to 1 nM soman and subsequently to 1 nM soman plus 100 nM PB. Soman-induced inhibition of IPSCs remained after 10-min washing of the preparations with drug-free ACSF solution. Analyses of the results were done according to the protocol described in A. Each graph and error bar represent mean and S.E.M., respectively, of results obtained from four neurons. All experiments were carried out in the presence of 20 μM CNQX and 50 μM APV. Holding potential, −60 mV. Wherever indicated in the graphs of A, B, and C, amplitudes of events recorded in the presence of a given drug were significantly different from those of events recorded under control conditions; *p < 0.05 and **p < 0.01, according to the unpaired Student's t test.
The present study demonstrates that the nerve agent soman and the carbamate PB at clinically relevant concentrations selectively affect GABAergic transmission between the Schaffer collaterals and CA1 pyramidal neurons in rat hippocampal slices. Although sparing glutamatergic transmission, 1 to 100 nM soman inhibits and 100 nM PB facilitates GABAergic transmission. As discussed hereafter, the effects of both agents are the result of their direct interactions with different muscarinic receptors present on GABAergic axons/terminals synapsing onto the neurons from which recordings were obtained and correlate well with their reported behavioral effects.

**Mechanism of Action Underlying the Effects of Soman and PB on GABAergic Transmission in Rat Hippocampal Slices.** At 1, 10, and 100 nM, soman reduced by approximately 20, 35, and 36%, respectively, the amplitude of IPSCs evoked by field stimulation of Schaffer collaterals and recorded from CA1 pyramidal neurons. Soman also decreased the amplitude and frequency of spontaneous IPSCs recorded from CA1 pyramidal neurons. In contrast, PB potentiated evoked and spontaneous GABAergic transmission in the CA1 field of hippocampal slices; these effects were evident at 100 nM and became negligible at ≥300 nM PB. Whereas the effects of soman on GABAergic transmission could not be reversed during washout, those of PB were promptly reversed. The finding that soman had no effect on the amplitude or kinetics of miniature IPSCs and the fact that only at ≥3 μM can soman or PB interact directly with GABA_A receptors (Gant et al., 1987; Swanson et al., 1997) indicate that soman-induced inhibition and PB-induced potentiation of GABAergic transmission result from presynaptic actions of the drugs.

Cholinergic mechanisms regulating synaptic transmission in the CNS are mediated by ligand-gated nAChRs and metabotropic muscarinic receptors (Table 3). Different nAChR subtypes regulate synaptic transmission in the hippocampus. In general, activation of α4β2 and α7 nAChRs in GABAergic fibers/neurons facilitates spontaneous and inhibits evoked release of GABA (Alkondon et al., 1999, 2000; Radcliffe et al., 1999), whereas activation of α7 and α3β4 nAChRs in glutamatergic fibers/neurons facilitates glutamate release (Radcliffe et al., 1999; Alkondon and Albuquerque, 2002). In the hippocampus, m1 and m3 receptors have been detected in pyramidal neurons and in a small population of interneurons, whereas m2 receptors have been found predominantly in interneurons (Levey et al., 1995; Hájos et al., 1998). Activation of m1 or m3 receptors in interneurons increases GABAergic activity impinging onto CA1 pyramidal neurons in hippocampal slices (Martin and Alger, 1999). On the other hand, activation of m2 receptors decreases GABAergic activity impinging onto the perisomatic region of CA1 pyramidal neurons in the rat hippocampus (Pitler and Alger, 1992; Hájos et al., 1998). Muscarinic agonists also inhibit glutamatergic transmission between the Schaffer collaterals and CA1 pyramidal neurons.
rons; this effect has been attributed to activation of m1 receptors in glutamatergic fibers (Sheridan and Sutor, 1990).

The effects of soman and PB on GABAergic transmission cannot be solely explained by increased ACh concentration arising from cholinesterase inhibition. First, the maximal effect of soman on GABAergic transmission was observed at 10 nM, whereas 1 nM soman was sufficient to inhibit cholinesterase activity by 99.5% in hippocampal slices. Second, 15-min exposure of intact hippocampal slices to 100 nM PB facilitated GABAergic transmission, but caused no significant change in cholinesterase activity. Third, increased availability of ACh resulting from cholinesterase blockade by soman or PB would have altered the activity of nACHRs and muscarinic receptors, and consequently, affected both glutamatergic and GABAergic transmissions. If cholinesterase inhibition underlies the effects of soman or PB on GABAergic transmission, they should also have been sensitive to nicotinic and muscarinic receptor antagonists. Instead, soman- and PB-induced changes in GABAergic transmission were selectively blocked by muscarinic receptor antagonists. Therefore, potentiation and inhibition of GABAergic transmission by PB and soman, respectively, are most likely mediated by direct interactions of these compounds with muscarinic receptors present in GABAergic neurons/fibers synapsing onto the pyramidal neurons studied.

Previous binding and functional studies have indicated that soman and PB interact directly with m2 and m3 receptors. In the rat heart, soman displaces binding of $[^3H]$cis-methyldioxalane from m2 receptors; the reported $K_{i}$ value is 0.8 nM (Silveira et al., 1990). At concentrations >100 nM, soman also acts as an m3 receptor antagonist as indicated by its ability to inhibit mucus secretion in the ferret trachea (McBride et al., 1991; Ramnarine et al., 1996). Soman-induced inhibition of GABAergic transmission was blocked selectively by the m2 receptor-prefering antagonist AFDX-116 and showed a concentration dependence similar to that of soman-induced displacement of $[^3H]$cis-methyldioxalane binding from m2 receptors (Silveira et al., 1990). These findings, in addition to the fact that selective inhibition of m2 receptors by AFDX-116 alone caused no significant change on GABAergic transmission, indicate that the effect of soman on GABA release results primarily from its direct action as an agonist on presynaptic m2 receptors. However, the possibility that m3 receptor block contributes to soman-induced inhibition of GABAergic transmission cannot be ruled out, because the effect of soman on spontaneous IPSCs was more pronounced in slices where cholinergic activity was enhanced, as previously reported, by field stimulation of Schaffer collaterals (Pitler and Alger, 1992; Alkondon et al., 1998; Araque et al., 2002).

In rat brain membranes, PB displaces binding of the m2/m4 ligand $[^3H]$oxotremorine with an $IC_{50}$ value of approximately 1 μM (Lockhart et al., 2001) and in the rat trachea, PB activates m3 receptors with an $EC_{50}$ value of about 2.8 μM (Shibata et al., 1998). PB-induced activation of m3 receptors on GABAergic fibers synapsing onto the CA1 pyramidal neurons under study can explain the finding that potentiation of GABAergic transmission by 100 nM PB was selectively blocked by the m3 receptor-prefering antagonist 4-DAMP. The finding that PB at ≈300 nM had no significant effect on GABAergic transmission can be accounted for by the fact that direct activation of m2/m4 receptors by PB and consequent reduction of GABA release counteracts (and might eventually prevail over) the increased GABA release resulting from activation of m3 receptors. This notion is in agreement with the finding that in three of four neurons 100 nM PB caused a slight, but significant inhibition of GABAergic transmission when m3 receptors were inhibited by 4-DAMP.

**Pretreatment, but not Post-Treatment with PB Can Mask Soman-Induced Inhibition of GABAergic Transmission.** In hippocampal slices where GABAergic transmission had already been inhibited by 1 nM soman, 100 nM PB was devoid of any effect. An irreversible interaction of soman with m3 receptors could explain the ability of the organophosphate to inhibit the effect of PB. Alternatively, because there seems to be cross talk between m2 and m3 receptors at the level of second messenger (Dell’Acqua et al., 1993), it is possible that alterations induced by soman in the coupling of m2 receptors with $G_{o}$ proteins impair signaling of the m3 receptors that subserves the effects of PB on GABAergic transmission.

In hippocampal slices where GABAergic transmission had been potentiated by preexposure to 100 nM PB, the inhibitory effect of soman could still be detected. However, because the effects of 100 nM PB and 1 nM soman on GABA release were diametrically opposed, preexposure of the slices to 100 nM PB masked the effect of 1 nM soman. Pretreatment of the slices with PB at a higher concentration that had negligible effect on GABAergic transmission did not affect the magnitude of the inhibitory effect of soman on GABA release. These findings indicate that PB up to 1 μM is unable to reverse the pseudoirreversible/irreversible interaction of soman with its primary target, most likely, the m2 receptors. They also suggest that m3 receptor activation does not alter m2 receptor signaling.

**Toxicological Relevance.** Anxiety and hypolocomotion are common symptoms observed in animals treated with low doses of soman, and seizures are commonly observed in animals and humans exposed to high doses of soman (Moore, 1998). These symptoms correlate well with decreased GABA receptor activity in the CNS (Ungard et al., 2000; Trevitt et al., 2002) and can, therefore, be the result of the concentration-dependent inhibition of GABAergic transmission by soman. In addition, although the cationic structure of PB impairs its access to the brain, CNS-related symptoms have been reported in both laboratory animals and humans treated with clinically relevant doses of PB (Borland et al., 1985; Servatius et al., 1998; Abou-Donia et al., 2002). These symptoms, which include increased arousal in humans and increased startle response in rats, have also been observed under conditions of increased GABAergic activity (Fendt, 1998; Xi et al., 1999) and can thereby be the result of PB-induced facilitation of GABAergic transmission.

The finding that potentiation of GABAergic transmission by 100 nM PB functionally antagonized soman-induced inhibition of GABAergic transmission only when hippocampal slices were pretreated with PB lends support to the concept that PB can be an effective preventive countermeasure to the neurotoxicity induced by low doses of soman. It is concluded from the present study that, acting via m3 receptors present on GABAergic neurons, PB at 100 nM effectively prevents inhibition of GABAergic transmission induced by the interactions of soman with m2 receptors located on GABAergic
neurons. Thus, in the absence of the nonselective muscarinic antagonist atropine, PB can effectively counteract the toxic effects resulting from exposure to low doses of soman.

Acknowledgments

We thank Mabel Zelle, Barbara Marrow, and Bhagavathy Alkondon for technical support.

References


Chebabo SR, Santos MD, and Albuquerque EX (1999) The organophosphate sarin, atropine, PB can effectively counteract the toxic effects resulting from exposure to low doses of soman.