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Evaluation of cognitive and biochemical effects of low-level exposure to sarin in rhesus and African green monkeys☆

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Abstract

We investigated the potential of low-level exposures to the chemical warfare nerve agent, sarin, to produce adverse effects. Rhesus (*Macaca mulatta*) and African green monkeys (*Chlorocebus acthiops*) were trained on a serial probe recognition (SPR) task before IM administration of a low-level concentration (5.87 μg/kg or 2.93 μg/kg) of sarin. Blood was sampled before agent administration and at various times following administration. Sarin administration did not disrupt performance on the SPR task in either species. Major dependent measures characterizing performance (accuracy, number of completed trials per session, average choice response time) were largely unaffected on the day sarin was administered as well as on subsequent testing sessions occurring over several weeks following administration. Analyses of red blood cell (RBC) and plasma samples revealed that sarin administration produced a substantial degree of inhibition of circulating acetylcholinesterase (AChE) in RBC fractions and butyrylcholinesterase (BChE) in plasma fractions, which only slowly recovered. In this regard, AChE activity was inhibited to a greater extent than BChE activity. Blood samples were also evaluated for regenerated sarin, which was found in RBC and plasma fractions in both species and showed orderly elimination functions. More sarin was regenerated from RBC fractions than from plasma fractions. Elimination of regenerated sarin was much slower in RBC than plasma and exceeded the expected time of AChE aging, suggesting the presence of additional sarin binding sites. In general, effects were similar in both species. Taken together, our results show that while the concentrations of sarin administered were clearly biochemically active, they were below those that are required to produce a disruption of behavioral performance.

Keywords: Sarin; Primate; Rhesus; African green; GB; SPR; Cognition; Memory

☆ The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense, (para 4-3, AR 360-5). Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition. All procedures were reviewed and approved by the Institutes' Animal Care and Use Committees, and performed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Portions of this study were presented at the 2005 Scientific Conference on Chemical and Biological Defense Research, Timonium, MD, USA.

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1. Introduction

Sarin, or GB, is an organophosphorus chemical warfare nerve agent (CWNA) that is part of the "G" series of agents, which also includes GA (tabun), GD (soman) and GF (cyclosarin). The major effect of this highly potent agent is the rapid inhibition of acetylcholinesterase (AChE). The inhibition of AChE by sarin occurs via phosphorylation of active-site serine, which is followed by dealkylation resulting in irreversibly inhibited enzyme (Harris et al., 1966; Worek et al., 2005). The resulting increase in cholinergic activity produces a cholinergic crisis that, when exposure is sufficient, results in death. The clinical sequelae following sufficient CWNA exposure includes convulsions, salivation, nystagmus, confusion, anxiety, irritability, muscle fasciculation, and tremors (e.g., Grob and Harvey, 1953; Bowers et al., 1964; Sidell, 1974).

The effects of large exposures to CWNA have been well investigated but far less is known about the potential effects of low-level exposure to CWNA. In this regard, a low-level exposure is generally considered to be one that does not produce severe clinical signs of toxicity such as convulsions. While the effects of low-level CWNA exposures have received some attention (e.g., Somani and Romano, 2001), there is a paucity of studies of low-level CWNA exposure in higher-order species such as primates. We, therefore, evaluated the effects of low-level exposure to sarin in rhesus and African green monkeys.

To evaluate performance, we used a serial probe recognition (SPR) test. The SPR is an established procedure to measure cognitive functioning in non-human primates (Castro and Finger, 1991; Castro et al., 1992). Additionally, the procedure provides measures of general performance and motivation level. Biochemical evaluations consisted of measuring circulating cholinesterase (ChE) activity and regenerated sarin in blood. ChE inhibition is a standard procedure to evaluate the effect of CWNA exposure. The regeneration assay was also employed that provides a direct measure of the quantity of circulating agent, and thus, provides an additional means of comparing exposures across species, type of CWNA and route of exposure (Jakubowski et al., 2004). While evaluations focused on the time period immediately after exposure, measures were taken as long as 10 weeks following exposure.

2. Methods

2.1. Subjects

Five rhesus monkeys (*Macaca mulatta*, three male and two female) and two African green monkeys (*Chlorocebus acthiops*, female) were used. Male rhesus (N670, EPSI, Omega) aged between 15 years and 20 years and weighed 7.9–8.11 kg. Female rhesus (HPK, FGW) were 13–14-year old and weighed 4.3–4.9 kg. Female African green monkeys, 5041 and 5037 (adult of indeterminate age) weighed 4.1–4.3 kg. Subjects were individually housed and maintained in a temperature (20–22 °C) and humidity (50 ± 10%) controlled vivarium under a 12 h light–dark cycle (lights on at 0600).

Water was available ad libitum and all subjects were fed commercial primate rations supplemented with fresh fruit and vegetables, vitamins, and banana-flavored pellets (750 mg or 190 mg, Bio-Serv, Frenchtown, NJ) earned during behavioral sessions. Animals were mildly food restricted to maintain performance motivation by regulating food intake outside of that earned during behavioral sessions. All subjects had at least some previous experience with the SPR procedure. Subjects had not received any pharmacological agents for testing for at least 6 months before participation in the study.

2.2. Behavioral procedures

Behavioral sessions were conducted with subjects unrestrained in their home cages using a custom manufactured (BRS/LVE, Laurel, MD) behavioral test panel measuring 22 in. (h) × 27 in. (w). The panel contained a 15 in. (diagonal), touch-sensitive, flat-panel screen monitor (model FP15C1A0-12, Omni Vision, Glendale Heights, IL), mounted 7.5 in. from the sides and 5.5 in. from the top of the panel and was used to present visual stimuli and record responses. The panels also contained a speaker (mounted in the upper left corner) and a food trough (mounted in the center and 1 in. from the bottom). The trough was connected to a food dispenser capable of delivering 750 mg (rhesus) or 190 mg (African green) food pellets (Bio-Serv, Frenchtown, NJ). Experimental parameters were controlled and monitored using custom programs (written by Genovese) running on a laptop computer (e.g., Dell Lattitude C640) mounted on the back of each panel.

All subjects were tested behaviorally using a SPR task. The SPR task is a list memory task wherein sequences of stimuli (sample stimuli) are presented. Subsequently, the subject is presented with a choice stimulus (probe) and, based upon extensive training, responds differentially depending upon whether or not the probe stimulus appeared in the previous sequence. SPR methodology has been described in detail elsewhere (Castro and Finger, 1991; Castro et al., 1992; Castro, 1995). Briefly, a trial contains an observation period and a choice period. In the observation period, a sequence of stimuli is presented individually in the center of the screen. For rhesus, the sequence consisted of six stimuli and for the African greens, three stimuli. Each stimulus was distinct and contained two alpha-numeric characters. Stimuli in the list were chosen randomly (without replacement for a given list) from a set of 197 possible. List stimuli were displayed until touched by the subjects or 5 s had elapsed. After all list stimuli were displayed, the choice period began. In the choice period, two stimuli were presented side by side on the screen (left–right positioning was