Muscarinic Receptor Dysfunction Induced by Exposure to Low Levels of Soman Vapor

Paul A. Dabisch, Michael S. Horsmon, William T. Muse, Robert J. Mioduszewski, and Sandra Thomson

Operational Toxicology Team, U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, Maryland 21010-5424

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In the eye, it has been previously reported that exposure to a cholinesterase inhibitor results in a reduced miotic response following prolonged exposure and a decreased miotic response to the cholinergic agonists. However, no studies exist that characterize the effect of a single low-level vapor exposure to a nerve agent on parasympathetic function in the eye or determine the threshold dose for such an effect. The present study investigated the hypotheses that a single low-level exposure to soman vapor would result in dysfunction of the parasympathetic pathway mediating the pupillary light reflex resulting from a loss of muscarinic receptor function on the pupillary sphincter muscle. Adult male rats were exposed to soman vapor in a whole-body dynamic airflow exposure chamber. Rats exposed to low levels of soman vapor dose-dependently developed miosis (threshold dose between 4.1 and 6.1 mg-min/m³). Pupil size returned to preexposure levels within 48 h due to desensitization of pupillary muscarinic receptors, as assessed by the pupillary response to the muscarinic agonist oxotremorine. An attenuated pupillary light reflex was also present in miotic animals (threshold dose near 6.1 mg-min/m³). While pupil size recovers within 48 h, other measures of pupillary function, including the light reflex, acetylcholinesterase activity, and muscarinic receptor responsiveness, did not return to normal for up to 10 days postexposure. Recovery of the light reflex coincided with the recovery of pupillary muscarinic receptor function, suggesting that the attenuation of the light reflex was due to receptor desensitization.

Key Words: miosis; soman; organophosphates; muscarinic receptors; pupillary light reflex.

However, at lower doses, few if any of these severe toxic signs are observed. Pupil constriction, or miosis, is one of the threshold clinical effects that can be observed following less-than-lethal dosages of nerve agent vapor exposure and is due to direct contact of nerve agent vapor with the ocular surface (Sim, 1956).

In the eye of the rat, both the sympathetic and parasympathetic nervous systems are involved in the regulation of pupillary size. Activation of the sympathetic nervous system results in dilation of the pupil through the release norepinephrine onto alpha-adrenergic receptors located on the radial muscles of the iris (Yu and Koss, 2002, 2003). Activation of the parasympathetic nervous system results in constriction of the pupil through the release of acetylcholine onto muscarinic acetylcholine receptors present on the pupillary sphincter. Predictably, administration of muscarinic antagonists results in pupillary dilation, demonstrating the role for muscarinic receptors and the parasympathetic nervous system in the control of pupil diameter (Furuta et al., 1998; Smith et al., 1996). Nerve agent-induced miosis is due to excessive stimulation of the parasympathetic nervous system and its associated muscarinic receptors located on the pupillary sphincter muscle.

It is well known that tolerance to a cholinergic agonist develops following prolonged stimulation, and this tolerance has been reported in several tissues, including the eyes (Bito and Banks, 1969; Smith and Smith, 1980), the heart (Shui et al., 2002; Vallette et al., 1997; Zhu et al., 1991), and the gastrointestinal tract (Mita et al., 1997). It has also been reported that exposure to an OP compound, an indirect cholinergic agonist, results in downregulation of muscarinic acetylcholine receptors in the retina (Tandon et al., 1994), and brain (Tang et al., 1999) of the rat, suggesting that cholinergic tolerance may be a result of receptor downregulation or internalization. However, in the iris of guinea pigs, it has been reported that exposure to the OP compound soman results in a decreased miotic potency of soman upon subsequent exposure without a change in the number of muscarinic receptors present in the iris (Soli et al., 1980), suggesting that receptor downregulation and/or internalization is not responsible for the observed tolerance. Further studies have demonstrated that phosphorylation of muscarinic receptors by a G

The nerve agent α-pinacolyl methylphosphonofluoridate, also known as soman or by its military designation GD, is a highly toxic organophosphorous (OP) compound that exerts its effect by inhibiting the enzyme acetylcholinesterase (AChE). Inhibition of AChE results in the accumulation of the neurotransmitter acetylcholine and excessive stimulation of cholinergic receptors. At higher doses, effects of exposure include salivation, muscle twitches, tremors, convulsions, seizures, and death usually attributable to respiratory failure (Taylor, 2006).

1 To whom correspondence should be addressed at Operational Toxicology Team, U.S. Army Edgewood Chemical Biological Center, ATTN: AMSRD-ECB-RT-TT, 5183 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5424. Fax: (410) 436-7129. E-mail: paul.a.dabisch@us.army.mil.

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protein–coupled receptor kinase activated following excessive stimulation of the receptor results in loss of receptor responsiveness (Shui et al., 2002). Thus, the mechanisms mediating cholinergic tolerance can vary from tissue to tissue, but commonly involve either internalization of the receptors or phosphorylation and subsequent inactivation of the receptors.

Previous studies in the human eye have demonstrated that administration of a cholinergic agonist results in an attenuated pupillary light reflex after the eye has recovered from miosis (Smith and Smith, 1980), suggesting dysfunction of the parasympathetic branch of the autonomic nervous system. Additionally, it has been reported that exposure to a cholinesterase inhibitor results in a decrease in the miotic response following administration of a cholinergic agonist (Bito and Banks, 1969). However, these effects utilized repeated exposures (Dabisch et al., 2005; Soli et al., 1980) or applied the chemical directly to the surface of the eye (Bito and Banks, 1969; Smith and Smith, 1980; Soli et al., 1980). Arguably, the most relevant route of exposure to a cholinesterase inhibitor, such as a pesticide or nerve agent, is exposure to vapor or aerosol. However, no studies exist that characterize the effects of a single low-level whole-body vapor exposure to a nerve agent on parasympathetic function in the eye or determine the threshold dose necessary to produce these effects. The role muscarinic receptor desensitization plays in mediating the decreased response to cholinergic agonists in the eye following prolonged stimulation is also unclear. Therefore, the present study investigated several hypotheses aimed at elucidating the effects of low-level exposures on parasympathetic function in the eye of the rat. The first hypothesis was that a single low-level exposure to soman vapor would result in dysfunction of the parasympathetic pathway mediating the pupillary light reflex resulting from a loss of muscarinic receptor function on the pupillary sphincter muscle. Additionally, since loss of muscarinic receptor function would prevent contraction of the pupillary sphincter muscle, it was hypothesized that the “recovery” of pupil size following exposure to soman was due to loss of muscarinic receptor function and not due to recovery of AChE activity in the eye. If this hypothesis is correct, it would also be expected that the loss of muscarinic receptor function would persist after the miotic response to soman had disappeared.

MATERIALS AND METHODS

Animal model. Autonomic control of pupil size in humans is dependent upon muscarinic and alpha-adrenergic receptors located in the iris (Henderer and Rapuano, 2006). The human eye also possesses a rapid, measurable, and reproducible pupillary response to light (Bergamin et al., 2003; Lee et al., 2005; Yasukouchi et al., 2007). The eye of the rat also displays a large, rapid pupillary response to light, and autonomic control of pupil size is also mediated by muscarinic and alpha-adrenergic receptors (Dabisch et al., 2005; Fuuta et al., 1998; Smith et al., 1996; Yu and Koss, 2002, 2003). Therefore, adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 200–300 g were used in this study. All experiments and procedures were approved by the U.S. Army Edgewood Chemical Biological Center Institutional Animal Care and Use Committee and conducted in accordance with the requirements of the National Research Council’s “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, 1996).

Soman vapor generation. Soman vapor generation was accomplished using a glass saturator cell as described previously (Muse et al., 2006). Briefly, neat liquid soman (Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD) was placed into a glass multipass saturator cell. The saturator cell contains a hollow ceramic cylinder that serves to increase the contact surface area between the liquid soman and the nitrogen carrier gas that flows through the saturator cell. The nitrogen carrier gas makes three passes along the surface of the wetted ceramic cylinder before exiting the outlet arm of the glass cell. As the nitrogen carrier gas flows through the saturator cell, it becomes saturated with soman vapor. The temperature of the water-jacketed saturator cell and the flow rate of the nitrogen determine the amount of soman vapor generated. The flow rate of the nitrogen carrier gas through the saturator cell ranged from 10 to 100 ml/min. This carrier gas is subsequently drawn into a Rochester-style 750-liters-per-minute airflow chamber that has an airflow rate of approximately 900 liters/min. The interior of the exposure chamber was maintained under negative pressure (−0.30 to −0.40” H2O) and monitored with a calibrated manometer (Dwyer, Michigan City, IN). A thermo-anemometer (Model 8565, Anhorn, Skokie, IL) was used to monitor chamber airflow at the chamber outlet. Soman vapor from the chamber atmosphere was collected onto a 10-mm Tenax TA—Haysep D solid sorbent tube (CDS Analytical, Oxford, MA). Soman concentrations were then determined using a gas chromatograph equipped with a flame photometric detector (Agilent Technologies Inc., Wilmington, DE).

Exposure protocol. Previous studies have used 60-min exposures to assess the effects of OP compounds, including nerve agents, on the eye and other organ systems of the rat (Benton et al., 2005; Whalley et al., 2004). In order to make potency comparisons between the present and past studies, similar exposure conditions were used in the present study. Male rats were exposed to either soman vapor or air in a whole-body dynamic flow inhalation chamber for 60 min. Exposure groups consisted of 10 soman-exposed rats and five air-exposed rats. Eight groups were exposed to doses of soman vapor ranging from 2.1 to 24.0 mg-min/m3 in order to determine the dose-response relationship between soman concentration and the degree of pupil constriction. Measurements of pupil size and the pupillary light reflex were made preexposure and at 20 min, 22 h, 28 h, 48 h, 4 days, 6 days, and 10 days postexposure in order to determine the magnitude of any response elicited by soman exposure, as well as to estimate the rate of recovery of any changes seen.

An additional group of rats was exposed to 24.0 mg-min/m3 of soman (0.4 mg/m3 for 60 min). A portion of this group (30 rats) was used for the determination of AChE and butyrylcholinesterase (BChE) activities in the anterior eye at 20 min, 2 days, 6 days, 14 days, and 21 days postexposure. The remainder of the group (five rats) was used for the determination of muscarinic receptor functionality using oxotremorine at 2, 6, and 9 days postexposure.

Ocular measurements. The right eye of all rats in the study was digitally photographed in order to determine the degree of miosis produced by exposure to soman vapor. This technique has been described previously (Dabisch et al., 2005). Briefly, animals were temporarily hand restrained (<20 s) to minimize movement of the head, and the pupil was illuminated with an infrared spotlight (SL1236, Advanced Illumination, Rochester, VT). Several images were digitally recorded using an infrared capable video camera (model XC-ST30, Sony Corp., Park Ridge, NJ) equipped with a 75-mm/F2.7 lens (model LMV7527) and an image acquisition PC card (model PCI-1411, National Instruments Corp., Austin, TX). All images were taken under controlled low-light conditions (<5 lux) and only after the animals had been allowed to adjust to the darkness for at least 5 min. Pupil and iris radii were determined using custom image analysis software written using LabView 6 (National Instruments Corp.). For each animal, the pupil and iris measurements from two to three images.
were averaged. The results were expressed as the ratio of the pupil radius to the iris radius in order to correct for any variability between animals in the distance from the camera to the eye. Pupil size was measured at the times mentioned previously.

The pupillary light reflex in the right eye of all rats in the study was also measured in order to determine whether a given dose of soman produced alterations in parasympathetic function. A similar technique has been described previously (Dabisch et al., 2005). Briefly, animals were temporarily hand restrained (< 90 s) to minimize movement of the head, and the pupil was illuminated with an infrared spotlight (model SL1236, Advanced Illumination). A 25-W light illuminated the right eye of the rat for 13 s, resulting in a light intensity of 15 lux at the eye of the rat. Digital images of the eye were taken at a rate of 1 per second until the pupil had returned to its baseline diameter. Pupil images were acquired and analyzed using the same acquisition and analysis system mentioned previously. All light reflexes were recorded under controlled low-light conditions (< 5 lux) and only after the animals had been allowed to adjust to the darkness for at least 5 min. The light reflex was measured at the times mentioned previously.

Rats exposed to 24.0 mg-min/m3 of soman vapor were euthanized at either 2, 4, 6, 14, or 21 days postexposure in order to determine the degree of inhibition of AChE and BChE in the anterior portion of the eye postexposure, as well as the rate of recovery of AChE and BChE activity. Immediately following euthanasia, both eyes were removed and snap frozen in liquid nitrogen. Each eye was then removed from the liquid nitrogen, and a circumferential scalpel incision was made to separate the anterior and posterior halves. The anterior portion, containing the pupillary sphincter muscle, was removed from the posterior portion and the lens. The anterior portion was washed with 0.69mM phosphate buffer and placed in a fresh volume of 0.69mM phosphate buffer so that the final weight to volume ratio was 100 mg tissue per 1 ml of saline. The tissue was then homogenized using a Sonicator 3000 ultrasonic grinder (Misonix Inc., Farmingdale, NY). The sample was cleared of cell debris by centrifugation at 15,000 rpm for 5 min at 15°C. The cleared sample was diluted 1:200 in 0.69mM phosphate buffer, and the activities of AChE and BChE in the anterior eye were determined using an assay kit based on the Ellman assay (Cholinesterase Chemistry Set—Model 700, EJM Research, Cincinnati, OH). AChE and BChE activities were also determined in whole blood drawn from a tail snap at 20 min postexposure.

To determine the functionality of muscarinic receptors in the eye, the magnitude of the pupil constriction induced by the muscarinic receptor agonist oxotremorine was assessed. This method was adapted from a previously published study (Hagan et al., 1988). Briefly, rats were anesthetized with a combination of ketamine (50 mg/kg im) and xylazine (10 mg/kg im). Baseline infrared images of the pupils were taken under low-light conditions (< 5 lux) as described previously. A 20-μl drop of the muscarinic receptor agonist oxotremorine (1.6 mg/ml; Sigma Aldrich, St Louis, MO) dissolved in saline was placed on the surface of the eye. Infrared images of the pupil were taken every 10 min to monitor the change in pupil size.

Data analysis. Statistical analysis was done by ANOVA with a Bonferroni/Dunn posttest. A p value of < 0.05 was used as the criterion for statistical significance. All numerical values are reported as mean ± SEM.

RESULTS

The effect of a single 60-min exposure to soman vapor on pupil size in the rat is summarized in Figure 1. Soman vapor dose-dependently produced pupil constriction. The first significant decrease in pupil size occurred at a concentration of 0.101 mg/m3 for 60 min or a dose of 6.1 mg-min/m3. No significant pupil constriction was seen at a concentration of 0.068 mg/m3 for 60 min or a dose of 4.1 mg-min/m3. Thus, the threshold dose required to produce a significant decrease in pupil size in male Sprague-Dawley rats is between 4.1 and 6.1 mg-min/m3 for a 60-min exposure. Representative pupil images from several soman-exposed rats are shown in Figure 2. A dose of 4.1 mg-min/m3 did not result in a pupil size that was significantly smaller than air-exposed rats (Figs. 2A and 2B), while exposure to 8.1 and 24.0 mg-min/m3 of soman vapor produced dose-dependent decreases in pupil size (Figs. 2C and 2D).

The effect of a single 60-min exposure to soman vapor on the pupillary light reflex is shown in Figure 3. The light reflex
was recorded at 48 h postexposure, after the pupils of soman-exposed rats had recovered to the preexposure baseline size. Soman vapor dose-dependently attenuated the pupillary light reflex. Exposure to a dose of 6.1 mg-min/m³ of soman vapor resulted in a small, but significant, decrease in the magnitude of the pupil constriction induced by illumination of the eye. Exposure to doses of 17.0 and 24.0 mg-min/m³ produced a significantly greater attenuation of the pupillary light reflex (Fig. 3).

A dose of 24.0 mg-min/m³ (0.40 mg/m³ for 60 min) was chosen to explore the mechanisms involved in the loss of the pupillary light reflex, as well as the time course of the recovery. This dose was chosen because it produced the largest attenuation of the pupillary light reflex in the present study. The pupils of rats exposed to this dose of soman vapor were decreased to 8 ± 4% of the baseline size immediately after the exposure period (Fig. 4). The pupils of rats exposed to room air were 99 ± 10% of baseline following the exposure period (data not shown). Pupil size returned to baseline over the next 48 h (Fig. 4). The light reflex in animals exposed to a dose of 24.0 mg-min/m³ of soman vapor was greatly attenuated at 48 h postexposure (Fig. 5A). At 6 days postexposure, the light reflex had partially recovered but was still significantly decreased relative to air-exposed control animals (Fig. 5B). The light reflex was no longer significantly different from that of air-exposed rats at 10 days postexposure (Fig. 5C).

AChE and BChE activities in the anterior portion of the eye were also investigated, and these data are summarized in Figure 6. Following exposure to a dose of 24.0 mg-min/m³ of soman vapor, AChE activity in the anterior portion of the eye was decreased from a baseline value of 916 ± 63 to 390 ± 70 U/L. AChE activity in the anterior eye did not significantly recover during the first 6 days following the exposure. By 14 days postexposure, AChE activity in the anterior eye had recovered to the preexposure baseline level. Following exposure to a dose of 24.0 mg-min/m³ of soman vapor, BChE activity in the anterior eye was decreased from a baseline level of 361 ± 19 to 106 ± 22 U/L. BChE activity in the anterior eye did not significantly recover during the first 6 days following the exposure but completely recovered to the preexposure baseline level by 14 days postexposure. In the blood, AChE and BChE activities were not significantly decreased following exposure to soman vapor at doses less than 6.1 mg/m³ (data not shown). A small degree of AChE depression (< 15%) was detected at higher doses (data not shown).

The effect of the muscarinic receptor agonist oxotremorine on pupil size in ketamine/xylazine anesthetized rats was assessed at 2, 6, and 9 days postexposure in both soman- and air-exposed rats. The pupils of soman-exposed rats were not significantly different in size relative to air-exposed control rats at the time of testing. Administration of ketamine/xylazine slightly increased the baseline pupil size (data not shown). A 20-μl drop of oxotremorine (1.6 mg/ml) placed onto the surface of the eye resulted in a peak change in pupil size that occurred at approximately 40 min postadministration. This is consistent with previous studies (Hagan et al., 1998). In rats exposed to soman vapor, the peak change in pupil size following oxotremorine administration was significantly decreased relative to air-exposed rats at 2 days postexposure (Fig. 7). The response to oxotremorine recovered gradually over the next 7 days and was not significantly different from air-exposed control rats at 9 days postexposure (Fig. 7).
Nerve agents, including soman, are known to produce a myriad of effects due to their ability to inhibit the enzyme AChE. Some of the first effects seen upon exposure to low levels of nerve agent vapor include miosis, tightness in the eyelids, and a decrease in the pupillary light reflex.

**FIG. 5.** Recovery of the pupillary response to light following exposure to 0.40 mg/m³ of soman vapor for 60 min. At 48 h following the exposure, the pupil size of exposed rats was the same as air-exposed control rats. However, the pupillary light reflex was significantly attenuated. At 6 days postexposure, the pupillary response to light in soman-exposed rats had recovered but was still significantly attenuated relative to air-exposed rats.

**FIG. 6.** Recovery of AChE and BChE activities in the anterior eye following exposure to 0.40 mg/m³ of soman vapor for 60 min. (A) AChE activity was significantly decreased following exposure to soman vapor for at least 6 days. During this period, no significant recovery of AChE activity occurred. Between days 6 and 14 postexposure, AChE recovered and was not significantly different from the preexposure baseline level (BL) by 14 days postexposure. (B) BChE activity was significantly decreased following exposure to soman vapor for at least 6 days. During this period, no significant recovery of BChE activity occurred. Between days 6 and 14 postexposure, BChE recovered and was not significantly different from the preexposure BL by 14 days postexposure. Asterisk denotes $p < 0.05$ when compared to the appropriate preexposure baseline value.

At 10 days postexposure, the pupillary response to light was not significantly different between the soman- and air-exposed rats. At 10 days postexposure, the pupillary response to light was not significantly different between the soman- and air-exposed rats (C); asterisk denotes $p < 0.05$ when compared to the baseline pupil size (baseline level [BL]).

**DISCUSSION**

Nerve agents, including soman, are known to produce a myriad of effects due to their ability to inhibit the enzyme AChE. Some of the first effects seen upon exposure to low levels of nerve agent vapor include miosis, tightness in the eyelids, and a decrease in the pupillary light reflex.
Dabisch et al. (2004) have shown previously that exposure to nerve agent vapor on the eyes and respiratory tract (Brown and Brix, 1998; Sim, 1956). In the present study, miosis and large decreases in ocular AChE and BChE activities were present at soman doses that produced little or no decrease in whole blood AChE and BChE activities, supporting previous studies that suggest that nerve agents act directly on the eye and do not distribute to the eye through the blood. The miotic response to soman vapor was found to be dose dependent. At doses of 24.0 mg-min/m³ or less, the duration of the miotic response was less than 48 h. It was determined that the threshold dose required to produce a significant degree of pupil constriction in the male rat lies between 4.1 and 6.1 mg-min/m³ for a 60-min exposure. The EC₅₀ for miosis, or the dose of soman vapor required to produce a 50% decrease in pupil size, lies between 8.3 and 12.1 mg-min/m³. In male rats exposed to cyclosarin for 60 min, the EC₅₀ for miosis was reported to be 2.52 mg-min/m³, while the EC₅₀ for VX for a 60-min exposure was reported to be 0.38 mg-min/m³ (Benton et al., 2005; Whalley et al., 2004). Therefore, soman vapor is significantly less potent at producing miosis than either cyclosarin or VX.

As with the miotic response, exposure to soman vapor dose-dependently attenuated the pupillary light reflex, a response mediated by the parasympathetic nervous system in the eye (Dutsch et al., 2004). At a dose of 24.0 mg-min/m³, the duration of the attenuation produced by soman vapor lasted from 6 to 10 days. A dose of 6.1 mg-min/m³ produced only a slight attenuation of the pupillary light reflex, suggesting that this dose is near the threshold for attenuation in male rats for a 60-min exposure. While the threshold doses required to produce miosis and attenuation of the pupillary light reflex are similar, the duration of these responses is significantly different, with the attenuation of the pupillary light reflex persisting for up to 8 days after the pupil size has returned to normal in animals exposed to 24.0 mg-min/m³ of soman vapor. It is likely that the recovery of the pupillary light reflex is dose dependent, with higher doses of soman vapor resulting in a longer response than lower doses. However, additional studies are necessary to verify this since the recovery rate of the pupillary light reflex was not determined for doses other than 24.0 mg-min/m³. Cholinergic agonists and nerve agent vapor have been shown previously to alter the pupillary light reflex (Dabisch et al., 2005; Smith and Smith, 1980); however, the present study is the first to describe this effect following a single low-level exposure to nerve agent vapor and to determine a threshold dose for this effect.

It was hypothesized that the loss of the pupillary light reflex, a reflex mediated by the parasympathetic nervous system, is due to a loss of muscarinic receptor function on the pupillary sphincter muscle. Additionally, it was hypothesized that the recovery of pupil size following exposure to soman was also due to loss of muscarinic receptor function and not due to recovery of AChE activity in the eye. A dose of 24.0 mg-min/m³ of soman vapor produced a longer response than lower doses. However, additional studies are necessary to verify this since the recovery rate of the pupillary light reflex was not determined for doses other than 24.0 mg-min/m³. Cholinergic agonists and nerve agent vapor have been shown previously to alter the pupillary light reflex (Dabisch et al., 2005; Smith and Smith, 1980); however, the present study is the first to describe this effect following a single low-level exposure to nerve agent vapor and to determine a threshold dose for this effect.

FIG. 7. Effect of exposure to soman vapor on the miotic potency of oxotremorine. Rats, exposed to either soman vapor or air, were anesthetized with ketamine/xylazine. The baseline pupil size was similar following anesthesia in both soman- and air-exposed rats. However, the peak change in pupil size produced by administration of oxotremorine onto the surface of the eye (20 µl of 1.6 mg/ml oxotremorine) was significantly different between the two groups. Soman-exposed rats had a significantly blunted response to oxotremorine at 2 and 6 days postexposure, suggesting a decrease in the number of functional muscarinic receptors. Asterisk denotes significance when compared to the control response obtained in air-exposed rats.

chest, and rhinorrhea due to the direct actions of the nerve agent vapor on the eyes and respiratory tract (Brown and Brix, 1998; Sim, 1956). In the present study, miosis and large decreases in ocular AChE and BChE activities were present at soman doses that produced little or no decrease in whole blood AChE and BChE activities, supporting previous studies that suggest that nerve agents act directly on the eye and do not distribute to the eye through the blood. The miotic response to soman vapor was found to be dose dependent. At doses of 24.0 mg-min/m³ or less, the duration of the miotic response was less than 48 h. It was determined that the threshold dose required to produce a significant degree of pupil constriction in the male rat lies between 4.1 and 6.1 mg-min/m³ for a 60-min exposure. The EC₅₀ for miosis, or the dose of soman vapor required to produce a 50% decrease in pupil size, lies between 8.3 and 12.1 mg-min/m³. In male rats exposed to cyclosarin for 60 min, the EC₅₀ for miosis was reported to be 2.52 mg-min/m³, while the EC₅₀ for VX for a 60-min exposure was reported to be 0.38 mg-min/m³ (Benton et al., 2005; Whalley et al., 2004). Therefore, soman vapor is significantly less potent at producing miosis than either cyclosarin or VX.
including the eye (Bito and Banks, 1969) and heart (Shui et al., 2002).

In the rat iris, several subtypes of muscarinic receptors are known to be present. mRNAs coding for muscarinic receptor subtypes M2, M4, and M4 have all been found in the rat iris (Furuta et al., 1998). All of these subtypes are known to undergo desensitization following excessive stimulation with cholinergic agonists. M3 muscarinic receptor desensitization in SH-SY5Y cells has been shown to occur through a G protein–coupled receptor kinase–dependent pathway (Willetts et al., 2003). In cultured rat atrial cells and Chinese hamster ovary cells transfected with M2 muscarinic receptors, incubation with the muscarinic agonist carbachol depressed the response of muscarinic receptors to acetylcholine by greater than 90% through a similar G protein–coupled receptor kinase–mediated mechanism (Shui et al., 2002). M4 muscarinic receptors on NG108-15 cells have been shown to undergo desensitization mediated both by phosphorylation through a G protein–coupled receptor kinase–dependent pathway, as well as by internalization of the receptor (Holroyd et al., 1999). While all of these studies involve desensitization involving G protein–coupled receptor kinases, it is not possible to determine the role that these kinases may be playing in mediating the muscarinic receptor desensitization observed in the present study without additional experiments.

Since it was hypothesized that recovery of pupil size following exposure to soman was due to loss muscarinic receptor function, it was also expected that the loss of muscarinic receptor function would persist after the miotic response to soman had disappeared. Therefore, the present study also investigated that rate of recovery of the ocular effects of soman vapor exposure. Pupil size had returned to baseline level by 48 h following exposure to 24.0 mg-min/m³ of soman vapor. However, the response of the eye to oxotremorine in these animals took up to 9 days to completely recover. Similarly, the pupillary light reflex took up to 10 days to completely recover following exposure to 24.0 mg-min/m³ of soman vapor. These data suggest that muscarinic receptor function was slowly recovering during the postexposure period, either through synthesis of new receptors or reactivation of desensitized receptors, and that this recovery of muscarinic receptor function is responsible for the recovery of the pupillary light reflex. However, AChE and BChE in the eyes did not begin to recover until after 6 days postexposure. Thus, the muscarinic receptors mediating contraction of the pupil were recovering while inhibition of AChE and BChE, the stimulus that resulted in desensitization in the first place, was still present. This seems counterintuitive since it would be expected that acetylcholine would be present in high levels due to the inhibition of AChE and BChE, producing prolonged stimulation of muscarinic receptors and, subsequently, prolonged desensitization. However, it is known that presynaptic muscarinic autoreceptors are involved in a negative feedback loop that helps to regulate neuronal acetylcholine release in several tissues, including the eye (Mattio et al., 1984), lung (Fryer and Jacoby, 1998), and bladder (D’Agostino et al., 2000). In the rat eye, it has been previously demonstrated that the increase in acetylcholine levels induced by the cholinesterase inhibitor diisopropylfluorophosphate gradually decreases within 6 h of administration as a result of stimulation of muscarinic presynaptic autoreceptors (Mattio et al., 1984). Therefore, in the present study, it is possible that stimulation of muscarinic presynaptic autoreceptors resulted in decreased acetylcholine release from the nerve terminal over time, compensating for the loss of the ability of break down acetylcholine once it has been released due to the inhibition of AChE. This would result in a decreased amount of acetylcholine in the synaptic cleft and decreased stimulation of muscarinic receptors on the pupillary sphincter, thereby allowing the recovery of receptor function to occur. Additionally, it is possible that decreased acetylcholine release due to muscarinic autoreceptor stimulation may also contribute to the recovery in pupil size immediately following exposure to soman vapor. However, further experiments are necessary to investigate the involvement of muscarinic presynaptic autoreceptors in the responses observed in the present study.

As mentioned previously, the threshold doses of soman vapor required for pupil contraction and attenuation of the pupillary light reflex were similar. It was determined that the threshold dose required to produce a significant decrease in both pupil size and the pupillary light reflex is near 6.1 mg-min/m³ in the male rat for a 60-min exposure. It would be expected that the threshold doses for these effects should be similar since inhibition of pupillary AChE and subsequent excessive stimulation of pupillary muscarinic receptors are necessary to initiate both responses.

In summary, male Sprague-Dawley rats exposed to low levels of soman vapor dose-dependently developed miosis, with the threshold dose falling between 4.1 and 6.1 mg-min/m³. Recovery of pupil size from soman-induced miosis occurred within 48 h postexposure; however, the recovery of pupil size was due to muscarinic receptor desensitization and not recovery of AChE and BChE activities. Doses of soman that produced miosis also resulted in parasympathetic dysfunction in the eye characterized by an attenuated pupillary light reflex, with the threshold for the response falling near 6.1 mg-min/m³. While pupil size recovers within 48 h, other measures of pupillary function, including the pupillary light reflex, pupillary AChE activity, and pupillary muscarinic receptor function, do not return to normal for up to 10 days postexposure. This is the first study to demonstrate dose-dependent attenuation of the pupillary light reflex following low-level exposure to nerve agent vapor. Given the structural and functional similarities between the human eye and the rat eye, and given that a previous study in humans reported an attenuation of the pupillary light reflex following exposure to cholinergic agonists similar to that reported in the present study (Smith and Smith, 1980), it is likely that the results of the present study are relevant to cases of human exposure to nerve agents.
Additionally, this is the first study to demonstrate that the loss of the light reflex persists for a longer period of time than the miotic response to nerve agent exposure, demonstrating that pupil size alone cannot be used as an indicator of recovery of the eye following exposure. Recovery of the light reflex coincided with the recovery of muscarinic receptor function, as measured by the ability of oxotremorine to constrict the pupil, suggesting that the attenuation of the light reflex was due to desensitization of pupillary muscarinic receptors. As with the miotic response to soman vapor, the recovery of the pupillary light reflex was not a result of the recovery of AChE and BChE activities in the eye since recovery of AChE and BChE activity lagged recovery of the light reflex by several days.

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**REFERENCES**


