NeuroToxicology 31 (2010) 77–84

Contents lists available at ScienceDirect

NeuroToxicology

An acute exposure to a sub-lethal dose of soman triggers anxiety-related behavior in guinea pigs: Interactions with acute restraint

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ARTICLE INFO

Article history:
Received 21 September 2009
Accepted 23 October 2009
Available online 31 October 2009

Keywords:
Anxiety
Locomotion
Open field
Elevated plus maze
Organophosphorus compounds

ABSTRACT

In this study, we tested the hypothesis that a single exposure of guinea pigs to sub-lethal doses of soman triggers anxiety-related behavior that is modifiable by acute stress. Prepubertal male guinea pigs were subjected to one of the following treatments: (i) saline (0.5 ml/kg, sc), (ii) soman (0.6× or 0.8×LD50, sc), (iii) saline followed 30 min later by 2-h restraint, or (iv) soman followed 30 min later by 2-h restraint. Behavior of the animals was examined 2 and 3 months later in a large open field and in the elevated plus maze. Animals that had been exposed to restraint stress alone or soman alone showed decreased exploratory activity when tested in the open field with bare floor at light intensity of 20–30 lx. Total distance traveled and distance traveled in the center of the field were shorter for animals that were exposed to either restraint stress or soman than for saline-injected animals. In addition, animals challenged with soman or restraint stress remained immobile for a longer time in the open field than did saline-injected guinea pigs. Performance in the elevated plus maze test revealed that exposure of guinea pigs to soman or restraint stress decreased their number of entries and the time spent in the open arms of the maze (measures of anxiety) and reduced their overall locomotor activity. Soman exposure and restraint stress cancelled out each other's effect on locomotion, while only attenuating one another's effect on anxiety-related behavior. It is concluded that a single exposure to sub-lethal doses of soman triggers long-lasting anxiogenesis and decreased locomotor activity and that acute restraint stress modifies the magnitude of these effects.

1. Introduction

Nerve agents, including soman, sarin, tabun and VX, are organophosphorous compounds (OP) chemically related to, but far more toxic than the majority of pesticides used worldwide in agriculture and households. There have been reports of the catastrophic use of some of those agents as weapons of mass destruction in wars and in terrorist attacks (Romano and King, 2001).

The acute toxicity of nerve agents is well documented and results primarily from irreversible inhibition of acetylcholinesterase (AChE), the enzyme that breaks down the neurotransmitter acetylcholine (ACh). Immediately following an exposure to toxic doses of these agents, overactivation of muscarinic and nicotinic receptors by excess ACh, both within and outside the central nervous system (CNS), triggers a syndrome characterized by miosis, blurred vision, dyspnea, cough, nausea and vomiting, headache, muscle weaknesses, muscle fasciculations, decreased consciousness level, and convulsions. High doses result in death from respiratory failure, involving both central and peripheral mechanisms (Bajgar, 2004). Acute poisoning with soman is especially difficult to treat because of the rapid aging of the soman-inhibited AChE that renders the enzyme refractory to reactivation by oximes (Bajgar, 2004).

The terrorist attack with sarin in the Tokyo subway in 1995 represented the largest exposure of a civilian population to nerve agent. Twelve out of the possible 5500 victims of the attack died. It is reported that only 16% of the 640 patients admitted to one of the hospitals presented with moderate signs of acute intoxication requiring immediate therapeutic intervention; less than 1% of those patients had severe signs of acute intoxication requiring intubation (Okumura et al., 1996). However, 6 months to 7 years later, increased incidence of posttraumatic stress disorder and chronic cognitive impairments were reported among the victims of the sarin attack who did not necessarily present with signs of acute toxicity at the time of the incident (Araki et al., 2005; Yokoyama et al., 1998). Cognitive dysfunctions have also been observed in rescue team members 3 years after their initial exposure to sarin at

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doi:10.1016/j.neuro.2009.10.012

the catastrophe site (Miyaki et al., 2005). Finally, it has been suggested that the exposure of soldiers to sub-lethal doses of nerve agents during the Gulf War may have contributed to the delayed development of a group of clinical manifestations currently referred to as the Gulf War syndrome (Golomb, 2008).

Insufficient knowledge of the behavioral effects of sub-lethal doses of nerve agents has made it difficult to unequivocally assign a cause–consequence relationship between an initial exposure to low doses of these agents and specific neurological deficits in humans. Further, in all the circumstances described above, stress is a significant confounding factor. Stress is known to increase AChE release in some brain areas (Imperato et al., 1991) and to aggravate the negative acute effects of an exposure to lethal doses of nerve agents (Philippens et al., 2005). However, the impact of stress on the behavioral effects of low doses of these agents is not well known.

Immediate and long-term behavioral effects of acute intoxication with nerve agents are well characterized, and seizure activity is believed to be the main underlying cause (Filliat et al., 2007; Coubard et al., 2008). In contrast, only a few studies have examined the behavioral effects of a single exposure of rodents to sub-lethal doses of nerve agents (Kassa et al., 2001; Sirkka et al., 1990; Baille et al., 2001). Hypolocomotion has been detected in rats at various times (1 day to 5 weeks) following a single exposure to a low dose of sarin or soman (Kassa et al., 2001; Baille et al., 2001). Anxiety-related behavior has also been observed in rats following a single exposure to a low dose of soman (Sirkka et al., 1990; Baille et al., 2001).

The specific aims of the present study were to identify the long-term behavioral effects of a single exposure to sub-lethal doses of soman in guinea pigs, the best non-primate model of OP toxicity (Inns and Leadbeater, 1983; Maxwell et al., 1988), and to determine how these effects are modified by exposure of these animals to acute stress. Prepubertal male guinea pigs were subjected to different challenge doses of soman (0.6× or 0.8×LD50, sc) and/or a 2-h restraint stress. Two to three months later, locomotor activity and anxiety-related behavior of the animals were evaluated in the open field and in the elevated plus maze. Results presented herein demonstrate that a single exposure of guinea pigs to sub-lethal doses of soman triggers long-lasting anxiety-related effects that are significantly modified by acute restraint stress. Evidence is provided that interactions between soman and acute restraint stress on the exploratory activity of guinea pigs in the open field and in the elevated plus maze are not simply additive and vary according to the parameter analyzed.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs [Crl(HA)Br] were purchased from Charles River Laboratories (Wilmington, MA) and housed in stainless steel cages (60 cm × 60 cm × 25 cm), in a climate controlled animal-care unit with constant temperature of 21 ± 0.5 °C and a 12-h light/dark cycle. Animals were 28–30 days old (300–320 g) on arrival at the animal-care unit. Food and water were available ad libitum. In conducting the research described in this report, the investigators complied with the regulations and standards of the Animal Welfare Act and adhered to the principles of the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

2.2. Methodological study

In contrast to rats and mice, guinea pigs like humans and non-human primates express low levels of plasma carboxylesterases—the enzymes that metabolize OP compounds (Inns and Leadbeater, 1983; Maxwell et al., 1988). Consequently, the sensitivity of guinea pigs to the toxicity of OP compounds and their responsiveness to OP antidotes resemble those of primates (Inns and Leadbeater, 1983; Maxwell et al., 1988). Although guinea pigs have become the rodent species of choice for studies of OP toxicity, they have not been very often used in studies of spontaneous behavior because their high level of anxiety in a novel environment inhibits their exploratory activity. Guinea pigs have even been considered as unsuitable animals for behavioral research (Smart and Adlard, 1974).

Given the natural ability of guinea pigs to explore their home cages, a methodological study was conducted to determine whether decreasing the anxiogenic effects of the testing environment would disinhibit the exploratory activity of the animals in the open field. In this methodological study, four naive animals were tested at 2 months of age in a large open field apparatus. Tests were performed once during each of three consecutive days. The first and the third tests were performed in the open field with bare floor. The second test was performed in the same apparatus with the floor covered with the same type of paper bedding as used in the home cages. Light intensity in the open field was kept at a 80–100 lx during all three tests.

2.3. Exposure to soman and/or restraint stress

To examine the effects of soman on spontaneous behavior, guinea pigs received a subcutaneous (sc) injection between the shoulder blades of 0.6×LD50 (16.8 μg/kg) soman ($n = 8$), 0.8×LD50 (22.4 μg/kg) soman ($n = 9$), or saline ($n = 8$) at the age of 35–36 days. After the soman challenge, gross behavior of the guinea pigs was observed continuously for 30 min. In the groups of animals injected with 0.6×LD50 soman or saline, half of the animals were transported to another room with an ambient temperature of 21 ± 0.5 °C and placed in Plexiglas restrainers for 2 h. The other half was left undisturbed in the cages. Four of the nine guinea pigs exposed to 0.8×LD50 soman were moved to the room where they were subjected to the acute restraint stress, and the other five were left in their cages. At the end of the 2-h restraint, animals were removed from the restrainers and observed for an additional 2 h. Then, all animals were transported to the animal facility, where they were maintained for the next 3 months. Two stressed and two non-stressed animals were housed together for each test group, except for the animals challenged with 0.8×LD50, which were housed in groups of four (two stressed and two non-stressed) or five (two stressed and three non-stressed). At 48 h after the restraint session, one of the animals that had shown mild signs of intoxication when challenged with 0.8×LD50 soman was very ill, lost more than 20% of its initial body weight, and had to be euthanized (see Section 3).

2.4. Behavioral testing

Two months after the injection of soman or saline with or without the acute restraint stress challenge, exploratory and anxiety-related behaviors were assessed in the open field test. Based on the finding that lowering the anxiogenic effect of the novel environment increased the exploratory activity of the guinea pigs in the open field (see Section 3), the first test was performed under high-light conditions in an open field apparatus that had the floor covered with paper bedding. Two days later, guinea pigs were tested under low-light conditions in the open field with bare floor. One month later (i.e., three months after the initial injections), guinea pigs were tested again on the open field with bare floor at low-light conditions. High- and low-light conditions refer to light intensities of 80–100 and 20–30 lx, respectively, within the open fields.