Published in final edited form as:
Toxicol Appl Pharmacol. 2008 November 1; 232(3): 440–447. doi:10.1016/j.taap.2008.07.016.

Inhalation of the Nerve Gas Sarin Impairs Ventilatory Responses to Hypercapnia and Hypoxia in Rats

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Abstract

Sarin, a highly toxic nerve gas, is believed to cause bronchoconstriction and even death primarily through respiratory failure; however, the mechanism underlying the respiratory failure is not fully understood. The goals of this study were to ascertain whether sarin affects baseline ventilation (V_E) and V_E chemoreflexes as well as airway resistance and, if so, whether these changes are reversible. Four groups of F344 rats were exposed to vehicle (VEH) or sarin at 2.5, 3.5, and 4.0 mg h m^{-3} (SL, SM, and SH, respectively). V_E and V_E responses to hypercapnia (7% CO_2) or hypoxia (10% O_2) were measured by plethysmography at 2 h and 1, 2, and 5 days after VEH or sarin exposure. Total pulmonary resistance (R_L) also was measured in anesthetized VEH- and SH-exposed animals 2 h after exposure. Our results showed that within 2 h after exposure 11% of the SM- and 52% of the SH-exposed groups died. Although the SM and SH significantly decreased hypercapnic and hypoxic V_E to similar levels (64 and 69%), SH induced greater respiratory impairment, characterized by lower baseline V_E (30%; $P < 0.05$), and total loss of the respiratory frequency response to hypercapnia and hypoxia. V_E impairment recovered within 1–2 days after sarin exposure; interestingly, SH did not significantly affect baseline R_L. Moreover, sarin induced body tremors that were unrelated to the changes in the V_E responses. Thus, LC_{50} sarin causes a reversible impairment of V_E that is not dependent on the sarin-induced body tremors and not associated with changes in R_L.

Keywords

acetylcholine; respiratory failure; chemoreflexes

Introduction

Sarin (O-isopropyl methylphosphonofluoridate, also known as GB) is an organophosphorus nerve agent that binds and irreversibly inactivates acetylcholinesterase (Grob and Harvey, 1958). Inhibition of acetylcholinesterase elevates the synaptic level of acetylcholine (ACh),

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Conflict of Interest statement
The authors declare that there are no conflicts of interest.

Some of the findings reported in this paper were presented at Society for Neuroscience Meeting, San Diego, CA, 2007.

causing cholinergic shock and clinical symptoms of sarin intoxication. Sarin is more volatile than other nerve gases such as soman, tabun, and VX (Lee, 2003), and combined with its lethality and low cost of production, it is the nerve agent of choice in the hands of terrorists and rouge nations. In the 1980s, Iraq used sarin against Iranian soldiers and Kurds, resulting in thousands of deaths; and a Japanese terrorist group used sarin in two attacks in the city of Matsumoto in 1994 and on a Tokyo subway in 1995, killing dozens and injuring over 6000 people (Okumura *et al.*, 2003). At or near LC_{50}, sarin causes miosis, bronchoconstriction, excessive secretions, vomiting, body tremors, seizures, and may lead to respiratory and cardiac arrest (Sidell and Borak, 1992; Sidell, 1994; Abu-Qare and Abou-Donia, 2002). The respiratory system is the major route of entry and absorption for nerve gases and plays a major role in the pathogenesis of nerve gas toxicity. Respiratory failure is the most common cause of death following nerve gas exposure (Rickett *et al.*, 1986; Colwill *et al.*, 2004), and it is generally thought that the severe bronchoconstriction from nerve agent exposure is involved in respiratory failure (Johnson *et al.*, 1958; Holstege *et al.*, 1997). However, there are no critical data to prove this premise.

Exposure to nerve agents has been reported to affect ventilatory (V_E) responses (Adams *et al.*, 1976; Rickett *et al.*, 1986), and several lines of evidence suggest that V_E changes in response to hypercapnia and hypoxia are critical in maintaining V_E rhythm. First, CO_2-chemoreception is critical for maintaining the V_E rhythm in mammals, and reduction of the CO_2-respiratory drive by lowing arterial blood CO_2 or blunting CO_2-chemosensitivity may lead to ventilatory arrest (Bruce and Cherniack, 1987; Nattie, 2000). Second, children with congenital central hypoventilation syndrome (CCHS), characterized by severely blunted or even absent V_E responsiveness to hypercapnia, require artificial ventilation (Shea *et al.*, 1993; Gozal *et al.*, 1996). Similarly, severe chronic obstructive pulmonary disease (COPD) patients who exhibit blunted V_E response to hypercapnia and hypoxia are likely to develop respiratory failure (Schaefer, 1949; Fahey and Hyde, 1983; Franciosi *et al.*, 2006; Ucgun *et al.*, 2006). Third, blunted V_E response to hypoxia may lead to V_E dysfunction, as was shown in an animal model of sudden infant death syndrome (SIDS) (Milerad *et al.*, 1995; St-John and Leiter, 1999; Hafstrom *et al.*, 2002; Murai *et al.*, 2003).

Although respiratory failure is the major cause of sarin-induced mortality, the precise role of V_E chemoreflexes in sarin-induced respiratory failure is essentially unknown. In this communication we present evidence that sarin decreases baseline V_E and the V_E responses to hypercapnia and hypoxia, and that the changes in the V_E responses are not dependent on body tremors and not associated with significant changes in baseline airway resistance.

Materials and methods

Animals

Pathogen-free, 8-week-old male Fischer 344 rats were purchased from Harlan Sprague-Dawley Farms (Indianapolis, IN). Animals were quarantined for 2 weeks, and food and water were provided *ad libitum*. At the age of approximately 12–14 weeks, animals were exposed to sarin via inhalation. All studies were conducted at Lovelace Respiratory Research Institute (LRRI), a facility fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Sarin exposure

Sarin in isopropyl alcohol was obtained from the United States Army. Animals (n = 84) were exposed to either isopropyl alcohol (vehicle) or to various concentrations of sarin by the nose-only inhalation method developed at LRRI as described previously (Henderson *et al.*, 2002). Briefly, prior to exposure animals were acclimatized to the nose-only inhalation

chambers for 120 min day^{-1} for 3 consecutive days. Rats were divided into 4 groups and exposed to either vehicle at 35 ppm in fresh air (VEH, n = 23) or three doses of sarin: 2.5 (SL, n = 12), 3.5 (SM, n = 9), or 4.0 mg h m^{-3} (SH, n = 40) at the rate of 2.0 mg m^{-3} h^{-1}. After degassing, animals were removed from exposure chambers and experiments began 2 h post exposure. Essentially, all sarin-related deaths occurred within 2 h of sarin exposure. Among 84 rats, one in SM and 21 in SH groups died after sarin exposure, the 62 surviving rats (23, 12, 8, and 19 for VEH, SL, SM, and SH groups, respectively) underwent the following tests.

Measurement of the V_E responses in conscious rats

40 rats out of the surviving rats (n = 12, 12, 8, and 8, for VEH, SL, SM, and SH, respectively) were placed in an unrestrained whole-body plethysmograph system (Buxco Electronics, Sharon, CT) two hours after exposure. Respiratory activity, including airflow, tidal volume (V_T), respiratory frequency (f_R), and V_E were continuously monitored and recorded. In addition to baseline V_E and V_E changes in response to 5 min of hypercapnia (7% CO_2 and 30% O_2 balanced with N_2) or hypoxia (10% O_2 balanced with N_2) were measured in these animals at 2 h, and 1, 2, and 5 days after VEH or sarin exposure. The rectal temperature of the animals was monitored with a flexible temperature microprobe (MLT 1401 Thermocouple Probe; ADInstruments, Castle Hill, Australia) and maintained at 36–37°C by adjusting airflow temperature in the chamber and a placing a heating pad underneath the plethysmograph.

Measurement of the V_E responses in two groups of anesthetized rats

Anesthetic (Nembutal®, 50 mg kg^{-1} i.p.) was administrated 2 h and 5 days post the exposures in the first and second group, respectively. Each group contains 6 VEH and 6 SH rats. It should be noted that the 12 rats in the second group came from those after completion of the plethysmographic V_E response tests in conscious state. The left femoral artery was cannulated for monitoring arterial blood pressure (ABP) and heart rate (HR), and the femoral vein was used to administer supplemental anesthetic (combination of chloralose, 100 mg kg^{-1} and urethane, 500 mg kg^{-1}) if the ABP, HR and f_R responses to pinching the hind limb paw were 15% greater than the control values. The trachea was cannulated and connected to a pneumotachograph (Frank's Mfg. Co., Albuquerque, NM) to record airflow. The pneumotachograph, as previously reported (Wang and Xu, 2006), was made of stainless steel with a linear flow-pressure relationship in the range of 0–10 ml s^{-1} and having a flow resistance equal to 0.046 cm H_2O ml^{-1} s^{-1} with a dead space of 0.08 ml. The other end of the pneumotachograph was placed (~3 mm deep) in a plastic tube with a diameter five-fold greater than the pneumotachograph. A three-way stopcock was attached to the other side of the plastic tube and connected to a supplemental gases device that controlled the inhaled gas composition. Baseline V_E and V_E response to 2 min of hypercapnia (7% CO_2) or 1 min hypoxia (10% O_2) were measured. It should be noted that because the route of exposure to 7% CO_2 or 10% O_2 challenge was different between conscious and anesthetized rats (plethysmograph vs. tracheal cannulation), the duration of exposure was shorter in the latter. The interval between two stimulation-episodes was at least 5 min. The animal was supine and its body temperature was monitored with a rectal probe and maintained at approximately 36.5°C by a heating pad and a radiant heat lamp.

Measurement of arterial blood gases and pH

In the 24 anesthetized rats mentioned above, ~120 μl arterial blood was sampled from each animal before hypercapnia and hypoxia challenges. Blood samples were taken from 12 VEH- and 12 SH-exposed rats at 2 h or 5 days after exposure, and blood gases were measured immediately after collection by a blood gas analyzer (GEM Premier 3000, Instrumentation Lab., Lexington, MA).

Measurements of total pulmonary resistance (R_L)

To measure airway resistance and responsiveness to methacholine, the R_L was measured in 10 surviving rats (n = 5 each from VEH and SH groups) 2 h after exposure. The animals were anesthetized, tracheotomized, paralyzed and ventilated, and a small incision was made in the trachea through which a 20-gauge needle hub was inserted. The tracheal cannula was secured by suture thread and the skin was pulled back over the cannula and secured by cyanoacrylate adhesive. A small saline-filled catheter was placed in the thoracic esophagus via the mouth to obtain transthoracic pressure measurements. The rat was placed on the Flexivent apparatus (Scireq, Montreal, Canada) and ventilated through the tracheal cannula. Once on the ventilator, paralytics (doxicurium, 0.5 mg kg^{-1}) were administered via the intraperitoneal route. Aerosolized methacholine was delivered in increasing doses (0, 1, 3, 6, 12, 25, and 50 mg ml^{-1}) with a 6-min interval between two doses.

Data acquisition and analysis

Raw data from the conscious or the anesthetized (paralyzed) rats were recorded by a PowerLab/8sp data acquisition system connected to a computer employing the PowerLab Chart 5 software (ADInstruments, Australia). Respiratory variables including V_T, f_R, and V_E were derived by the on-line calculation functions of the software. The baselines values were collected and averaged for 2 min immediately before 7% CO_2 or 10% O_2 challenge. The maximal responses during the challenge period and the percent change from the baseline values were calculated. Percent mortality following sarin exposure represents the number of dead animals divided by total number of animals at a given concentration of sarin multiplied by 100. Baseline R_L and R_L responses were determined by R_L values 1 min before methacholine challenge and the peak response during each methacholine challenge, respectively.

Statistical analysis

Values for V_E parameters, R_L, and body weights were calculated as mean ± SE. Because the baseline V_E and V_E responses as well as the arterial blood gas data in anesthetized animals obtained from VEH rats at 2 h and on day 5 after exposure were not different statistically, they were combined into one group (VEH) in the tables and figures for simplicity. Comparison between mortality rates among various groups was evaluated by the Pearson's Chi-Square test. Paired t-test was used to evaluate if a V_E response was different from the baseline value. One-way analysis of variance (ANOVA) was used to examine the effects of various doses of sarin exposure on respiratory parameters, while two-way ANOVA with repeated tests was used to test the differences in the recovery times among the sarin groups and the R_L responses to different levels of methacholine challenge between the VEH and SH groups. If the overall ANOVA had a P value less than 0.05, the Tukey's method for multiple comparisons was followed. STATISTICA 6.0 software (StatSoft, Inc., Tulsa, OK) was employed for statistical analysis. Difference is considered significant at a P value < 0.05.

Results

Mortality and morbidity is associated with acute sarin exposure

Among the rats exposed to VEH or SL, there was no apparent abnormal sign observed during the exposures. At higher levels of sarin exposures, however, the rats showed body tremors, hypersecretion of saliva, and seizures that were more severe in SH than SM group. Two h after the exposures, significant body tremor was still observed in SM and SH rats, while other signs were minimal. Of the 84 rats tested for sarin-related mortality, no deaths were observed among the rats exposed to VEH or SL. However, as shown in Fig. 1, 1 out of 9 rats exposed to SM and 21 out of 40 rats exposed to SH died within 2 h after sarin

exposure, representing 11% and 52% mortality, respectively. Compared with the VEH rats, only the SH group showed significant mortality ($P < 0.01$). Mortality was defined as loss of heartbeat and respiratory rhythm, and usually occurred between 45–95 min after sarin exposure. Among the surviving animals there were no significant differences in the body weight between the SH and VEH groups before (212 ± 6 and 207 ± 3 g) and 5 days post sarin exposure (220 ± 9 and 215 ± 7 g). These results suggest that 4.0 mg h m^{-3} sarin approximates the LC_{50} for sarin in male F344 rats.

High-dose sarin affects baseline V_E

Two hours after sarin or VEH exposure, surviving rats were placed in the whole body plethysmograph. Among these rats, body tremors were noticed in the SM and SH groups and the severity of tremors was greater in latter than the former, but no tremors were observed in SL and VEH rats. The baseline f_R in the rats exposed to the three sarin doses was similar to that of the VEH animals (Fig. 2, right panel). However, the baseline V_E and V_T of the SH animals were consistently lower (approximately 33%; $p < 0.01$) than those of the animals in the VEH, SL, or SM groups (Fig. 2, left and middle panel). Moreover the animals' body temperature was not statistically different among the four groups. Thus, SH rather than SL and SM is able to suppress baseline V_E and V_T in conscious rats.

Sarin depresses V_E responses to hypercapnia and hypoxia

Normally, the increase in the partial pressure of CO_2 (P_{CO2}) leads to elevated V_E, characterized by increasing V_T and f_R (Xu and Frazier, 2002). However, sarin exposure even at SL blunted the V_E response to hypercapnia (7% CO_2 for 5 min), as shown in Fig. 3 (an example of the recording data) and Fig. 4 (the group data). Compared with VEH, the SL, SM, and SH attenuated the V_E responses by 28%, 64%, and 69%, respectively ($P < 0.01$). We observed an interesting correlation between the V_E response to hypercapnia and the level of sarin exposure. While all the three doses of sarin attenuated V_E responses through reducing the V_T response, the f_R response was unaffected by SL, reduced by SM, and totally abolished by SH. Exposure to hypoxia (10% O_2 for 5 min) also significantly increased the ventilatory parameters in the VEH rats, with V_E, V_T, and f_R increasing by 142 ± 16%, 42 ± 5%, and 69 ± 7%, respectively, ($P < 0.01$)(Fig. 5). The SM and SH attenuated the V_E response by 56% and 68% ($P < 0.01$) as compared with VEH; however, differently from the V_E response to hypercapnia, the V_E response to hypoxia was not significantly affected by SL. The most surprising finding was that the SM diminished the hypoxia-induced V_E response that resulted mainly from the decreased V_T response. On the other hand, changes in V_E in SH animals were primarily contributed by a marked decrease in the f_R response, which is similar to the V_E response to hypercapnia. Together, these results suggest that sarin blunts the V_E responses to hypoxia and hypercapnia; however, while the SM-induced attenuation is achieved by impairing the V_T response, in SH animals it is achieved by eliminating the f_R response.

Sarin also changes V_E in anesthetized rats

As mentioned above, the surviving SH rats exhibited body tremors 2 h post sarin exposure and severe respiratory impairment (essentially eliminating the f_R response). Sarin-induced body tremors may impair respiratory activity (Shih *et al.*, 2007), thereby blunting the V_E responses. To evaluate the possible contribution of tremors to the sarin-induced changes in V_E, we compared the baseline V_E and V_E responses to hypercapnia and hypoxia challenges in anesthetized VEH and SH rats. Interestingly, the anesthetized SH animals' tremors ceased and their body temperatures were consistent in both VEH and SH rats. Moreover, sarin exposure did not significantly alter baseline V_E but profoundly attenuated the V_E responses to hypercapnia and hypoxia by 47% and 57%, respectively (Fig. 6). In addition, as in conscious rats, SH caused the loss of the f_R response in the anesthetized rats. The absence of

baseline V_E depression and relatively smaller inhibitory V_E responses in the anesthetized compared with conscious rats is very likely due to the anesthetic effect on V_E (Wixson *et al.*, 1987) and/or a relative shorter hypercapnic/hypoxic exposure. In the anesthetized state at 2 h and 5 days post sarin exposure, the baseline cardiovascular activities and their responses to hypoxia and hypercapnia were not significantly different between VEH and SH animals (Table 1).

Sarin-induced V_E changes are relatively short-lived in surviving animals

To ascertain the duration of sarin-induced V_E changes, VEH and surviving SH animals were retested on days 1, 2, and 5 for baseline and hypercapnic/hypoxic V_E. Results presented in Fig. 7 suggest that the baseline changes in V_E and V_T in SH animals observed 2 h post sarin exposure were resolved within 24 h after sarin exposure. Similarly, hypoxia-induced changes in V_E, V_T, and f_R in SH animals also recovered within 24 h after sarin inhalation (Fig. 8B). However, the hypercapnia-stimulated changes in the f_R response in SH animals remained significantly lower even at 24 h, but returned to normal within 2 days after sarin exposure (Fig. 8A). Thus, as in humans (Yanagisawa *et al.*, 2006), the respiratory effects of sarin in rats are relatively short-lived.

Sarin alters arterial blood gases

We measured the arterial blood CO_2 partial pressure (Pa_{CO2}), oxygen saturation (Sa_{O2}), and pH in anesthetized rats. Data presented in Table 2 suggests that at 2 h after exposure, compared with VEH, SH animals exhibited higher Pa_{CO2} and lower Sa_{O2}, although the differences did not reach statistical significance. This insignificant higher Pa_{CO2} is likely due to that arterial blood samples were collected from anesthetized animals, in which the hypoventilation observed in conscious SH rats were presumably masked by the anesthesia. On the other hand, compared with the VEH group, the arterial blood pH, bicarbonate (HCO_3^-), and base excess (BE) were significantly lower in SH rats at 2 h after sarin exposure, suggesting a metabolic acidosis in rats post acute sarin exposure. At day 5 after sarin treatment, all the arterial blood gas parameters were similar in VEH and SH rats.

SH exposure fails to change baseline R_L

Sarin is a potent cholinergic compound that causes bronchoconstriction (Grob and Harvey, 1958). To ascertain the effects of SH on airway resistance, we determined the baseline R_L in VEH and SH animals and the effects of seven different concentrations of methacholine on R_L. Results indicated that the baseline R_L and the R_L response to methacholine at concentrations less than 6 mg ml^{-1} were not significantly different between VEH and SH rats; however, the R_L of SH rats at methacholine concentrations greater than 6 mg ml^{-1} were significantly higher than VEH rats (Fig. 9). These data indicated that sarin does not significantly alter the baseline R_L, but makes the airways more sensitive to the bronchoconstricting effects of muscarinic receptor agonists.

Discussion

The respiratory system is the major route of sarin entry and absorption and plays an important role in the pathogenesis of nerve agent toxicity. In animal experiments, even at low doses, sarin causes long-term neurological and immunological deficiencies (Abu-Qare and Abou-Donia, 2002; Kalra *et al.*, 2002; Pena-Philippides *et al.*, 2007); however, immediate death from high doses of nerve gas exposure is invariably due to respiratory failure. The latter is suggested to be resulted from respiratory muscle paralysis, increased lung secretion-induced airway obstruction, and dysfunction of central respiratory centers as a result of ACh over stimulation and disruption of the nervous system (Rickett et al., 1986; Colwill et al., 2004). To understand the mechanism of acute respiratory failure in sarin-

exposed animals, we first established the LC_{50} dose for sarin in F344 rats. We compared mortality in the rats exposed to three different doses of sarin, SL, SM, and SH, and found that a significant mortality (52%) occurred within 2 h after exposure in SH animals. Our results also indicated that most animals that were alive at 2 h post sarin exposure lived until sacrificed at the next 2 weeks post sarin exposure. Similarly, in Japan, most human fatalities emanating from sarin terrorism occurred within a few hours of the attacks, and were attributed to respiratory failure. However, following respiratory stabilization, subsequent deaths among the sarin victims resulted primarily from neurotoxicity or causes indirectly related to sarin exposure (Yanagisawa *et al.*, 2006).

While chemoreflexes to hypercapnia and hypoxia are critical for normal respiratory rhythm and life, the role of V_E chemoreflexes in sarin-induced respiratory failure is essentially unknown. An important finding of this study is that sarin significantly blunts the V_E responses to hypercapnia and hypoxia in conscious as well as anesthetized rats. The results unequivocally indicate that even an acute exposure to sublethal doses of sarin (SL and SM) blunts the V_E responses, and the changes in V_E from SH reflected mainly the effects of sarin on the f_R response. Although, we did not present direct evidence demonstrating that sarin-induced V_E dysfunction leads to respiratory failure and mortality, we observed that SH exposure not only caused significant mortality but it also produced a marked impairment of V_E characterized by baseline V_E inhibition and the absence of the f_R responses to hypercapnia and/or hypoxia. To our knowledge, this is the first research showing that sarin affects the V_E responses to hypercapnia and hypoxia. The loss of the f_R responses to hypoxia and/or hypercapnia strongly suggests that sarin blunts the respiratory central drive, which could lead to respiratory failure and death. Indeed, it has been documented that high doses of physostigmine, another acetylcholinesterase inhibitor causes death through centrally mediated respiratory arrest (Anzueto *et al.*, 1990; Futagawa *et al.*, 2000; Duncan *et al.*, 2001). Blunted chemoreflexes have been correlated to respiratory failure and death in other diseases, including COPD and CCHS, and in animal model of SIDS (Schaefer, 1949; Fahey and Hyde, 1983; Shea *et al.*, 1993; Milerad *et al.*, 1995; Gozal *et al.*, 1996; St-John and Leiter, 1999; Hafstrom *et al.*, 2002; Murai *et al.*, 2003; Franciosi *et al.*, 2006; Ucgun *et al.*, 2006).

An interesting finding from this study is that although the magnitude of inhibition of V_E by SH and SM is essentially similar, mortality is primarily associated with SH exposures. It is likely that this difference in lethality arises from the SH's more potent effects on the central respiratory chemical drive, and this assumption is supported by the following observations: (1) Only SH significantly inhibited the baseline V_E in conscious rats. (2) The f_R responses to hypoxia and hypercapnia were almost completely abolished by SH exposures, but much less affected by SM. Several studies indicate that decreased f_R responses (appearance of apnea) correlate with respiratory failure. For example, apnea (reduction in f_R) caused by the stimulation of pulmonary C-fibers is strongly aggravated by respiratory syncytial virus, causing death in infected animals (Peng *et al.*, 2007). Similarly, increased mortality associated with the blunted V_E responses to hypercapnia and hypoxia in an animal model of COPD and SIDS is also characterized by a depressed f_R response and respiratory arrest (St-John and Leiter, 1999; Xu *et al.*, 2007). (3) Compared with SM, the slower recovery of V_E impairment in SH rats suggests a more severe damage to the central respiratory system in the latter. That the V_T responses to hypercapnic and hypoxic challenges were seemingly higher in SH than SM rats might stem from a compensatory response to SH-depressed f_R responses, and/or a more severe acidic response to hypoxia and hypercapnia in SH rats (Kussmaul's respiration).

Sarin-induced body tremors could impair the respiration and in the present study the sarin-induced changes in V_E in response to hypercapnia and hypoxia were invariably associated

with body tremors. Therefore, it was important to determine whether the V_E abnormality was secondary to the inception of body tremors. However, a significant reduction in the V_E response to hypercapnia was also observed in the SL-exposed rats that had no tremors. Moreover, because the blunted V_E responses also exist in anesthetized SH rats that do not exhibit tremors, it is unlikely that the two events (body tremors and V_E changes) are causally related.

Our results indicated that SH exposures decrease arterial pH and HCO_3^-concentration. These changes may result from metabolic acidosis. Metabolic acidosis due to accumulation of lactic acid has been reported in sarin-exposed animals (Gold *et al.*, 1957). Cerebral acidosis stimulates V_E (Van de Ven *et al.*, 2001); however, in spite of increased chemical drive in SH animals, V_E was still significantly lower than that in VEH-exposed rats. This finding argues again that the SH-induced substantial respiratory impairment. V_E function could also be affected by cardiovascular function (Turner, 1991; Dick *et al.*, 2005); therefore, we compared the cardiovascular response at baseline and in response to hypercapnia and hypoxia in anesthetized VEH and SH rats. Our results did not indicate a significant difference in these responses between the two groups, suggesting that the V_E abnormalities did not arise from sarin-induced cardiovascular dysfunction. Similarly, we did not find that the V_E changes in SH animals were related to changes in sarin-induced airway resistance, because SH exposures failed to increase the baseline R_L and, more importantly, the blunted V_E responses to hypercapnia and hypoxia was also seen in anesthetized rats with tracheal cannulation that greatly limits R_L changes.

It is plausible that sarin-induced V_E changes arise from the effects of sarin on the function of the chemoreceptors. The central and peripheral chemoreceptors play a key role in control of breathing (Gonzalez *et al.*, 1994; Nattie and Li, 1996; Burton *et al.*, 1997; Xu and Frazier, 2002), and there is abundant evidence showing the importance of cholinergic mechanisms in the central CO_2 chemoreception of mammals (Fukuda and Loeschcke, 1979; Loeschcke, 1982; Burton *et al.*, 1989; Burton *et al.*, 1990; Monteau *et al.*, 1990; Lydic *et al.*, 1991). For example, multiple brainstem areas with CO_2-chemoreception express a high density of cholinergic receptors (Hyde *et al.*, 1988; Mallios *et al.*, 1995; Rosin *et al.*, 2006), and the respiratory responses to focal stimulation of chemosensitive neurons are similar to those evoked by focal application of ACh agonists in the same regions (Fukuda and Loeschcke, 1979; Noakes *et al.*, 2006). ACh is also involved in facilitating the carotid chemoreceptor-mediated V_E response to hypoxia through nicotinic ACh receptors (van Lunteren *et al.*, 1984). Taken together, these studies suggest that cholinergic stimulation increases the V_E responses to hypercapnia and hypoxia and, therefore, the effects of sarin on the V_E responses to hypercapnia and hypoxia seem to be paradoxical. While the precise mechanism is still not clear, it is conceivable that excessive synaptic levels of ACh from high-dose sarin treatment are toxic to target neurons, including those in the medulla oblongata — the seat of the major respiratory control network (Gonzalez *et al.*, 1994; Nattie and Li, 1996; Burton *et al.*, 1997; Xu and Frazier, 2002). In fact, neuronal damage in the central nervous system, including the cortex, hippocampal formation, and cerebellum has been observed in animals exposed to high doses of sarin (Shih, 1982; Li *et al.*, 2000; Henderson *et al.*, 2002; Abou-Donia, 2003). While some neuronal injury was observed in the cortex and hippocampus of SH rats, we were unable to find significant neuronal damage in the medulla oblongata of these animals (Razani-Boroujerdi et al., unpublished observation). Therefore, the sarin-induced blunted V_E response to hypercapnia and hypoxia might reflect functional rather than overt loss of the target neurons in the central respiratory network. This possibility is further strengthened by the observations that the V_E changes in SH animals as well as the humans surviving the Japanese sarin terrorism (Yanagisawa *et al.*, 2006), were transitory and normalized within hours after the exposure.

While exposure of guinea pigs to the nerve agent VX was reported to cause pulmonary edema and increase emigration of inflammatory cells into the lung (Wright *et al.*, 2006), we were unable to find any histopathological evidence of lung injury in SH-exposed rats at 24 or 7 days after sarin exposure (Razani-Boroujerdi et al., unpublished observation). Moreover, several arguments exclude a significant contribution of possible lung inflammation in the sarin-induced blunted V_E responses. First, sarin exposure did not significantly change the baseline Pa_{CO2}, SaO_2, and R_L, suggesting that the sarin exposures did not affect pulmonary diffusion and pulmonary fluid exudation. Second, we found that the f_R response, which is controlled by the neurons, was almost eliminated by SH. Third, while bleomycin-induced acute lung injury elevates hypoxic V_E responses, it does not affect the hypercapnic V_E response (Jacono *et al.*, 2006).

In summary, these studies present three major observations: (1) The LC_{50} dose of sarin in adult Fischer 344 rat is approximately 4.0 mg h m^{-3}. (2) While the concentrations of sarin that induce tremors also lower the baseline V_E and the V_E responses to hypercapnia and hypoxia, the changes in the V_E are independent of tremor activity and predominantly contributed by the decreased f_R response. (3) The blunted V_E response is not associated with airway resistance and cardiovascular abnormality. (4) The altered baseline V_E and the V_E responses to hypercapnia and hypoxia by sarin recover within 2 days. Our results suggest that the sarin-induced respiratory impairment emanates, at least primarily, from a transitory dysfunction of the central respiratory network.

Acknowledgments

The study is supported by National Heart, Lung, and Blood Institute Grant 74183; and U.S. Army grant W81XWH-04-C-0071.

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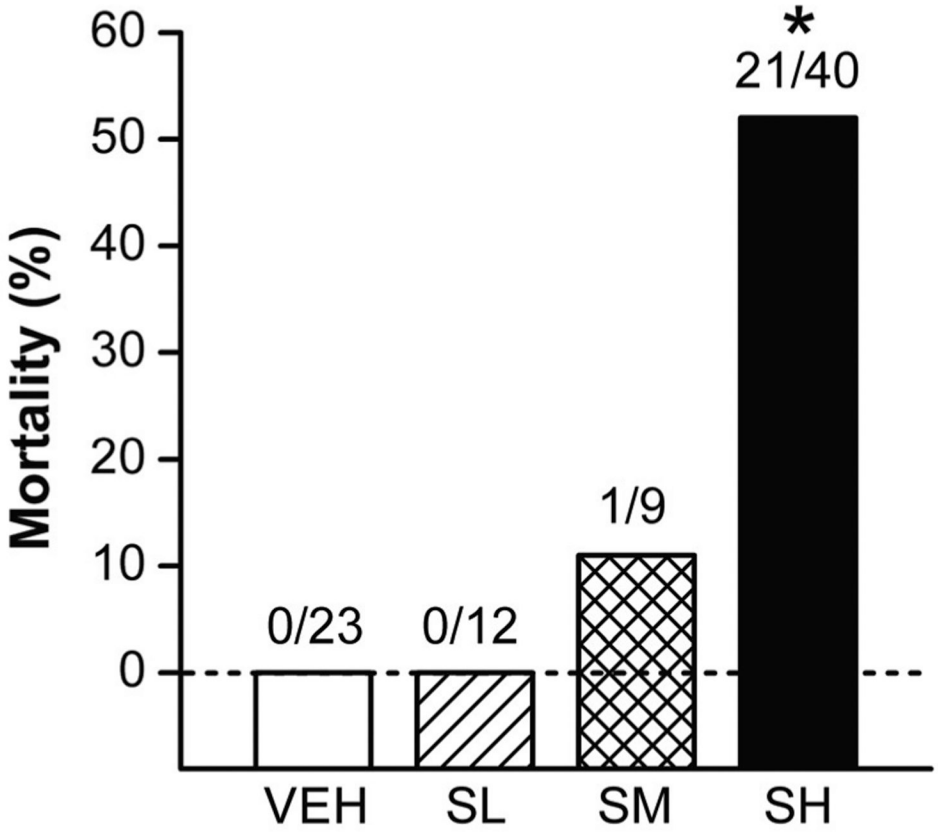

Figure 1.

Dose-dependency of acute sarin exposure-induced mortality. No death occurred in the rats exposed to vehicle only (VEH) or a low dose of sarin (SL). In contrast, 11% and 52% mortality was observed in the rats within 2 hours immediately after exposure to moderate (SM) and high doses (SH) of sarin, respectively. * $P < 0.01$, compared with VEH, SL and SM groups.

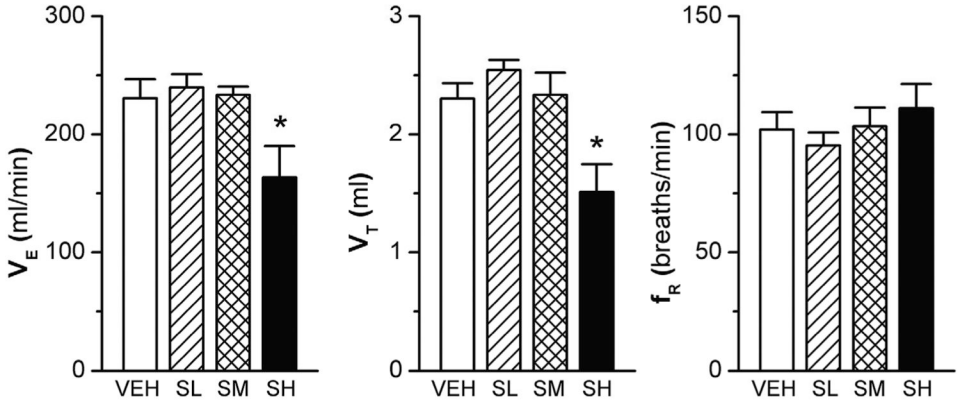

Figure 2.

Baseline ventilation (V_E) in the surviving conscious rats 2 h after sarin exposure. The baseline V_E, as compared with that in the vehicle (VEH)-exposed rats, was not changed by low and moderate levels of sarin (SL and SM), but was significantly reduced by high-level sarin (SH) due to a reduction of tidal volume (V_T) with little effect on respiratory frequency (f_R). Mean ± SE; n = 12, 12, 8, and 8 for VEH, SL, SM, and SH animals, respectively. * $P < 0.01$, SH vs. three other groups.

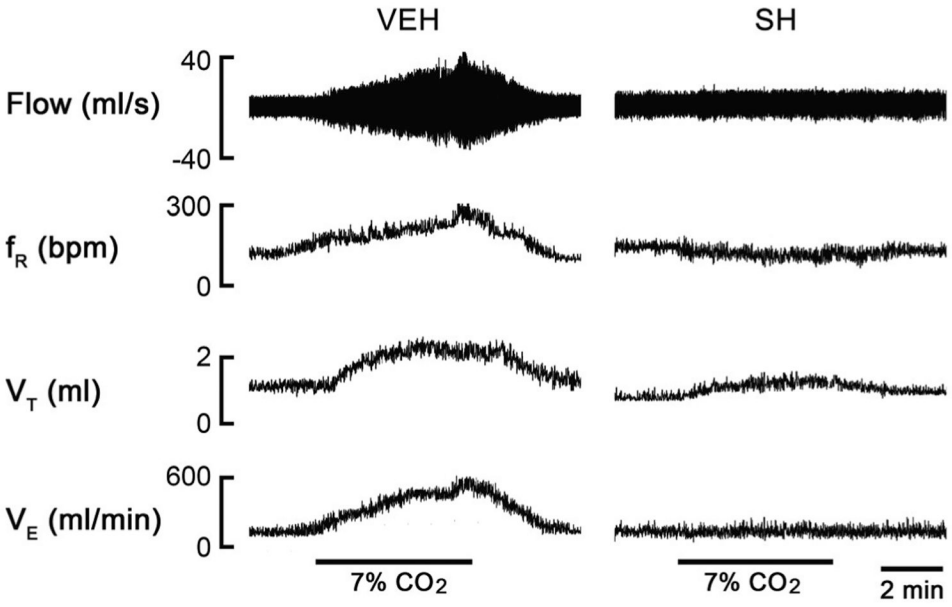

Figure 3.
Examples of experimental recordings showing the hypercapnic ventilatory responses in a vehicle (VEH)- and a high-dose of sarin (SH)-exposed rats 2 h after exposure by plethysmorgraphy. The traces from the top to bottom are airflow (Flow), respiratory frequency (f_R), tidal volume (V_T), and minute ventilation (V_E). SH almost eliminated the V_E response to hypercapnia.

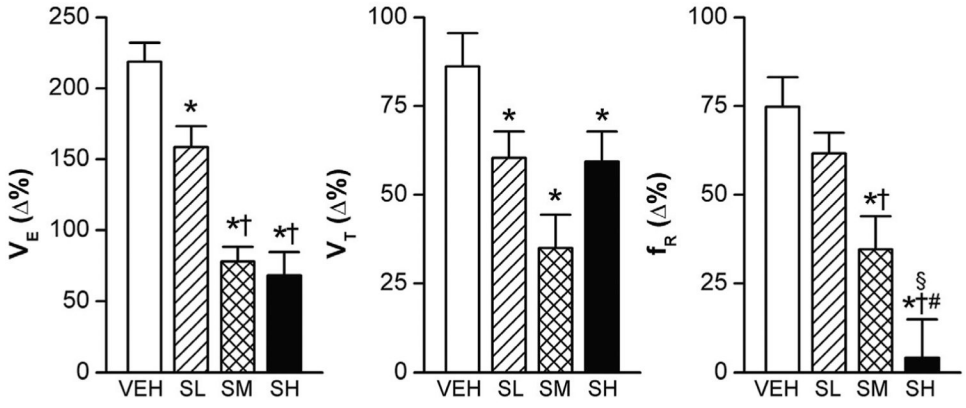

Figure 4.

The hypercapnic ventilatory (V_E) responses (7% CO_2 for 5 min) in rats exposed to three different levels of sarin. As compared with vehicle only (VEH), high and moderate sarin exposure (SM and SH) decreased the V_E responses more than the low level of sarin (SL). The respiratory frequency (f_R) response was absent in SH-exposed rats. Mean ± SE; n = 12, 12, 8, and 8 for VEH, SL, SM, and SH animals, respectively. * $P < 0.05$, compared with VEH group; † $P < 0.05$, SM and SH vs. SL; # $P < 0.05$, SH vs. SM group; § $P > 0.10$, compared with baseline "0".

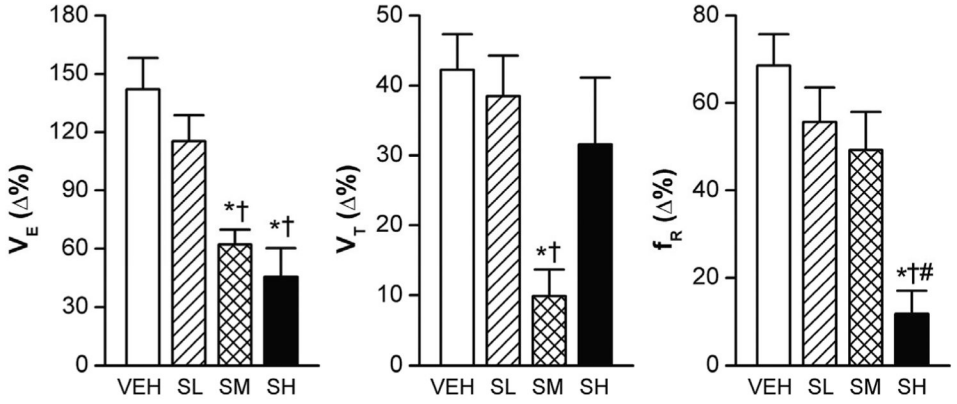

Figure 5.

Group data of the hypoxic ventilatory (V_E) responses (10% O_2 for 5 min) in rats exposed to sarin. High- and moderate- (SM and SH), but not low-dose sarin exposure (SL), attenuated the V_E responses to 10% O_2 due to a reduction in either tidal volume (V_T) or respiratory frequency (f_R). Mean ± SE; n = 12, 12, 8 and 8 for vehicle (VEH), SL, SM and SH animals, respectively. * $P < 0.05$, compared with VEH; † $P < 0.05$, SM and SH vs. SL; # $P < 0.05$, SH vs. SM group.

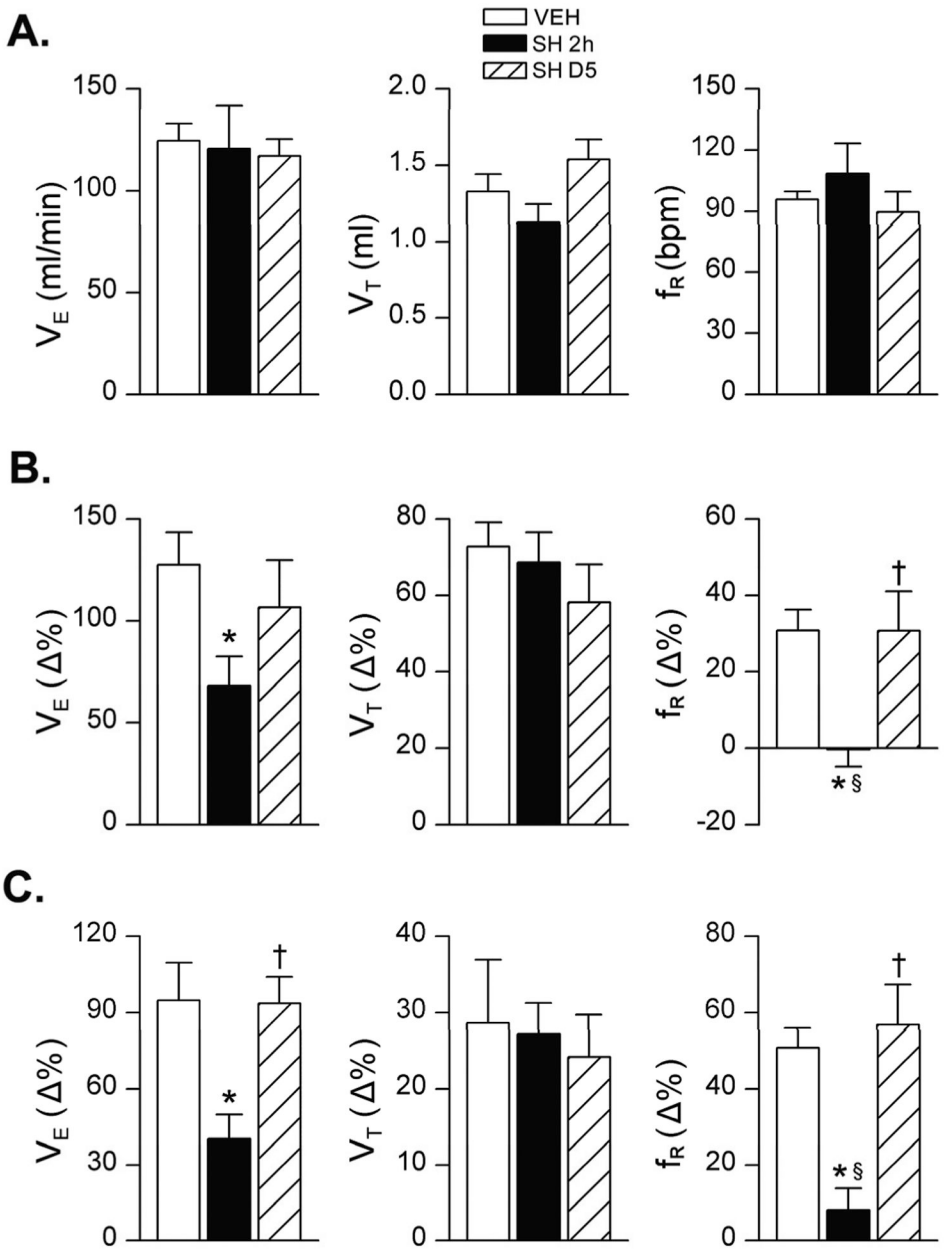

Figure 6.

Group data of the baseline V_E (panel A) and hypoxic V_E responses to hypercapnia (7% CO_2 for 2 min, panel B) and hypoxia (10% O_2 for 1 min, panel C) in the anesthetized rats exposed to vehicle only (VEH) or high-dose sarin (SH), respectively. Mean ± SE; n = 12, 6, and 6 for VEH, 2 h after SH (SH 2h), and 5 days after SH (SH D5) groups, respectively. * $P < 0.05$, compared with VEH group; † $P < 0.05$, SH D5 vs. SH 2h groups; § $P > 0.10$, compared with baseline "0".

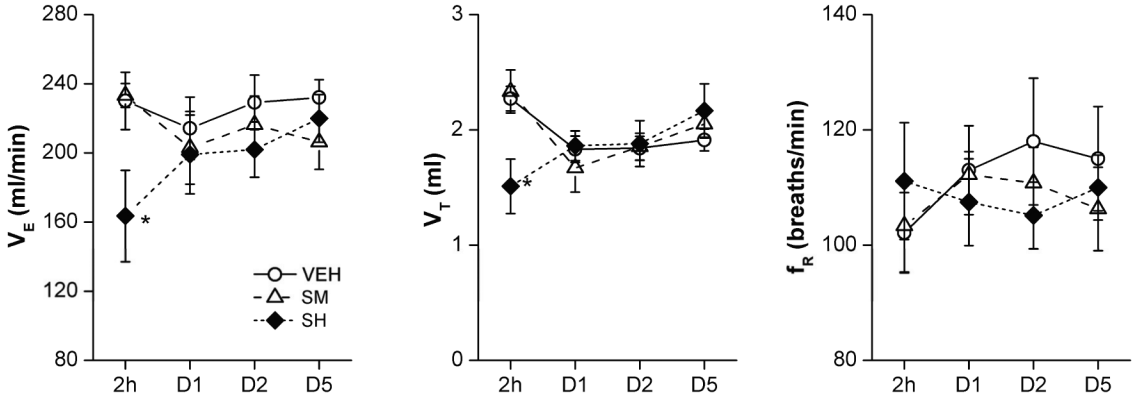

Figure 7.

The recovery of baseline ventilation (V_E) after sarin exposure in conscious rats. As compared with vehicle (VEH), the moderate- (SM) and high-dose sarin (SH)-induced reduction of baseline V_E and tidal volume (V_T) 2 h after exposure was recovered 1 day after sarin exposure. D1–D5 represents the tests applied 1, 2, and 5 days after exposure. Mean ± SE; n = 12, 8, and 8 for VEH, SM, and SH animals, respectively. * $P < 0.01$, SH vs. VEH and SM groups.

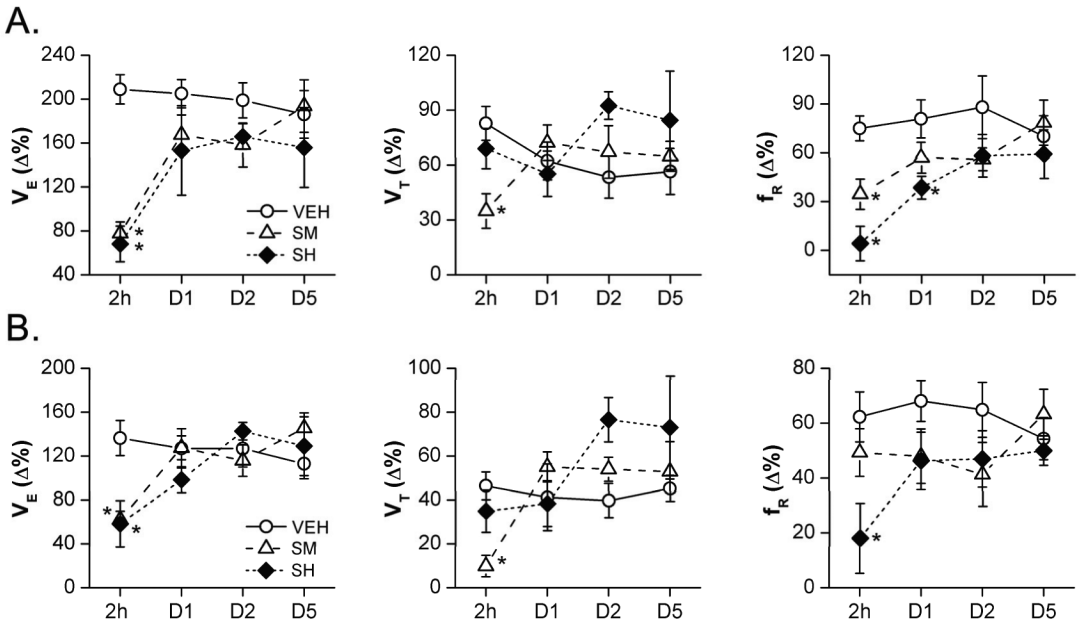

Figure 8.

The ventilatory (V_E) responses to hypercapnia (7% CO_2, panel A) and hypoxia (10% O_2, panel B) in conscious rats 2 h, and 1, 2, and 5 days after exposure to vehicle (VEH), moderate-, or high-dose of sarin (SM and SH). The reductions of V_E, tidal volume (V_T) and/or respiratory frequency (f_R) responses to hypercapnia or hypoxia immediately (2 h) after SM and SH exposure were recovered 1–2 days after sarin exposure. D1–D5 represents the tests applied 1, 2, and 5 days after exposure. Mean ± SE; n = 12, 8 and 8 for VEH, SM and SH animals, respectively. * $P < 0.05$, SH or SM vs. VEH group.

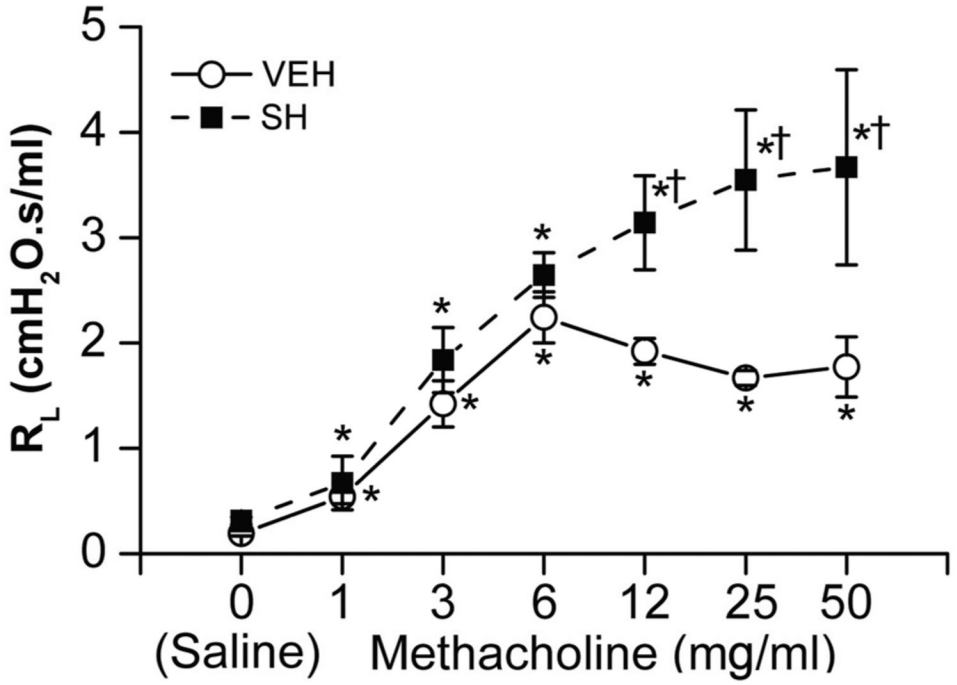

Figure 9.

Total pulmonary resistance (R_L) during different levels of methacholine challenge (0, 1, 3, 6, 12, 25 and 50 mg ml^{-1}) in vehicle (VEH)- and high dose sarin (SH)-exposed rats (2 h after exposure). Baseline R_L was not different between VEH and SH rats (see the variables after saline injection). R_L responses to higher dose of methacholine (12, 25 and 50 mg ml^{-1}) were significantly greater in the SH than VEH rats. Mean ± SE; n = 5 for each group. * $P < 0.05$, compared with saline; † $P < 0.05$, between SH and VEH group.

Table 1

Effects of SH exposure on cardiovascular responses to hypercapnia and hypoxia in anesthetized rats

		2h		D5	
		VEH	**SH**	**VEH**	**SH**
MABP	Baseline (mmHg)	106 ± 7	98 ± 11	108 ± 8	112 ± 12
	Hypercapnia (Δ%)	3 ± 2	5 ± 2	4 ± 2	6 ± 4
	Hypoxia (Δ%)	−42 ± 4*	−44 ± 5*	−44 ± 4*	−41 ± 7*
HR	Baseline (bpm)	394 ± 11	406 ± 7	398 ± 12	412 ± 25
	Hypercapnia (Δ%)	0 ± 1	−3 ± 3	−1 ± 0	−2 ± 1
	Hypoxia (Δ%)	−2 ± 1	2 ± 2	−1 ± 1	−1 ± 2

2h and D5 represent the data obtained at 2 h and on day 5 after vehicle (VEH) or high-dose sarin (SH, 4.0 mg h m^{-3}) exposure. Data are expressed in percentage change from baseline “0” (Δ%) in response to hypercapnia or hypoxia.

*$P < 0.05$, compared with baseline “0”; MABP, mean arterial blood pressure; HR, heart rate.

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Table 2

Baseline parameters of arterial blood gas in anesthetized rats exposed to VEH and SH

	VEH	SH 2h	SH D5
$Paco_2$ (torr)	36.8 ± 2.7	41.8 ± 0.5	36.8 ± 2.8
Sao_2 (%)	93.5 ± 0.5	87.5 ± 3.3	92.5 ± 1.9
pH (unit)	7.41 ± 0.02	7.22 ± 0.04 *	7.43 ± 0.03 †
HCO_3^- (mM)	22.6 ± 1.5	15.9 ± 1.4 *	24.5 ± 0.5 †
BE (mM)	−0.4 ± 1.4	−10.8 ± 2.1 *	0.6 ± 0.5 †

SH 2h and SH D5 represent the data obtained at 2 h and on day 5 after high-dose (SH, 4.0 mg h m^{-3}) exposures.

* $P < 0.05$, compared to VEH

† $P < 0.05$, compared to SH 2h. $Paco_2$, arterial CO_2 partial pressure; Sao_2, arterial O_2 saturation; HCO_3^-, bicarbonate; BE, base excess.