Bull. Environ. Contam. Toxicol. (1988) 40:365–372
© 1988 Springer-Verlag New York Inc.

Bulletin of Environmental Contamination and Toxicology

Effects of Daily Soman Administration on Rabbit Blood Pressure, Temperature, Body Weight, and Erythrocyte and Plasma Cholinesterases

C.-Y. Hu, C.-Y. Hung, and C. P. Robinson

College of Pharmacy, University of Oklahoma, Health Sciences Center, Oklahoma City, Oklahoma 73190

Soman (pinacolyl methylphosphonofluoridate), and other organophosphate acetylcholinesterase (AChE) inhibitors, in toxic doses usually cause a marked reduction in blood pressure. With some AChE inhibitors (AChEI) this is due primarily to a bradycardia-induced reduction in cardiac output (Holmstedt, 1951; Daly and Wright 1956; Fukuyama and Stewart, 1961). However, these organophosphate AChEI also constrict peripheral blood vessels to increase vascular resistance, thus opposing the usually predominant decrease in blood pressure (Holmstedt, 1951; Daly and Wright, 1956; Fukuyama and Stewart, 1961). Hypotension, however, can also be observed in anesthetized rabbits in which bradycardia is blocked by atropine (Preston and Heath, 1972 a,b). This was attributed to decreased central vasomotor output. In several species including rats and dogs, and in human subjects, sub-lethal doses of AChEI increase blood pressure through a central mechanism (Buccafusco and Brezenoff, 1979; Phillippu, 1981; Brezenoff and Giuliano, 1982; Brezenoff et al., 1984). Effects of repeated sub-lethal soman injections on blood pressure and correlation with plasma and erythrocyte cholinesterases have not been done.

The purpose of the present study was to examine the effects of the daily administration of sub-lethal soman doses on the blood pressure of rabbits, and to determine whether or not blood pressure alterations correlated with plasma or erythrocyte cholinesterase concentrations. Effects on body temperature and weight were also noted.

MATERIALS AND METHODS

Male New Zealand White rabbits were weighed, then given 5 μg/kg of soman each morning for seven days.

Send reprint requests to Casey Robinson at the above address.

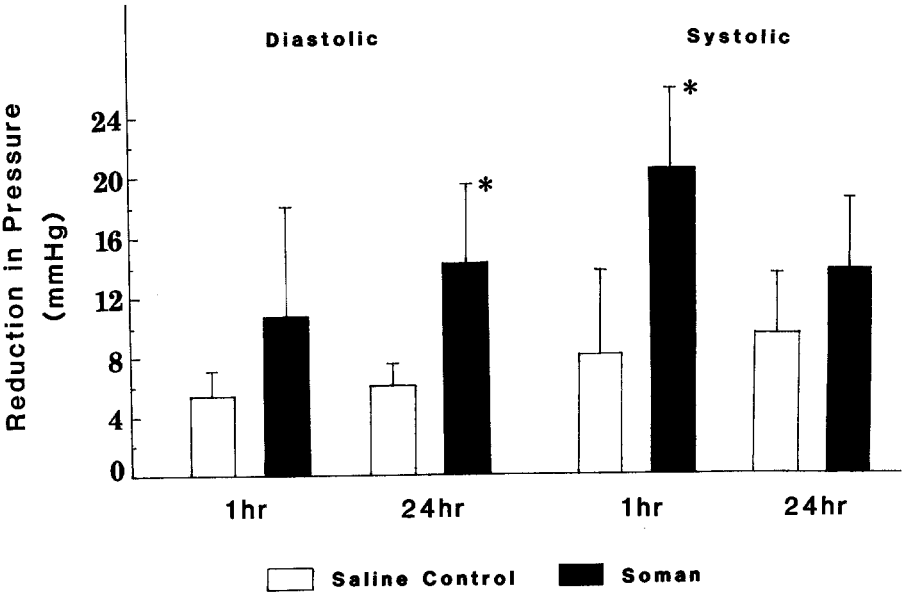

Figure 1. Reduction in blood pressure of rabbits one hour and 24 hours after a single s.c. injection of 5 μg/kg of soman. Control rabbits received saline. Each point is the mean ± SEM of observations in 6 rabbits *p<0.05.

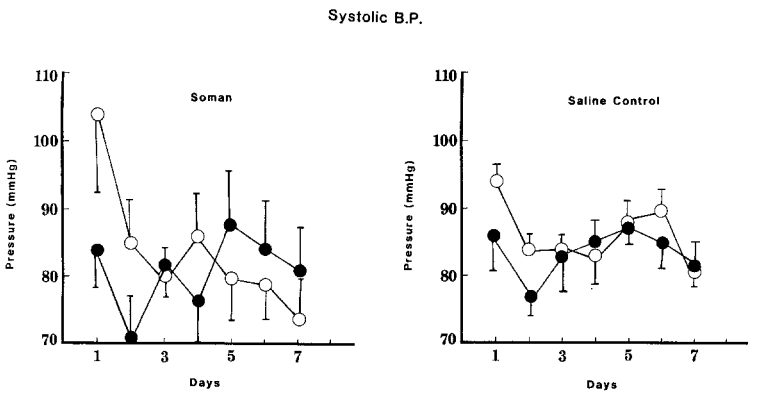

Figure 2. Effects of the daily s.c. injection of 5 μg/kg of soman on the rabbit systolic blood pressure before (○) and one hour after (●) injections. Each point represents the mean ± SEM from observations in 6 rabbits.

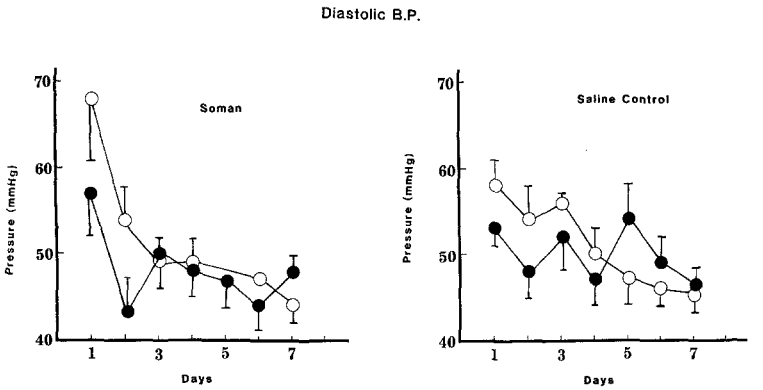

Figure 3. Effects of the daily s.c. injection of 5 μg/kg of soman on the rabbit diastolic blood pressure before (○) and one hour after (●) injections. Each point represents the mean ± SEM from observations in 6 rabbits.

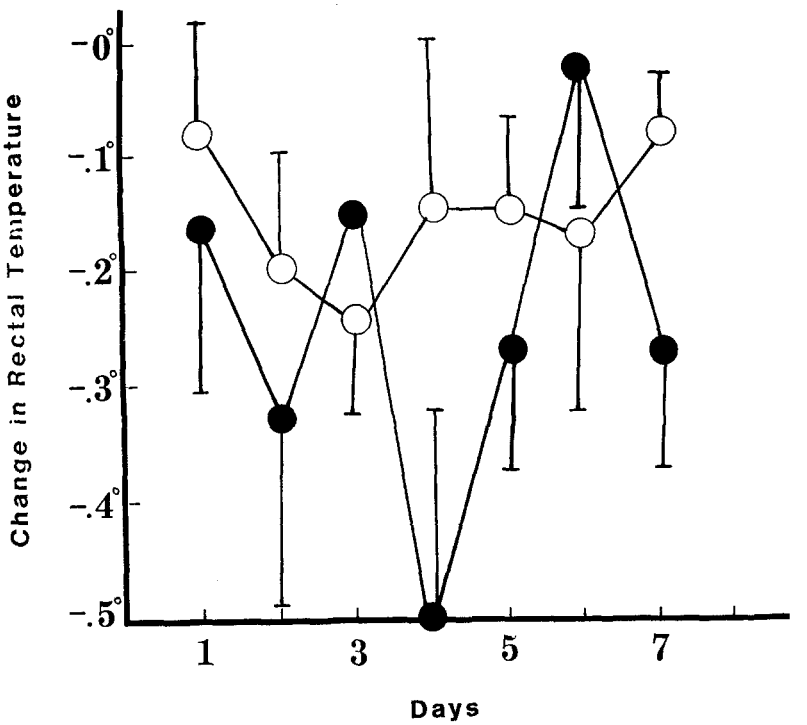

Figure 4. Decrease in rectal temperature one hour after the s.c. injection of 5 μg/kg of soman (●) or saline control (○). Each point represents the mean ± SEM of observations in 6 rabbits. Decreases are significantly different ($p<0.05$) on days 4, 5 and 7.

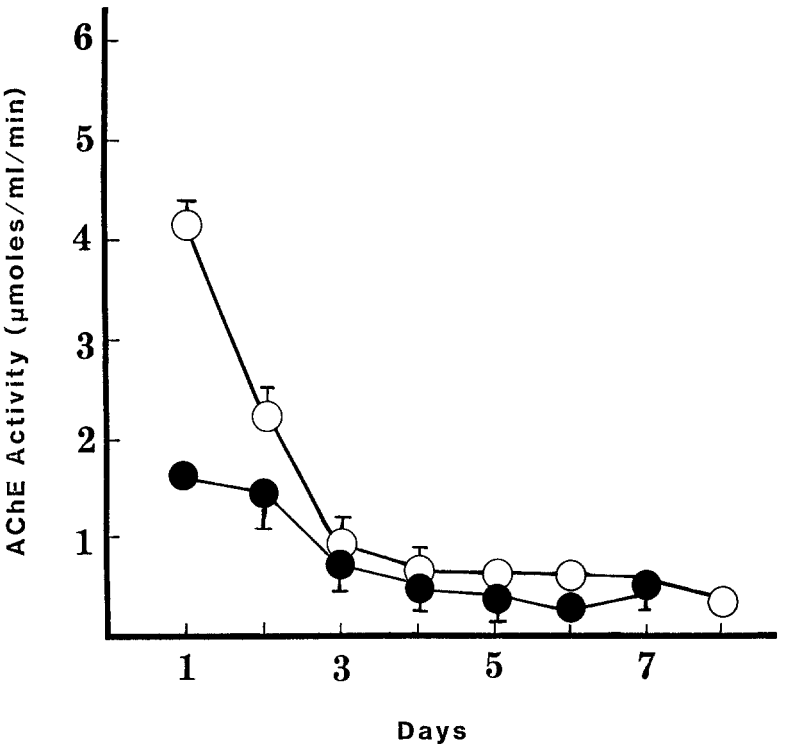

Figure 5. Cholinesterase activity of erythrocytes from rabbits given 5 μg/kg of soman s.c. each day for seven days. Each point represents the mean ± SEM of observations in 6 rabbits.
○ = Before soman injection.
● = One hour after soman injection.

The blood pressure changes determined immediately before soman injection each day were not different in sham-injected and soman-injected rabbits. [systolic blood pressure, $F(dF\ 5,50) = 1.96; p>0.05$), diastolic blood pressure, $F(dF\ 5,50) = 0.70; p>0.05$]. Changes in systolic blood pressure during the first hour after the daily soman injections were significantly different than those occuring in the sham-injected animals ($F(dF\ 5,50) = 3.05; p<0.05$) but in diastolic blood pressure were not significant ($F(dF\ 5,50) = 1.25; p>0.05$).

Soman administration significantly decreased body temperature measured one hour after soman injection on days four, five and seven (Fig. 4).

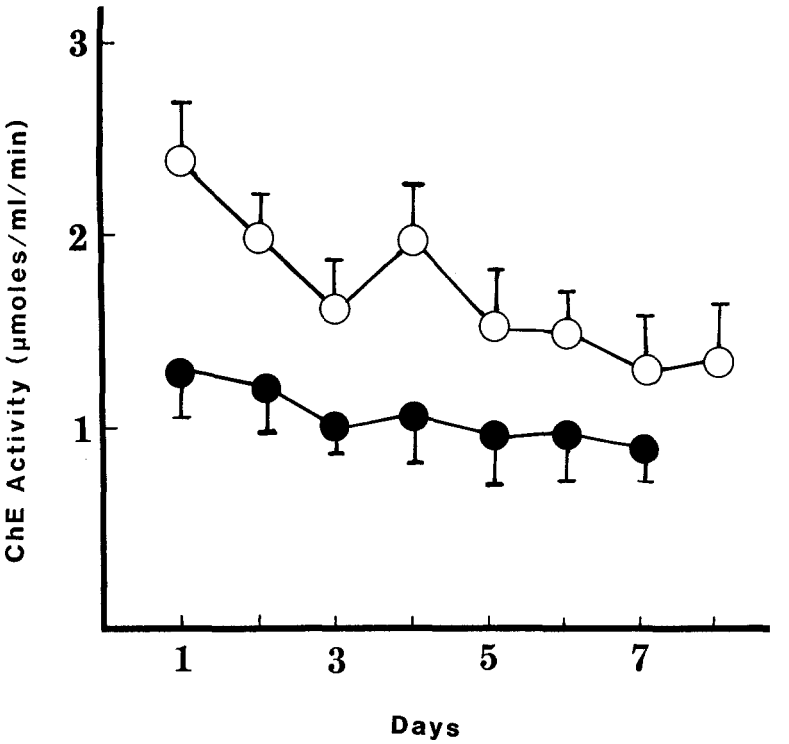

Figure 6. Cholinesterase activities of plasma from rabbits given 5 μg/kg of soman s.c. each day for seven days. Each point represents the mean ± SEM of observations in 6 rabbits.

○ = Before soman injection.

● = One hour after soman injection.

Cholinesterase (ChE) activities of both erythrocytes and plasma of control animals varied little throughout the seven-day treatment period. Control erythrocyte ChE activity varied from 4.16 ± .49 to 5.1 ±.39 (mean 4.6 ± .38) moles of AcThCh hydrolyzed per ml per min over the eight day period. Control plasma ChE activity varied from 2.34 ± .15 to 2.61 ± .20 (mean 2.49 ±.09) moles AcThCh hydrolyzed per ml per min during the eight day period.

Erythrocyte cholinesterase values were reduced greatly with the first dose of soman, and continued to decline throughout the treatment period (Fig. 5). The greatest inhibition observed was 93% on day 8. There was little or no increase in activity between samples obtained one hour after soman was injected and those taken 23 hours later, i.e., immediately before soman was injected the next day.

Blood pressures, rectal temperatures and blood samples were taken before and one hour after injections. For these determinations, rabbits were placed in a restraining box from which their heads protruded.

Both systolic and diastolic blood pressures were determined in the rabbit pinna using a device and method described by Grant and Rothschild (1934). In their studies, these indirectly obtained pressure measurements were somewhat lower than those recorded directly in a larger artery.

Rectal temperatures were taken with a rectal thermister probe inserted to a 6 cm mark and read by a Type 402 Tele-Thermometer, Model 44TE (Yellow Springs Instrument Company, Yellow Springs, Ohio).

One-half ml of blood was taken from the marginal ear vein into a heparinized syringe directly before and one hour after soman injection, and on the eighth day when no soman was given. Plasma and erythrocytes were separated by centrifugation. Erythrocytes were washed twice with normal saline. The cholinesterase activity of both were determined on the same day the blood was taken using the Technicon Auto Analyzer (Technicon Corp., Terrytown, NY) colorimetric method of Fowler and McKenzie (1967) measuring the rate of hydrolysis of acetylthiocholine (AcThCh). This method is an adaptation of an earlier method by Levine et al., (1966).

Plasma samples were diluted in five volumes of normal saline for assay. Erythrocytes were diluted in ten volumes of 5% Triton X-100 in normal saline to lyse the red cells, before assay.

RESULTS AND DISCUSSION

The daily injection of soman did not cause significant changes in body weight ($p>.05$). Rabbits were less active during the first few hours after injection of the first soman dose, and had diarrhea for several days.

There was a general decline in blood pressure of the saline-injected animals over the treatment period. One hour after the first soman injection diastolic blood pressure fell 6 mm Hg more than control (not significant, $p>0.05$) and systolic pressure fell 13 mm Hg more ($p<0.05$) (Fig. 1) than control.

Twenty-four hours later diastolic pressure fell 8 mm Hg more than control ($p<0.05$) and systolic pressure fell 4 mm Hg more than control (not significant, $p>0.05$).

Plasma ChE activity was reduced less than erythrocyte ChE activity (Fig. 6). Also, there was a much greater recovery of enzyme activity during the 23 hours after soman injection. The lowest value recorded was 34% of the initial activity, which was in the sample obtained one hour after soman injection on day seven. Plasma ChE activity was up to 44% at the time of sacrifice the next day (Day 8).

As observed in studies with acutely applied soman or other ChE inhibitors (Holmstedt, 1951; Daly and Wright, 1956; Fukuyama and Stewart, 1961, Preston and Heath, 1972a, b; Edery and Berman, 1985) the administration of soman reduced the blood pressure. There was no significant reduction in blood pressure after the first day. This is in contrast to erythrocyte ChE, which continued to fall over the seven-day period. Perhaps the reason that there was no significant reduction in blood pressure after the first day was that cholinergic effects (to reduce blood pressure) may have been opposed by increased sympathetic activity. Increased sympathetic capacity following repeated soman administration has been reported (Hu and Robisnon, 1987). In that study there was an increase in NE stores, a decrease in activity of enzymes that metabolize NE, and an increased release of NE during transmural nerve stimulation. Thus the ability of soman to either directly or indirectly release NE in the heart could be increased, increasing cardiac output to increase the systolic blood pressure.

During a four week period of daily soman injection, rats become tolerant to hypothermia (Hoskins et al., 1987). In the present study there was only a suggestion of reversal following the lowest point reached on the fourth day.

Acknowledgments. Supported by USAMRDC contract DAMD17-85-5114. The opinions and/or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

REFERENCES

Brezenoff HE, Giuliano R (1982) Cardiovascular control by cholinergic mechanism in the central nervous system. Ann Rev Pharmacol Toxicol 22:341-381

Brezenoff HE, McGee J, Knight V (1984) The hypertensive response to soman and its relation to brain acetylcholinesterase inhibition. Acta Pharmacol et

Toxicol 55:270-277
Buccafusco JJ, Brezenoff HE (1979) Pharmacological study of a cholinergic mechanism within the rat posterior hypothalamic nucleus which mediates a hypertensive response. Brain Res 165:295-310
Daly M de Burgh, Wright PG (1956) The effects of anticholinesterases upon peripheral vascular resistance in the dog. J Physiol 133:475-497
Edery H, Berman HA (1985) Yohimbine antagonism of the vasodepression elicited by organophosphates applied on ventral medulla oblongata. J Autonom Nerv Sys/4:229-238
Fowler PR, McKenzie JM (1967) Symposium: Automation in analytical chemistry. Mediad Inc, New York, pp 155-159
Fukuyama GS, Stewart WC (1961) The effect of intravenous sarin on systemic blood pressure and peripheral vasomotor tone. Arch int Pharmacodyn 130:9-17
Grant RT, Rothschild P (1934) A device for estimating blood pressure in the rabbit. J Physiol 81:265-269
Holmstedt B (1951) Synthesis and pharmacology of dimethylamido ethoxy-phosphoryl cyanide (tabun) together with a description of some allied anticholinesterase compounds containing the N-P bond. Acta Physiol Scand 25(Suppl)90:1-120
Hoskins B, Fernando JCR, Dulaney DK, Lim DK, Liu DD, Watanabe HK, Ho IK (1987) Tolerance to soman, sarin and tabun. Fed Proc 46:3162
Hu CY, Robinson CP (1987) Effects of soman on norepinephrine content, release and metabolism and cholinesterase activity in rabbit blood vessels. Sixth Annual Bioscience Review 499-502
Levine JB, Scheidt RA, Nelson VA (1965) An automated micro determination of serum cholinesterase. Technicon Symposium, Automation in Analytical Chemistry, New York, NY
Philippu A (1981) Involvement of cholinergic systems of brain in central regulation of cardiovascular functions. J Autonom Pharmacol 1:321-330
Preston E, Heath C (1972a) Atropine-insensitive vasodilatation and hypotension in the organophosphate-poisoned rabbit. Arch Int Pharmacodyn 200:231-244
Preston E, Heath C (1972b) Depression of the vasomotor system in rabbits poisoned with an organophosphate anticholinesterase. Acta Pharmacol et Toxicol 200:245-254
Received August 10, 1987; accepted November 3, 1987.