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Research Article

Dramatic Increase in Cerebral Blood Flow following Soman Intoxication If Signs of Symptoms Can Be Seen

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Organophosphate poisoning is associated with adverse effects on the central nervous system such as seizure/convulsive activity and long term changes in neuronal networks. This study report an investigation designed to assess the consequences of Soman, a highly toxic organophosphorus compound, exposure on regional blood flow in the rat brain and peripheral organs. We performed repeated blood flow measurements in the same animal, using the microspheres technique, to characterize changes in regional blood flow at different times after Soman intoxication. In addition, the cardiopulmonary effects of Soman were followed during the intoxication. Administration of Soman (1 LD_{50}; 90 μg/kg, s.c.) to anaesthetized rats produced a decrease in blood acetylcholinesterase activity in all animals tested. Although, only six out of ten rats showed signs of poisoning like a decrease in respiratory rate, the results show that only animals with significant signs of poisoning demonstrated an increase in cerebral blood flow. We conclude that it is of great importance to treat all data individually. An overall mean can easily be misinterpreted and conceal important effects. We also conclude that the increase in cerebral blood flow has an important role in the effect on respiration and that this effect is independent of the blood acetylcholinesterase activity.

1. Introduction

Organophosphorus compounds (OP) irreversibly inhibit the enzyme acetylcholinesterase (AChE), which is responsible for terminating the neurotransmitter action of acetylcholine (ACh) at the various cholinergic nerve endings. This results in the accumulation of cholinergic receptor sites producing continuous stimulation of cholinergic fibers throughout the central and peripheral nervous systems [1, 2]. The clinical signs and symptoms of OP poisoning are commonly divided into three groups: muscarinic, nicotinic, and central. Death is normally caused by respiratory paralysis, which may be of central or peripheral origin [3]. Antidotal treatment of OP-induced poisoning usually consists of two steps: anticholinergic drug to block the overstimulation of the cholinergic receptors by ACh and oximes to reactivate OP-inhibited AChE. Reactivation of OP-inhibited AChE by oximes can generate enough active AChE to restore normal cholinergic neurotransmission after exposure to OP [2, 4].

Soman is a highly toxic organophosphorus compound that is rapidly distributed throughout the body after its administration [5]. We have earlier shown that clearly measurable amounts of the highly toxic C(±)P(−) isomers of Soman reached the brain one minute after intravenous administration of different bolus doses of Soman in anaesthetized pigs [6]. This indicates that the toxic isomers of Soman rapidly penetrate the walls of the capillary vessels at the blood-brain barrier. The rapid absorption of C(±)P(−) isomers leads to increasing intracerebral levels of these isomers for a period of approximately 3 minutes, before the elimination phase begins to prevail.

Exposure to Soman causes a variety of signs of poisoning involving the cholinergic system. The inhibition of AChE causes an accumulation of ACh in the synaptic cleft, which generates frequent activation of ACh receptors [7]. This activation causes a progression of toxic signs that include hypersecretions, convulsions, respiratory depression, and death [8, 9]. It is also shown that ACh triggers the initiation

of seizures and gliosis following Soman intoxication, mainly by activation of muscarinic receptors [10].

In the present experimental set-up, the integrated physiological response to Soman (1 LD_{50}; 90 μg/kg, s.c.), including both effects on the cardiorespiratory system and blood flow regulation, was evaluated in anaesthetized rats. Tissue blood flows were determined from the distribution of radioactivity labelled microspheres as described by Peeters et al. [11]. Three subsequent injections of differently labelled (^{141}Ce, ^{103}Ru, and ^{98}Sn) microspheres were given to each animal, which has the advantage that the animal can serve as its own control. Since only 2-3% of the capillaries are blocked upon each injection, it is possible to perform more than one injection per animal without significant impairment of the blood flows.

Our aim of this investigation was to evaluate how the cardiorespiratory system and cerebral and regional blood flow are affected by Soman intoxication. The role of cerebral blood flow (CBF) in Soman-induced convulsions may lead to improved treatment of Soman intoxication and a better understanding of the role of CBF in other forms of seizures, including human epilepsy.

2. Materials and Methods

2.1. Animals. The experiments were performed on 16 male, Wistar rats (Möllegaard, Denmark), with body weight (BW) ranging between 325 and 399 g. The animals were acclimatized in the animal department for at least 1 week prior to the experiments. The room temperature was 21–24°C and humidity 50 ± 5%. Artificial light was the only source of light, and the animals were set on a 12 h light/dark cycle with lights on at 6.00 a.m. Prior to the experiment, all rats had free access to food and water ad libitum. All the animal experiments were approved by the Regional Research Ethical Committee in accordance with Swedish laws (SFS 1988:539, LSFS 1989:41).

2.2. Drugs. Soman (pinacolyl methylphosphonofluoridate; >95% pure) was synthesized at the Department of Chemistry, FOI CBRN-Defense and Security, Sweden, and diluted to its final concentration with sterile water on the day of the experiment.

2.3. General Surgical Procedure. At the day of microsphere studies, the animals were anaesthetized by injecting thiobutabarbital (Inactin, Byk-Gulden, Konstanz, West Germany) 120 mg kg^{-1} intraperitoneally (i.p.) and tracheotomized for spontaneous ventilation. Body temperature was kept at about 37.5°C by a rectal thermistor and servocontrolled heating pad (Atew, Sweden). To replace fluid losses during the experiment, a polyethylene catheter was inserted into a femoral vein for the administration of a Ringer solution, 0.5 mL h^{-1} 100 g BW^{-1}. For microsphere injections, a catheter was retrogradely introduced in the left ventricle via the right carotid artery. The position was confirmed by pressure measurement. Both femoral arteries were cannulated. The left femoral artery was used for arterial blood sampling and the right artery for continuous measurements of heart rate (HR) and mean arterial pressure (MAP) with a Gould P2310 transducer (Gould Inc., CA, USA) and a ABB SE 120 recorder (ABB Goerz AG, Vienna, Austria). To assure free urine flow, the bladder was catheterized through a suprapubic incision. Arterial pO_2, pCO_2, and pH were determined at intervals with an ABL 520 acid-base analyzer (Radiometer, Copenhagen, Denmark). Heparin (Løvens kemiske Fabrik, Ballerup, Denmark) at 500 I.U. kg^{-1} i.v. was administered as an anticoagulant. For registration of the respiratory rate (RR), a probe was connected around the chest of the animals.

2.4. Microsphere Procedure. Microspheres (15 ± 3 μm diameter), labelled with either ^{141}Ce, ^{113}Sn, or ^{103}Ru to a nominal specific activity of 10 mCi/g, suspended in saline containing 0.01% Tween 80 (Du Pont, NEN Products, Boston, MA, USA) were used. The microspheres were injected into the ventricular catheter and mixed with blood in the left ventricle. In the first pass through tissue vasculature, the microspheres were entrapped in direct proportion to the blood flow to that tissue. The number of spheres in an arterial sample collected during microsphere distribution was used as a reference to determine cardiac output and absolute regional blood flows. Administering spheres with different labels enabled multiple blood flow determinations in a single rat, and each animal serves as its own control. On the day of the study, the tubes were agitated just before injection, and the spheres were injected slowly (15 sec) into the left ventricle. The reference samples were obtained by free flow of blood from a femoral artery for 1 min, giving a reference blood sample that was weighed to obtain a precise reference blood flow (in g/min). After the last microsphere injection, rats were sacrificed with an overdose of sodium pentobarbital injection (60 mg i.v., Apoteksbolaget AB, Sweden), and the position of the ventricular cannula below the aortic valve is verified. Various tissues as abdominal skin, facial skin, cardiac muscle, diaphragm, kidney, liver, lung, pancreas, and spleen were autopsied. The brain was bilaterally divided into the hemispheres, caudate nucleus, di- and mesencephalon, and cerebellum. The most proximal part of the spinal cord (C1–3) was dissected out. Total cerebral blood flow (CBF_{tot}) was calculated to include all regions except cerebellum and spinal cord. The tissues were blotted on filter paper and weighed, and microsphere radioactivity of blood and tissue samples was counted and presented as CPM (counts per minute) in a multichannel gamma counter (model 5230, Packard Autogamma Spectrometer). In order to obtain correct activity, background activity and crossover between energy channels were considered for each sphere measurement. Tissue blood flow (Qt, g × min^{-1} per g tissue) was calculated by multiplying tissue CPM with the reference flow and dividing by reference CPM. The vascular resistance (VR) in various organs was calculated as $VR = MAP \times Qt^{-1}$, where MAP is mean arterial blood pressure (mmHg, measured during the microsphere injection).

2.5. Experimental Protocol. An identical protocol was used for both Soman intoxicated animals ($n = 10$) and control ($n = 6$) rats. A control period of at least 15 min was recorded prior to the first sphere injection (^{141}Ce) which measured

Table 1: Flow chart of the experiment.

Actions	AChE + acid-base	^{141}Ce	Soman or saline	AChE + acid-base	^{113}Sn	AChE + acid-base	^{103}Ru
Time (min)	−2	0	10	13	15	38	40

Table 2: The absolute baseline values for mean arterial pressure (MAP), heart rate (HR), respiratory rate (RR), and partial pressure of carbon dioxide in the blood (pCO_2). No significant difference was seen between the three groups studied.

	MAP (mmHg)	HR (beats/minute)	RR (breath/minute)	pCO_2 (mmHg)
Control ($n = 6$)	125 ± 5.9	410 ± 15.5	92 ± 6.3	6 ± 0.3
No signs ($n = 4$)	129 ± 6.1	422 ± 24.5	94 ± 9.2	6 ± 0.2
Signs ($n = 6$)	127 ± 5.1	395 ± 9.3	89 ± 3.9	6 ± 0.1

control blood flow for each rat. Ten min later, the rats were intoxicated by 1 LD_{50} Soman (90 μg/kg, s.c.). Additional blood flow measurements were performed 5 min (^{113}Sn) and 30 min (^{103}Ru) after the intoxication. Two minutes before each sphere injection, physiological parameters were measured and blood samples collected to analyze AChE activity and acid-base status (Table 1). In the control group, Soman was replaced by the same volume of saline (0.3–0.4 mL).

2.6. Determination of AChE Activity. Blood samples (0.4 mL) were drawn from the femoral artery in heparinized syringes 2 min before each injection of microspheres. Part of each sample (0.3 mL) was used for measurements of pO_2, pCO_2, and pH; the other 0.1 mL was used for determination of AChE activity. The acetylcholinesterase activity was measured in the blood using a modified method of Augustinsson et al. [12]. The 50 μL blood sample was added to 950 μL 0.1% Triton. Twenty-five μL of blood solution was then mixed with 75 μL of PDS buffer (0.28 mM 4,4′-dithiodipyridine diluted in methanol) and incubated for 15 min on a shaker at room temperature. After incubation, the reaction was started by adding 100 μL acetylthiocholine iodide buffer (2 mM) and the changes in absorbance were measured for 10 min on a Labsystems EMS Reader MF (wavelength 324 nm). A software program calculated the slope from the four points that had the highest influence on the slope.

2.7. Clinical Observations. The animals were observed at regular intervals throughout the experiment with respect to muscle tremors, seizures, salivation, respiratory rate, heart rate, and mean arterial pressure. The animals were classified into two groups: no signs: no clinical signs of poisoning; signs: marked respiratory depression and salivation.

2.8. Statistics. For comparisons between animal groups, Student's unpaired t-test was applied, while the paired t-test was used within each group. Values were expressed as means ± SEM and $p < 0.05$ was regarded as significant.

3. Results

Our results show that when male Wistar rats are intoxicated with 1 LD_{50} Soman, two distinguished groups are obtained: one with clear signs of poisoning and another group without any symptoms. In the group with significant decrease in respiration rate, total cerebral blood flow was increased by about 290%, while no change in cerebral blood flow could be seen in the rats showing no signs of poisoning. Remarkably, blood AChE activity is depressed in all animals intoxicated with Soman.

Each animal served as its own control and a control period of at least 15 min was recorded prior to administration of saline (control group) or Soman (1 LD_{50}). Signs of cardiovascular and respiratory impact were evaluated throughout the course of intoxication

3.1. Sphere-Control Experiments. The influence of the sphere procedure was evaluated by giving saline instead of Soman in separate experiments in control rats ($n = 6$). None of the parameters measured were affected. Three subsequent injections of differently labelled (^{141}Ce, ^{103}Ru, and ^{98}Sn) microspheres were given to each animal, which has the advantage that the animal can serve as its own control. Since only 2-3% of the capillaries are blocked upon each injection, it is possible to perform more than one injection per animal without significant impairment of the blood flows [11].

3.2. Physiological Parameters. As can be seen in Table 2, there were no significant differences in mean arterial pressure (MAP), heart rate (HR), respiratory rate (RR), or partial pressure of carbon dioxide in the blood (pCO_2) at the start of the experiment. The absolute baseline values were between 89 ± 3.9 and 94 ± 9.2 breaths/min (respiratory rate), 125 ± 5.9 and 129 ± 6.1 mmHg (mean arterial pressure), and 395 ± 9.3 and 422 ± 24.5 beats/min (heart rate).

In four of ten animals intoxicated with Soman, the respiratory rate remained stable despite the decrease in blood AChE activity. In six out of ten animals, the respiratory rate decreased to about 40% of baseline after 25 minutes and remained stable during the rest of the experimental period (Figure 1, Table 3).

As seen in Figure 1, the change in RR was not consistent with the decrease in AChE activity for all rats intoxicated by Soman. The rats that show signs of poisoning progressed rapidly to apnea, manifested by the absence of regular respiratory efforts. At 30 minutes, RR was reduced to 40%

Table 3: Physiological parameters presented as percent of baseline value (100%) in control rats ($n = 6$), rats intoxicated with Soman not showing signs of poisoning ($n = 4$), and rats showing signs of poisoning ($n = 6$). Values represent mean ± SEM.

Parameter	Symptoms	–10 min	0 min	5 min	20 min	30 min	35 min
% MAP	Control	100 ± 0	87 ± 5.6	92 ± 3.5	90 ± 5.5	82 ± 7.0	83 ± 6.6
	No signs	100 ± 0	91 ± 5.8	84 ± 8.8	78 ± 5.6	80 ± 3.6	78 ± 6.9
	Signs	100 ± 0	98 ± 2.6	93 ± 1.9	109 ± 7.8	135 ± 7.3[a]	130 ± 4.8[a]
% RR	Control	100 ± 0	103 ± 2.5	103 ± 3.9	100 ± 2.1	103 ± 3.5	109 ± 3.4
	No signs	100 ± 0	97 ± 6.6	101 ± 3.6	97 ± 7.4	86 ± 5.5	91 ± 4.5
	Signs	100 ± 0	107 ± 3.0	105 ± 2.0	93 ± 5.9	46 ± 5.1[a]	42 ± 8.7[a]
% CBF	Control	100 ± 0		109 ± 4.5			98 ± 7.9
	No signs	100 ± 0		99 ± 5.0			80 ± 9.1
	Signs	100 ± 0		111 ± 8.0			290 ± 43.0[a]
% AChE	Control	100 ± 0	100 ± 0	97 ± 4.1	95 ± 2.8		93 ± 4.6
	No signs	100 ± 0	100 ± 0	57 ± 22.1[b]	17 ± 8.9[a]		8 ± 3.6[a]
	Signs	100 ± 0	100 ± 0	67 ± 10.4[b]	6 ± 2.6[a]		7 ± 1.4[a]
% HR	Control	100 ± 0	97 ± 2.4	98 ± 2.0	99 ± 2.6	97 ± 2.6	97 ± 2.8
	No signs	100 ± 0	93 ± 3.8	92 ± 4.3	93 ± 3.2	94 ± 2.8	91 ± 2.9
	Signs	100 ± 0	100 ± 2.0	102 ± 1.9	105 ± 1.4	107 ± 3.2	102 ± 3.3
% pCO_2	Control	100 ± 0	100 ± 0	93 ± 0.6	90 ± 1.4		89 ± 1.8
	No signs	100 ± 0	100 ± 0	96 ± 2.9	96 ± 3.4		92 ± 4.7
	Signs	100 ± 0	100 ± 0	94 ± 2.0	98 ± 2.9		105 ± 3.7[a]

[a] $p < 0.001$, paired comparisons from baseline value.
[b] $p < 0.01$, paired comparisons from baseline value.

of the baseline value and it remained at this level without ventilatory assistance.

The mean changes in MAP, HR, RR, AChE activity, pCO_2, and CBF from baseline values are depicted in Figure 1. Thirty minutes after intoxication, there was a significant increase in blood pressure in rats showing signs of poisoning, 140% compared to base line value of 100% ($p < 0.001$). Control rats and rats showing no signs of poisoning did not show a significant increase in MAP. The heart rate was unaffected in all animals by the challenges.

3.3. Blood Cholinesterase Activity. The change in blood cholinesterase activity was the same for all rats intoxicated with Soman (Figure 1, Table 3). In the first blood sample drawn, 5 min after injection of Soman, the activity had decreased below 60% of the baseline value, after 20 minutes the activity had decreased below 80%, and it remained depressed throughout the experiment.

3.4. Cerebral Blood Flow. Cerebral blood flow increased to 290 ± 43.0% ($p < 0.001$) from baseline in animals intoxicated by Soman showing a decrease in respiratory rate (Figure 1, Table 3). Control animals and animals with no signs of poisoning did not alter the cerebral blood flow significantly.

3.5. Regional Blood Flow to Peripheral Organs. The effect of Soman on regional blood flow to different peripheral organs is shown in Figure 2 and Table 4. Our results demonstrate that the vascular resistance decreases significantly in the brain and cardiac muscle in animals showing signs of symptoms. Concomitantly, these animals show a significant increase in vascular resistance in the diaphragm, facial skin, abdominal skin, spleen, pancreas, and the kidney.

A significant increase in vascular resistance was also observed in the facial and abdominal skin in animals intoxicated with Soman showing no signs of symptoms, but not to the same extent as for animals showing signs of poisoning.

The time dependence of Soman (1 LD_{50}, s.c.) and saline (control) administration is shown in Figure 1. The animals given Soman were divided into two groups according to experiencing or not experiencing decrease in respiratory rate.

4. Discussion

Our results demonstrate that although administration of 1 LD_{50} Soman to anaesthetized rats produced a significant decrease in blood AChE activity in all animals tested, only six out of ten rats showed signs of poisoning like a decrease in respiratory rate. The results also show that it was only in the animals with significant signs of poisoning that an increase in cerebral blood flow occurred.

Since all the intoxicated rats showed the same degree of inhibition of AChE in the blood, measurement of blood AChE activity is not a good candidate as a biomarker to confirm that Soman intoxication will prevail. The results demonstrate the importance to distinguish between animals showing signs of symptoms and animals not showing signs of symptoms. A mean in the whole group does not give the same result as if you divide them into two groups. As can be seen in Figure 1 and Table 3, there was a dramatic increase in cerebral blood flow of about 290% in animals showing signs of symptoms with a decrease in respiratory rate of about 60%

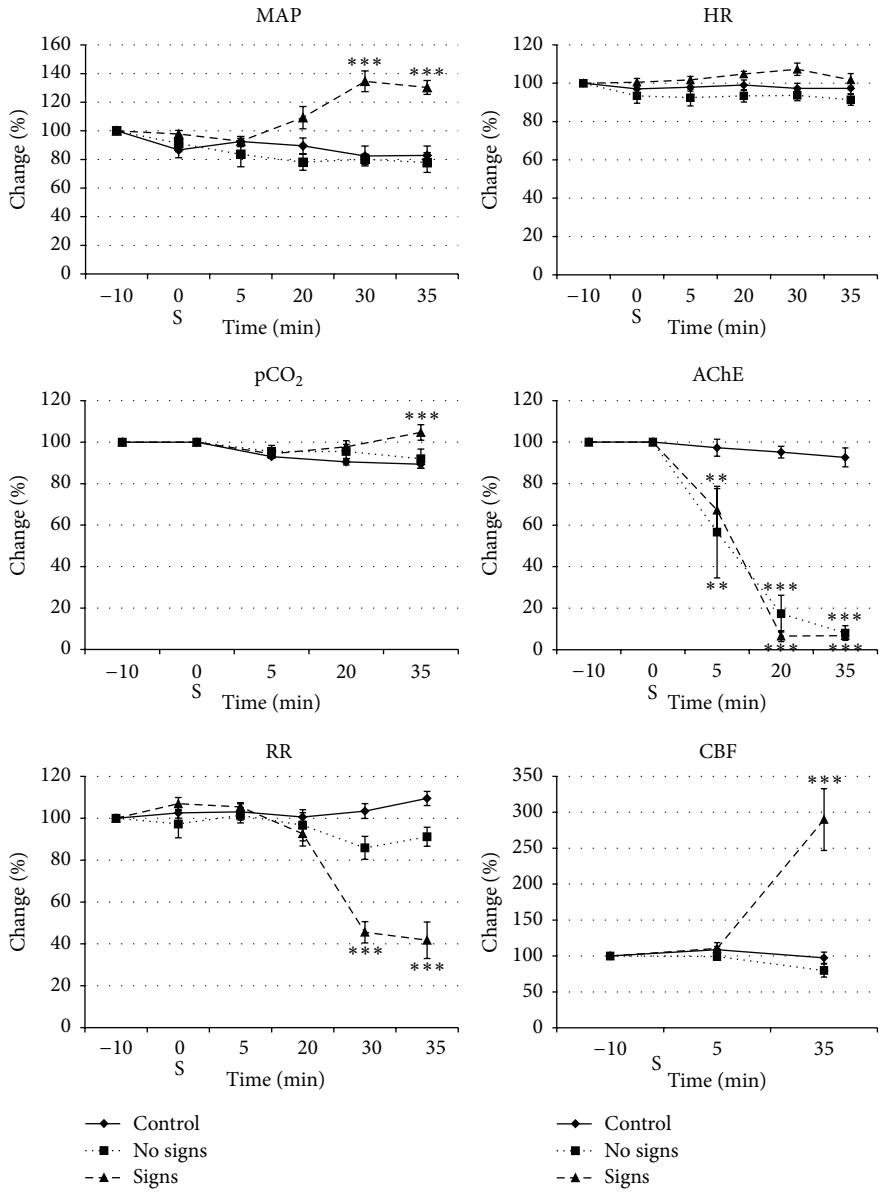

Figure 1: Physiological parameters presented as percent of baseline value (100%) in control rats ($n = 6$), rats intoxicated with Soman not showing signs of poisoning ($n = 4$), and rats showing signs of poisoning ($n = 6$). Percent changes in mean arterial pressure (MAP), heart rate (HR), partial pressure of carbon dioxide in the blood (pCO_2), acetylcholinesterase (AChE) activity, respiratory rate (RR), and cerebral blood flow (CBF) from baseline values, plotted against time. Values represent mean ± SEM. $^{**}p < 0.01$ and $^{***}p < 0.001$, paired comparisons from baseline value.

compared to baseline value. On the contrary, the animals that did not show any decrease in respiratory rate and had no significant increase in cerebral blood flow.

It has been reported by others [13, 14] that Soman increases the cerebral blood flow, but not to the same extent as in the present study. Perhaps they did not distinguish between animals showing signs of symptoms and animals not showing signs of symptoms.

That intoxication with Soman does not always lead to seizure and signs of poisoning despite a decrease in blood AChE activity which has earlier been reported [15–17].

In an earlier study we have shown that signs of poisoning correlate positively to acetylcholinesterase inhibition in the brain and demonstrated that the more severe convulsions, the more inhibition of AChE in the brain [16]. The concentration of AChE in the blood, however, is inhibited independently of degree of signs of poisoning if the rats are intoxicated by Soman. The decrease in AChE activity in the brain after Soman intoxication leads to an increase in ACh in the brain. This increase in ACh might have a direct effect on cerebral blood flow as demonstrated by A. Sato and Y. Sato [18].

Prolonged centrally mediated convulsions are one of the major signs that occur following poisoning with organophosphorus anticholinesterase nerve agents such as Soman [19]. Soman toxicity is generally believed to be due to its prolonged

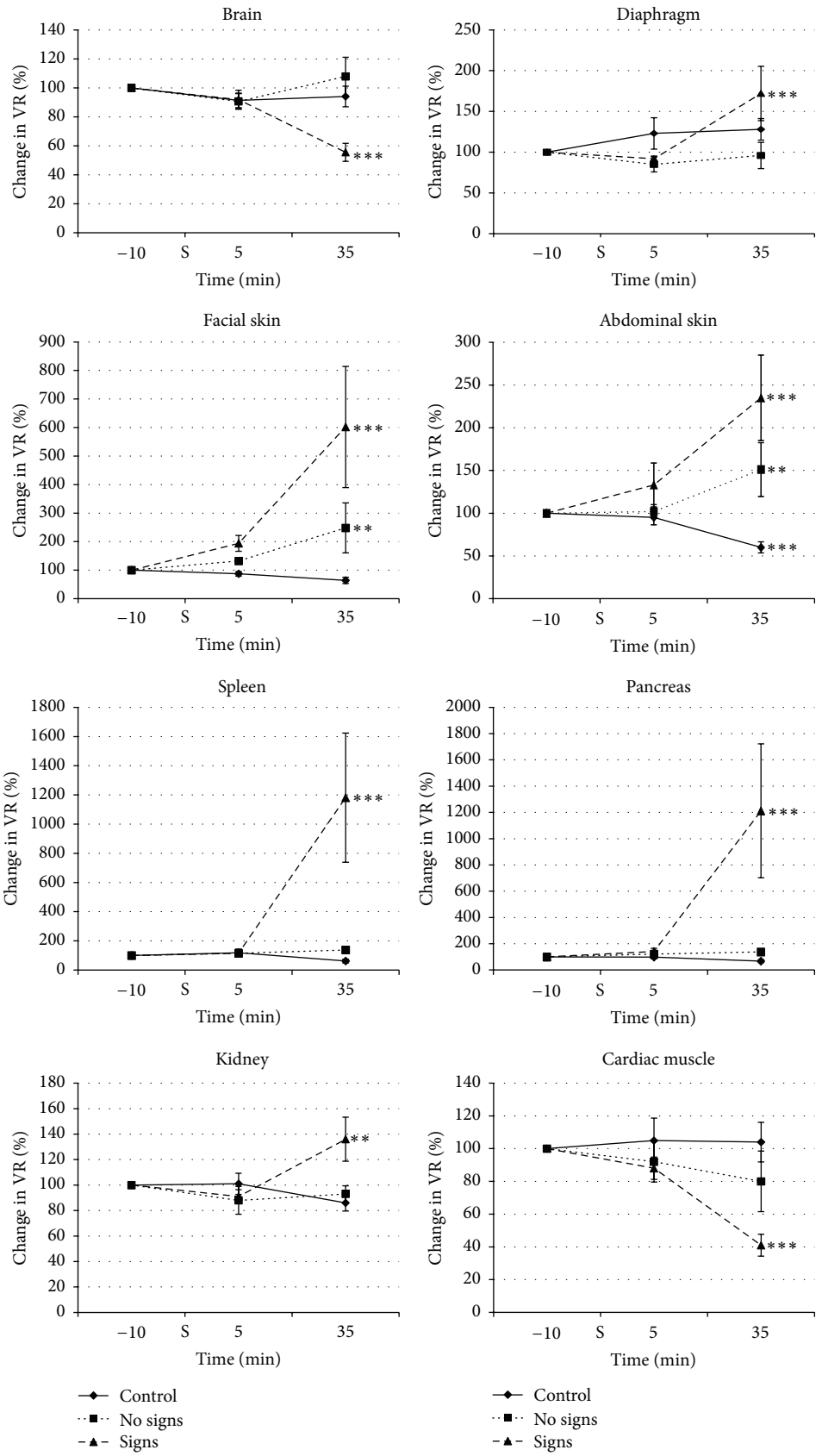

Figure 2: Vascular resistant (VR) presented as percent of baseline value (100%) in control rats ($n = 6$), rats intoxicated with Soman not showing signs of poisoning ($n = 4$), and rats showing signs of poisoning ($n = 6$). Percent changes from baseline values, plotted against time. Values represent mean ± SEM. $^{**}p < 0.01$ and $^{***}p < 0.001$, paired comparisons from baseline value.

Table 4: Vascular resistance (VR) presented as percent of baseline value (100%) in control rats ($n = 6$), rats intoxicated with Soman not showing signs of poisoning ($n = 4$), and rats showing signs of poisoning ($n = 6$). Values represent mean ± SEM.

Organ	Symptoms	−10 min	5 min	35 min
Brain	Control	100 ± 0	91 ± 5.0	94 ± 7.1
	No signs	100 ± 0	91 ± 5.4	108 ± 13.3
	Signs	100 ± 0	93 ± 6.4	51 ± 6.2[a]
Diaphragm	Control	100 ± 0	123 ± 19.2	128 ± 13.2
	No signs	100 ± 0	85 ± 9.2	96 ± 16.1
	Signs	100 ± 0	92 ± 3.3	172 ± 33.4[a]
Facial skin	Control	100 ± 0	87 ± 8.4	64 ± 11.2
	No signs	100 ± 0	132 ± 8.5	248 ± 87.6[b]
	Signs	100 ± 0	194 ± 27.7	602 ± 212.4[a]
Abdominal skin	Control	100 ± 0	95 ± 8.6	60 ± 6.5[a]
	No signs	100 ± 0	102 ± 8.0	151 ± 31.5[b]
	Signs	100 ± 0	133 ± 25.7	235 ± 50.0[a]
Spleen	Control	100 ± 0	118 ± 26.1	62 ± 13.0
	No signs	100 ± 0	115 ± 11.7	138 ± 19.1
	Signs	100 ± 0	116 ± 16.0	1181 ± 442.8[a]
Pancreas	Control	100 ± 0	99 ± 12.6	68 ± 11.3
	No signs	100 ± 0	121 ± 7.1	138 ± 7.4
	Signs	100 ± 0	141 ± 23.6	1212 ± 509.8[a]
Kidney	Control	100 ± 0	101 ± 8.3	86 ± 6.4
	No signs	100 ± 0	88 ± 10.9	93 ± 6.5
	Signs	100 ± 0	91 ± 5.4	136 ± 17.3[b]
Cardiac muscle	Control	100 ± 0	105 ± 13.6	104 ± 12.1
	No signs	100 ± 0	92 ± 12.4	80 ± 18.5
	Signs	100 ± 0	88 ± 6.8	41 ± 6.7[a]

[a] $p < 0.001$, paired comparisons from baseline value.
[b] $p < 0.01$, paired comparisons from baseline value.

inhibition of AChE and subsequent increase of acetylcholine at central and peripheral synapses [9]. Originally, convulsions were considered a factor that complicated the more immediate life-threatening effects that nerve agents have on the respiratory system [20]. However, evidence indicates that OP-induced convulsions rapidly progress to status epilepticus and contribute to profound irreversible brain damage. Therefore, effective management of OP-induced convulsions is critical for both immediate casualty treatment and minimization of neuropathology [21]. It has been shown that Soman initiated convulsions and associated treatment may involve noncholinergic neurotransmitter systems as well [8]. Kubek et al. [22] have demonstrated that generalized seizures produce significant and prolonged increases of the neuropeptide thyrotropin-releasing hormone (TRH) in seizure-susceptible subregions of the brain.

Acetylcholine is an important regulator of CBF in man and in many other species [18, 23]. There is, however, limited information available on the possible sites of action of this neurotransmitter on brain intraparenchymal microvessels. Results by Elhusseiny et al. [24] indicate that microvessels are able to respond to neurally released acetylcholine and that the muscarinic acetylcholine receptors, distributed in different vascular and astroglial compartments, could regulate cortical perfusion and, possibly, blood-brain barrier permeability, functions that could become jeopardized in neurodegenerative disorders such as Alzheimer's disease.

The effect of Soman on regional blood flow to different peripheral organs has, to our knowledge, never been published. Our results demonstrate that the vascular resistance decreases significantly in the brain and cardiac muscle in animals showing signs of symptoms. Concomitantly, these animals show a significant increase in vascular resistance in the diaphragm, facial skin, abdominal skin, spleen, pancreas, and the kidney. This can explain the huge increase in cerebral blood flow that was observed at the expense of a decrease in blood flow to these peripheral organs.

A significant increase in vascular resistance was also observed in the facial and abdominal skin in animals intoxicated with Soman showing no signs of symptoms. That this did not affect the cerebral blood flow has to be further elucidated.

Why the rats responded differently to the same dose of Soman has to be further elucidated. Other enzymes like butyrylcholinesterase (BuChE) and carboxylesterase (CarbE) might be involved [25].

It has been stated that respiratory paralysis following exposure to Soman is the result of a direct action of the agent on certain cholinergic synapses (inhibitory synaptic sites) of the respiratory centers in the brainstem [26, 27]. Most investigators agree that Soman-induced bronchoconstriction and neuromuscular blockade increase respiratory depression but are not the cause of the fatal depression [28, 29].

The huge increase in CBF demonstrated in the present study did probably lead to a higher concentration of Soman in the brain and as a direct effect decreased the respiration rate centrally.

During the past decades, there has been a continuous discussion on the mechanism of respiratory failure in Soman poisoning [26, 30, 31]. Central respiratory depression, neuromuscular blockade, or both have been considered by different investigators to be the causal mechanisms leading to death. The relative contributions of peripheral neuromuscular and CNS components in the respiratory failure remain unclear. Peripheral respiratory system toxicity is manifested as airway obstruction by secretions, laryngospasm, or bronchoconstriction and as depression of the muscles of respiration through actions at the neuromuscular junction. Central respiratory toxicity is reflected as a loss of respiratory drive, that is, an interruption or alteration in the patterned activity of those neurons controlling respiration. Rickett et al. [20] reported that, at the time of respiratory arrest after Soman intoxication, the phrenic nerve could be stimulated to contract the diaphragm muscle tetanically. They concluded that loss of central respiratory drive is the predominant cause of nerve agent-induced respiratory failure. We have demonstrated earlier that the respiratory failure during Soman intoxication can be reversed by antidotal treatment with HI 6 and atropine without reactivation of blood AChE activity

[32]. These results indicate that a noncholinergic mechanism is involved in the respiratory effect of Soman. This, however, does not exclude an effect on the brain AChE activity.

In conclusion, our results demonstrate thatwhen Wistar rats are intoxicated with 1 LD_{50} Soman, two distinguished groups are obtained: one with clear signs of poisoning and another group without any symptoms. Remarkably, AChE activity is depressed in all animals intoxicated with Soman. These results show that it is not advisable to rely on AChE activity in the peripheral blood at assessment of the severity of Soman intoxication. We conclude that it is of great importance to treat all data individually. An overall mean can easily be misinterpreted and conceal important effects.

Conflict of Interests

The authors declare no conflict of interests.

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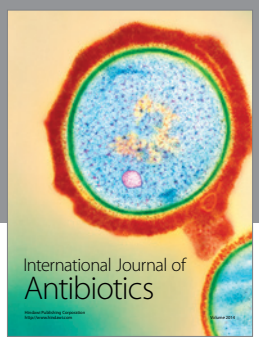

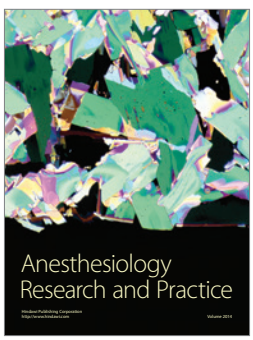

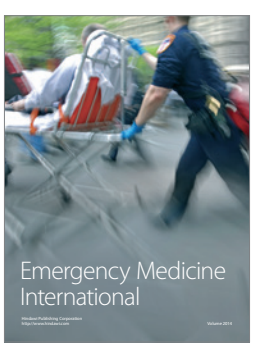

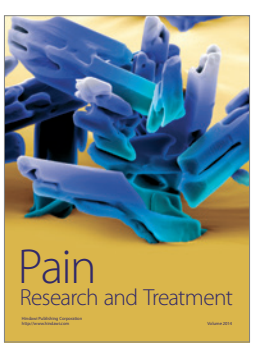

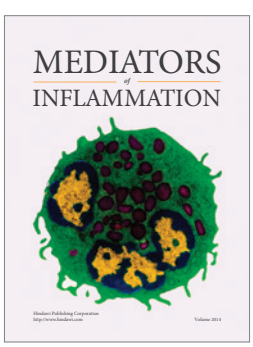

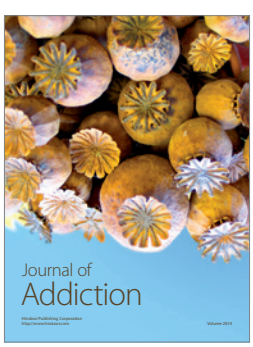

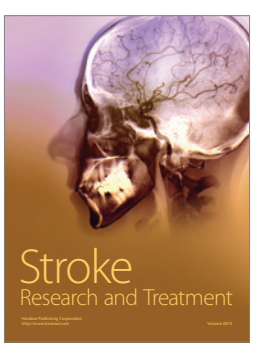

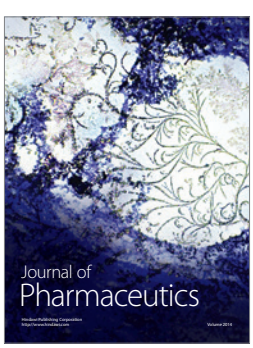

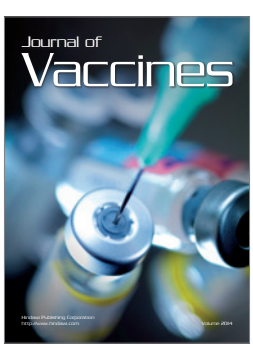

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