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Variability of sarin-induced hypothermia in mice: investigation into incidence and mechanism

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Sarin (isopropyl methylphosphonofluoridate) is a potent acetylcholinesterase inhibitor. The signs of poisoning (e.g. salivation, lacrimation, diarrhea, and tremors) are typical of cholinergic overstimulation. In rodents, a transient hypothermia is evident following administration of an organophosphate anticholinesterase [1–4]. It was noted in my laboratory that the incidence of sarin-induced hypothermia was variable following administration of a sublethal dose of sarin to mice, i.e. there were mice which displayed hypothermia (responders) and those which did not display hypothermia (non-responders). The present study documents the incidence of non-responders following sarin administration and investigates the mechanism behind the variable response of sarin-induced hypothermia in mice.

Male CD-1 mice (25–30 g) obtained from Charles River Canada Ltd., St. Constant, Quebec, were used in this study. The animals were kept in the vivarium at Defence Research Establishment Suffield for at least 1 week, following their arrival, prior to experimentation. The animals were allowed access to food and water *ad lib*. The room temperature was 21–22°.

For the determination of the temporal response following sarin administration, core temperature was monitored using telemetry [2]. The mice were allowed to recover for 1 week following implantation of the telemetry transmitter, prior to use in an experimental situation, at which time the telemetry transmitters were activated, the mice were placed in individual cages, and the core temperature was monitored. Typically, the first three data points established a control baseline. Sarin was administered immediately after the acquisition of the third data point. The data were acquired at 30-min intervals for a total of 720 min. The entire time period, including the control interval, was then used in the calculation of the mean minimum temperature and the area under the curve (AUC).

Brain tissue (hypothalamus, hippocampus, and cortex) was isolated and homogenized using a glass–teflon homogenizer in 0.01 M Tris buffer, pH 7.4, containing 1 M NaCl, 0.05 M $MgCl_2$ and Triton X-100 (1%). The homogenate was centrifuged at 5000 *g* for 20 min, and the acetylcholinesterase activity was determined in a microplate assay using the procedure of Ellman *et al.* [5]. For determination of plasma carboxylesterase activity, blood was sampled from the orbital sinus using heparinized hematocrit tubes. These were centrifuged, to separate the plasma, and the carboxylesterase activity was determined by a spectrophotometric assay using *p*-nitrophenylacetate as the substrate [6].

Sarin was prepared at Defence Research Establishment Suffield. The 24-hr LD_{50} of the sarin used in this study was between 160 and 170 μg/kg (s.c.). Sarin was dissolved in saline prior to injection and the volume of injection was 1% of body weight. Significant differences of the means were determined by Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

In responders, the sarin-induced hypothermia was rapid in onset, reached a maximum in approximately 2 hr and returned to control levels by 10–12 hr after administration (Fig. 1). In a certain population of those mice exposed to sarin (130 μg/kg, s.c.) hypothermia did not develop. The incidence of hypothermia non-responders varied from 25 to 67% for any particular experiment with an overall value of 41.7 ± 14.6% (N = 10–12 repeated ten times for total of 112) following adminstration of sarin (130 μg/kg, s.c.). In the non-responders, the signs of poisoning appeared to be absent, i.e. there was no sign of tremors, salivation, lacrimation, hypothermia, etc. It was as though the animals were not exposed to sarin.

Acetylcholinesterase activity was determined in the hypothalamus, hippocampus and cortex 90 min after sarin administration (Table 1). Brain acetylcholinesterase activity in both responders and non-responders was severely inhibited compared to the unexposed control group. However, the acetylcholinesterase activity in the hypothalamus, hippocampus and cortex in the non-responders was significantly higher than that of the responders. From these results there appeared to be a threshold of brain acetylcholinesterase inhibition required for the appearance of hypothermia in mice, >52% in hypothalamus, > 65% in hippocampus and > 82% in cortex. Interestingly, even though there were different degrees of acetylcholinesterase inhibition in the various brain areas of non-responders, there were no overt signs of cholinergic overstimulation. Previous authors have reported that there is a critical level of brain acetylcholinesterase inhibition, in particular in the hypothalamus, which is required for hypothermia to be expressed [7, 8]. These results were confirmed in the present study following sarin poisoning in mice where hypothalamic acetylcholinesterase inhibition must be > 52% for the appearance of hypothermia.

The differences in the degree of inhibition of acetylcholinesterase in the various brain regions between

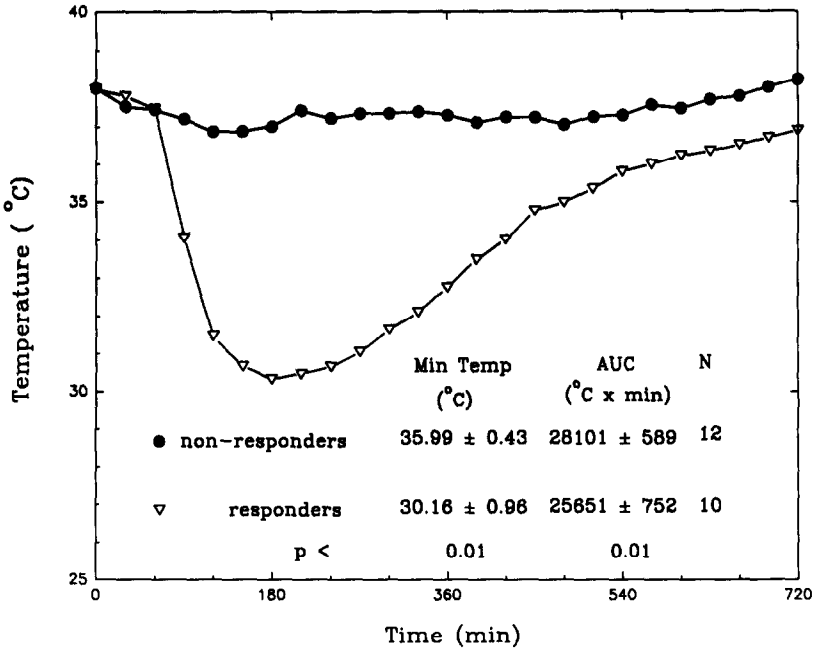

Fig. 1. Core temperature; temporal response following administration of sarin. Sarin (130 μg/kg, s.c.) was administered, and the core temperature was monitored, at 30-min intervals, for a total of 720 min. Those mice treated were separated into responders and non-responders by the presence of hypothermia at 90 min. Values in the table portion are means ± SD.

responders and non-responders, in particular the hypothalamus, indicated that there may be differences in the *in vivo* detoxification of sarin prior to it arriving at its locus of action, namely, acetylcholinesterase. Phosphorylphosphatase (sarinase) and carboxylesterase are enzymes which are involved in the *in vivo* detoxification of organophosphorus anticholinesterases [9–13]. Individual differences in the activity of the detoxification enzymes, such as sarinase and/or carboxylesterase, could be responsible for the variation encountered, i.e. responders and non-responders. Since sarinase hydrolyzes the non-toxic isomer of sarin [12], it is unlikely that differences in the levels of sarinase are important in the *in vivo* detoxification of the sarin in mice. Thus, it is most likely that differences in the level of plasma carboxylesterase are responsible for the differences in the inhibition of brain acetylcholinesterase and for the occurrence of responders and non-responders following sarin administration. This was confirmed experimentally. In a separate experiment, blood samples were collected from mice before sarin adminstration and the plasma was separated. Plasma carboxylesterase activity (mean ± SD; nmol *p*-nitrophenylacetate hydrolyzed/min/mL plasma) was found to be 92,579 ± 8049 (N = 4) and 81,866 ± 6776 (N = 15) ($P < 0.05$) in non-responders and responders, respectively. The difference in the response between responders and non-responders can be accounted for by the fact that sarin is detoxified more efficiently in the non-responders, due to the higher carboxylesterase levels. Thus, in non-responders less of the sarin administered reaches the site of action, which translates into less brain acetylcholinesterase inhibition. The latter was found experimentally.

In conclusion, the incidence of non-responders in mice following sarin administration can be explained by differences in the level of plasma carboxylesterase. A similar mechanism was proposed, but not substantiated experimentally, for the variability of soman and sarin poisoning in rats [14, 15]. The results of the present

Table 1. Brain acetylcholinesterase activity in responders and non-responders following sarin administration*

Treatment group	Acetylcholinesterase activity (nmol/mL/min)						
	Hypothalamus	% Control	Hippocampus	% Control	Cortex	% Control	N
Control	124.2 ± 22.7†	100	108.6 ± 27.7	100	84.6 ± 11.2	100	9
Responders	26.3 ± 11.3	21	16.2 ± 5.7	15	5.0 ± 3.6	6	5
Non-responders	59.5 ± 8.4‡	48	37.7 ± 13.0§	35	15.6 ± 9.0‖	18	12

* Mice were injected with sarin (130 μg/kg, s.c.). The animals were segregated into groups (responders and non-responders) based on the presence of hypothermia as determined using a rectal probe. The mice were killed 90 min after the sarin injection, and the acetylcholinesterase activity was determined.

† Mean ± SD.

‡–‖ Significantly different from responders: ‡$P < 0.001$, §$P < 0.01$, and ‖$P < 0.05$.

study once again demonstrate an important role of carboxylesterase in the detoxification of organophosphate anticholinesterases and the influence that this detoxification mechanism has on animal variation and the measured response, especially at sublethal doses.

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Identification of the novel rat liver IBMX-insensitive phosphodiesterase as a non-specific phosphodiesterase capable of hydrolysing cCMP

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Cyclic nucleotide phosphodiesterases (PDE*) may be classified into five isoenzyme families. These families may be characterized by different kinetic properties and sensitivity to physiological and pharmacological effectors. All known members of these families are selective for purine cyclic 3′:5′-nucleotides, Mg^{2+}-dependent, and are inhibited by IBMX [1, 2]. Lavan *et al.* [3] have reported that rat liver contains a PDE that is not inhibited by IBMX and does not require Mg^{2+} for activity. These properties made it distinct from any of the members of the five isoenzyme families, therefore it was suggested to be a novel activity. However, the properties of the rat liver IBMX-insensitive PDE are remarkably similar to those of a cCMP-PDE previously purified from pig liver [4–7]. In addition to insensitivity to IBMX and Mg^{2+}, both activities elute early from DEAE, have a size of 33 kDa on gel filtration and do not bind to Affigel blue. The pig liver cCMP-PDE will hydrolyse both cyclic 3′:5′-nucleotides and cyclic 2′:3′-nucleotides [5] and is inhibited by AMP and P_i [6, 7]. In this manuscript we report that the rat liver IBMX-insensitive PDE will also hydrolyse all these substrates and

* Abbreviations: EGTA, ethyleneglycol-bis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid; PMSF, phenylmethylsulphonyl fluoride; IBMX, 3-isobutyl-1-methylxanthine; PDE, phosphodiesterase; P_i, inorganic phosphate; cAMP, adenosine 3′:5′-cyclic monophosphate; cGMP, guanosine 3′:5′-cyclic monophosphate; cCMP, cytidine 3′:5′-cyclic monophosphate.