Pharmacology Biochemistry & Behavior, Vol. 39, pp. 305–312. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Hypothermia: Limited Tolerance to Repeated Soman Administration and Cross-Tolerance to Oxotremorine

JOHN G. CLEMENT[1]

Biomedical Defence Section, Defence Research Establishment Suffield, Ralston, Alberta, Canada

Received 27 September 1990

CLEMENT, J. G. *Hypothermia: Limited tolerance to repeated soman administration and cross-tolerance to oxotremorine.* PHARMACOL BIOCHEM BEHAV **39**(2) 305–312, 1991.—The effect of repeated administration of the organophosphate anticholinesterases, soman (pinacolyl methylphosphonofluoridate) and DFP (diisopropylfluorophosphate) on core temperature was investigated in mice. Mice were implanted with telemetry transmitters for the monitoring of core temperature. Following repeated administration of soman (3–10 injections), tolerance (as defined by a decrease in the organophosphate-induced hypothermia upon subsequent administration) to the organophosphate-induced hypothermia was evident after the 5th injection; however, there was cross-tolerance to oxotremorine hypothermia as early as after the 3rd injection of soman. Following repeated administration of DFP, there was no tolerance to the DFP-induced hypothermia following 5 injections, whereas cross-tolerance to oxotremorine was evident following the 5th injection. The organophosphate-induced hypothermia may have another component which contributes to the response. It is proposed that the cross-tolerance to oxotremorine hypothermia after subchronic administration of an anticholinesterase is representative of the functionality of muscarinic cholinergic receptor coupling.

Soman Organophosphate Anticholinesterase Diisopropylfluorophosphate Core temperature Body Tolerance Telemetry Oxotremorine Barbiturate Phenobarbital Hypothermia

INHIBITION of the enzyme acetylcholinesterase by soman (pinacolyl methylphosphonofluoridate) decreases the hydrolysis of the neurotransmitter acetylcholine. This condition produces an increase in the synaptic concentration of acetylcholine, which results in overstimulation of the postsynaptic cholinergic receptors and characteristic signs of anticholinesterase poisoning such as miosis, salivation, lacrimation, diarrhea, tremors, etc. Recovery of acetylcholinesterase activity after soman poisoning is due to synthesis of new enzyme (27) and is not due to dealkylation of the enzyme (16). Thus, following soman poisoning, the postsynaptic cholinergic receptors may be exposed to excessive concentrations of the neurotransmitter acetylcholine for various periods of time (41,47).

Subchronic administration of a cholinergic agonist or acetylcholinesterase inhibitor frequently results in tolerance to the physiological or behavioral effects. Tolerance to the organophosphate anticholinesterase diisopropylfluorophosphate (DFP) was characterized by a decrease in the symptoms of poisoning such as salivation, lacrimation, hypothermia (25, 30, 37) and a decrease in the number, but not affinity, of muscarinic receptors in various regions of the brain (4, 20, 40, 44–46). Similarly, following acute (1) or subchronic (5) administration of soman, there was a decrease in the number of muscarinic receptors in the brain, characteristic of receptor down regulation.

Drug receptor binding studies only indicate that the ligand binds to the receptor but do not provide information concerning the functioning of drug receptor coupling. Dilsaver and Alessi (17) stated that "binding data are adynamic measures which convey nothing about the function of a system." This concept was substantiated by the results of Baumgold et al. (2) which led to the conclusion "that agonist-induced sequestration of receptors does not always lead to desensitization of receptors." Oxotremorine-induced hypothermia is presumably the result of stimulation of muscarinic cholinergic receptors in the anterior hypothalamus (29). Thus, in the present study, oxotremorine-induced hypothermia is used as an in vivo estimate of the functionality of muscarinic cholinergic receptor coupling. The purpose of this investigation was to examine the development of tolerance to organophosphate-induced hypothermia following repeated administration of either soman or DFP and cross-tolerance to oxotremorine hypothermia.

METHOD

Animals

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 one week following their arrival

[1]Requests for reprints should be addressed to Dr. J. G. Clement, Defence Research Establishment Suffield, Box 4000, Medicine Hat, Alta, Canada T1A 8K6.

and prior to experimentation. The animals were allowed access to food and water ad lib. The room temperature was 21–22°C.

Subchronic Administration of Organophosphates

In the case of soman, mice were injected initially with soman (either 50, 70 or 100 μg/kg, SC); then 3 days after the initial injection and thereafter at 2-day intervals, mice were injected with the same dose of soman. In the case of DFP, mice were injected initially with DFP (1.0 mg/kg, SC). Three and 7 days after the initial injection and thereafter at 2-day intervals for injections 4 and 5, mice received the same dose of DFP (1.0 mg/kg, SC). These particular injection schedules were adopted to keep the mortality to an acceptable level.

On the second day after the last injection of either soman or DFP, mice were administered oxotremorine (either 625 or 156 μg/kg, IP) and the core temperature monitored by telemetry as described below.

The effect of repeated administration of soman on drug metabolism by the hepatic mixed function oxidases was assessed. Two days after the 5th injection of soman, sodium pentobarbital (75 mg/kg, IP) was administered and the sleep time recorded and compared to naive controls. In addition, the effect of induction of hepatic mixed function oxidases by sodium phenobarbital (100 mg/kg, IP; daily for 4 days) on the oxotremorine-induced hypothermia was also investigated. This treatment regimen is known to cause a liver hyperplasia and an increase in the mixed function oxidase levels which is manifested as an increase in drug metabolism and can be assessed by the pentobarbital sleep time. Oxotremorine was administered 24 h after the last phenobarbital injection and hypothermia monitored and compared to the control group.

Recording of Core Temperature

Core temperature was monitored using a telemetry system (10). The mice were anesthetized with sodium pentobarbital (75 mg/kg, IP). An abdominal incision was made, and the telemetry transmitter was implanted in the peritoneal cavity. The abdominal muscle incision was closed using sutures (000 plain gut), and the skin was closed using wound clips (9-mm Michel clips). The animal was then allowed to recover for at least 1 week prior to use in an experimental situation.

Analysis of Hypothermia Data

Following administration of oxotremorine, temperature was recorded at 10-min intervals, whereas, following administration of either soman or DFP, core temperature was recorded at 30-min intervals. Typically, the first 3 data points were collected to record the baseline. Immediately following the aquisition of the 3rd data point, the drug was administered, and the data were aquired for a total of 280 min for oxotremorine and 720 min for soman or DFP, and the mean minimum temperature and the area under the curve (AUC) were calculated (10). When one examines the data for the mean minimum temperature and the curve for the time course of the hypothermia, the mean minimum temperature on the graph may be different from the value in the table portion of the figure. The minimum temperature reported in the table is the mean of the lowest temperatures attained by individual mice during the entire observation period, irrespective of the time taken to attain this temperature, whereas the values used in plotting the graphs are the mean core temperatures at particular times following administration. If individual animals reached the minimum temperature at different times, then this is reflected in the apparent differences in the values between the graph and the table.

Data Analysis

The data were analyzed by one-way analysis of variance (ANOVA), and where a significant overall effect was found, the group means were compared using the multiple comparison test of Scheffe (23). A $p<0.05$ was considered significant.

Enzyme Determinations

Acetylcholinesterase. Acetylcholinesterase activity was determined at 37°C using the radiometric procedure (42) with ^{14}C-acetylcholine iodide (ACh) as the substrate. Mice were decapitated and exsanguinated. Brain tissues (hypothalamus, cerebellum, pons medulla, cortex, hippocampus and striatum) were isolated. A 1% (w/v) homogenate was prepared in a 0.1-M phosphate buffer (pH 7.4) containing 0.4 M sucrose using a Teflon pestle glass homogenizer. The homogenates were then frozen and stored at −80°C until analyzed for acetylcholinesterase activity, which was expressed as nmol ACh hydrolyzed/mg wet weight of tissue/min.

Carboxylesterase. Carboxylesterase activity was assessed by the pH-Stat technique using tributyrin as the substrate. To a reaction vessel (23°C), tributyrin (10 ml of 0.2% solution) was added, vigorously stirred and titrated to pH 7.9 with 0.01 N NaOH using a Radiometer titragraph. To start the reaction, 50 μl of serum was added. Rate of addition of 0.01 N NaOH from the first to the fifth minutes was used in determining the enzyme activity. The reaction was linear over this time period. All solutions were degassed by bubbling pure nitrogen through them for at least 5 min, prior to the start of the experiment, and the reaction vessel was purged with nitrogen while the reaction was in progress. Carboxylesterase activity was expressed as the nmol tributyrin (TBT) hydrolyzed/ml of serum/min.

Materials

Soman (pinacolyl methylphosphonofluoridate) was prepared at Defence Research Establishment Suffield. The following drugs were obtained from various commercial sources: sodium pentobarbital (MTC Pharmaceuticals); tributyrin and sodium phenobarbital (Sigma); and oxotremorine (Aldrich). In light of previous observations (28), the DFP (Sigma) was repurified before use by a Kugelrohr purification. All drugs were dissolved in saline immediately prior to injection. The volume of injection was 1% of body weight in all cases.

RESULTS

Effect of Metabolic Status on Oxotremorine Hypothermia

The minimum temperature and the time course of the oxotremorine-induced hypothermia may reflect either the sensitivity of the muscarinic receptor coupling or the metabolic status of the animal. The effect of metabolism on the oxotremorine parameters being measured (mean minimum temperature and AUC) was investigated in mice, following the induction of mixed function oxidases by the daily administration of the barbiturate sodium phenobarbital (100 mg/kg, IP). The pretreatment of mice with phenobarbital induced mixed function oxidases in the liver, as evidenced by a significant ($p<0.05$) reduction in the sodium pentobarbital-induced sleep time in mice from 122 ± 30 min (mean ± SD) in control mice to 31 ± 3 min in phenobarbital-pretreated mice. The metabolic status of mice following repeated injections of soman was evaluated using the sodium pentobarbital sleep time. The pentobarbital sleep time was 122 ± 30 (mean ± SD) min in control mice and 100 ± 17 min in soman-treated mice. This difference was not significant. Twenty-four h after the last injection of phenobarbital, mice were injected with oxotremorine (312.5 μg/kg, IP) and the hypothermia monitored. There was no significant difference between the mean minimum

TABLE 1

REPEATED ADMINISTRATION OF SOMAN: MINIMUM TEMPERATURE AND AUC*

Soman Dose (μg/kg)	Min. Temperature (°C)			AUC (°C × min)		
	50	70	100	50	70	100
Injection Number						
1	36.39 ± 0.99†	32.24 ± 2.08	30.96 ± 0.93	28373 ± 613	26904 ± 781	25625 ± 1164
2	34.86 ± 2.46	29.49 ± 1.85	31.07 ± 1.13	27787 ± 862‡	25417 ± 1205	26583 ± 529
3	32.24 ± 2.21‡	28.87 ± 1.33‡	28.92 ± 0.98‡§	26966 ± 875‡	25258 ± 1069	24721 ± 710§
4	32.47 ± 3.29‡	28.37 ± 1.65‡	28.98 ± 0.67‡§	26947 ± 1360‡	24618 ± 1211‡	24972 ± 671§
5	31.99 ± 3.24‡	28.92 ± 1.41‡	—¶	26853 ± 1443‡	25259 ± 1059	—¶
ANOVA	$F(4,45)=5.67$	$F(4,25)=5.01$	$F(3,32)=14.54$	$F(4,45)=3.92$	$F(4,25)=3.72$	$F(3,32)=9.61$
	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.05$	$p<0.01$

*Received 5 injections as described in the Method section.
†Mean ± SD.
‡Significantly different ($p<0.05$) from the lst soman injection.
§Significantly different ($p<0.01$) from the 2nd soman injection.
¶Data acquisition terminated prematurely due to power interruption.

temperature (28.41 ± 1.11 vs. 28.61 ± 0.32) or the AUC (9641 ± 346 vs. 9676 ± 99) in the phenobarbital-pretreated or control groups, respectively. Thus the change in the oxotremorine-induced hypothermia does not appear to be influenced by the metabolic status of the animal and thus is probably an indicator of the functionality of the muscarinic receptor coupling in the CNS.

Repeated Administration of Soman

In this study, tolerance was defined as a decrease in the organophosphate-induced hypothermia following repeated administration of the same dose, whereas cross-tolerance was defined as a decrease in the oxotremorine-induced hypothermia (compared to that found in naive control mice) following repeated administration of an organophosphate anticholinesterase.

The quantitation of the hypothermia parameters evaluated following repeated administration of soman are presented in Table 1. With the lowest dose of soman (50 μg/kg), the first 2 injections did not result in any significant degree of hypothermia. It was not until the 3rd injection that hypothermia became significant ($p<0.05$). Similarly, at the 70 μg/kg dose of soman, the subsequent injections appeared to result in a greater response compared to that found after the first injection. Only after injections 3–4 were the results significantly different ($p<0.05$) from the 1st soman injection. Following a 100 μg/kg dose of soman, the hypothermia increased significantly ($p<0.05$) by the 3rd injection. In addition, the 3rd soman (100 μg/kg) injection resulted in a significant ($p<0.01$) increase in the soman-induced hypothermia over the 2nd injection. However, tolerance, as previously defined, was not evident following repeated administration of soman. There was no mortality following repeated administration of the 50 and 70 μg/kg doses of soman, whereas there was approximately 40% mortality after the 5th injection of 100 μg/kg soman (Table 2). Signs of soman poisoning such as salivation, lacrimation, and tremors were evident following each injection of soman; however, quantitation of the severity or incidence of these particular signs was not performed.

Brain acetylcholinesterase activity was determined following repeated injections of soman (Table 2). After the 1st soman injection (100 μg/kg, SC), there was extensive inhibition of acetylcholinesterase in all brain regions except the striatum, where there was only a 30% inhibition of acetylcholinesterase activity compared to >94% inhibition in the cortex. Following the 5th soman injection, acetylcholinesterase inhibition in all the brain regions was >90%. Recovery of the acetylcholinesterase activity in the 2-day period following the last injection of soman was in the range of 18–32% for the various brain regions, whereas normal core temperature was reestablished within 12 h following soman administration. Serum carboxylesterase activity was inhibited following repeated injections of soman; however, in the 2-day recovery time following the last soman injection, serum carboxylesterase activity returned to 90% of control levels. The brain acetylcholinesterase activity following the 5th injection of 70 μg/kg soman was also determined (Table 2). The activities were in a similar range but generally greater than those found following 100 μg/kg soman. It may be that a certain degree of acetylcholinesterase inhibition is required before the soman-induced hypothermia becomes evident, which may be the reason that the lowest dose of soman required 3 doses to demonstrate hypothermia and that the 2nd injection of the 70- and 100-μg/kg doses appeared to produce a greater degree of hypothermia.

Additional experiments were performed in which the number of soman injections (70 μg/kg) was increased to a total of 10 to determine if the previous results, demonstrating a lack of tolerance to soman-induced hypothermia following repeated administration, were due to the fact that the number of injections was insufficient to produce a response. The dose of soman chosen for this experiment was approximately 70% of maximum and on the linear portion of the soman dose-response curve (11). The mean minimum temperature values had a maximum depression after the 2nd soman injection and tended to increase slightly in value for the remaining injections (Table 3). The ANOVA for the mean minimum temperatures was significant; however, when the significant differences between the various injections was analysed by the multiple comparison test of Scheffe, no significant differences were detected. The Scheffe multiple comparison test is very rigorous and occasionally results in no significant differences even when the ANOVA is significant (23). On the other hand, the Scheffe test detected significant differences of the AUC values. There was an increase in the hypothermic response following the second soman injection. Following the 5th soman injection and beyond, there were significant decreases in the soman-induced hypothermia. Thus it can be concluded that

TABLE 2

MORTALITY AND ACTIVITY OF ACETYLCHOLINESTERASE, IN VARIOUS BRAIN REGIONS, AND SERUM CARBOXYLESTERASE FOLLOWING REPEATED INJECTIONS OF SOMAN*

Time After Initial Soman Injection (days)	Soman Injection No.	% Mortality	Acetylcholinesterase Activity (nmol/mg/min)												Carboxyl-esterase (nmol TBT/ml/min)	
			Striatum		Hypothalamus		Hippocampus		Cortex		Cerebellum		Pons Medulla		Serum	
100 μg/kg																
0	1	1.6	24.00 ± 4.75†	**70.9‡**	0.95 ± 0.33	**16.3**	0.59 ± 0.16	**9.9**	0.22 ± 0.01	**5.7**	0.40 ± 0.04	**14.0**	2.28 ± 0.29	**22.2**	427 ± 117	**34**
3	2	3.3	14.40 ± 2.22	**42.5**	0.90 ± 0.14	**15.4**	0.22 ± 0.03	**3.7**	0.16 ± 0.03	**4.2**	0.27 ± 0.06	**9.4**	0.81 ± 0.14	**7.9**	168 ± 73	**13**
5	3	23.0	2.29 ± 0.58	**6.8**	0.86 ± 0.14	**14.8**	0.27 ± 0.12	**4.5**	0.16 ± 0.04	**4.2**	0.26 ± 0.03	**9.1**	0.47 ± 0.04	**4.6**	264 ± 95	**21**
7	4	34.4	1.12 ± 0.35	**3.3**	0.52 ± 0.06	**8.9**	0.23 ± 0.18	**3.9**	0.14 ± 0.03	**3.7**	0.22 ± 0.05	**7.7**	0.40 ± 0.08	**3.9**	293 ± 63	**23**
9	5	39.3	0.91 ± 0.49	**2.7**	0.52 ± 0.07	**8.9**	0.11 ± 0.04	**1.8**	0.13 ± 0.07	**3.4**	0.19 ± 0.02	**6.6**	0.45 ± 0.07	**4.4**	376 ± 171	**30**
11			6.86 ± 1.36	**20.3**	2.37 ± 0.25	**40.6**	1.13 ± 0.08	**18.9**	1.00 ± 0.15	**26**	1.08 ± 0.17	**37.8**	3.18 ± 0.14	**30.9**	1127 ± 436	**90**
70 μg/kg																
9	5	0	6.71 ± 3.79	**19.8**	0.75 ± 0.18	**12.9**	0.25 ± 0.03	**4.2**	0.13 ± 0.03	**3.4**	—		—		—	
Control			33.87 ± 7.48		5.83 ± 0.63		5.98 ± 0.71		3.84 ± 0.41		2.86 ± 0.17		10.28 ± 1.59		1251 ± 306	

*Mice were repeatedly injected with soman (100 or 70 μg/kg, SC) and were sacrificed 3 h following the soman injection for the determination of enzyme activity. Day 11 values were from mice which were sacrificed 48 h after the last soman (100 μg/kg, SC) injection.

†Mean ± SD; N = 5.

‡% control activity.

TABLE 3

REPEATED ADMINISTRATION OF SOMAN: EFFECT ON MEAN MINIMUM TEMPERATURE AND AUC*

Injection Number	Min. Temperature (°C)	AUC (°C × min)
1	31.38 ± 2.79†	26163 ± 1322§
2	28.42 ± 1.59	23393 ± 1088‡
3	28.91 ± 1.31	24657 ± 789
5	30.11 ± 0.84	25689 ± 585§
6	29.73 ± 1.08	25575 ± 660§
7	30.28 ± 1.44	26120 ± 733§
8	30.55 ± 1.56	26164 ± 670§
9	29.96 ± 1.25	25791 ± 427§
10	30.15 ± 1.28	25704 ± 755§
ANOVA	$F(8,54)=2.21$ $p<0.01$	$F(8,54)=8.53$ $p<0.01$

*Mice were injected with soman (70 μg/kg, SC) for a total of 10 injections as follows; starting on day 0, then again 3, 5, 7 and 9 days after the initial injection then at 2-day intervals during the week, with no injections on the weekend, for the remaining 5 injections. Data acquisition for the 4th injection was interrupted due to computer malfunction and therefore the parameters were not calculated.

†Mean ± SD. N = 7.

‡Significantly different from the 1st injection ($p<0.05$).

§Significantly different from the 2nd injection ($p<0.05$).

there was a certain degree of tolerance that developed starting after the 2nd injection of soman, but did not reach significance until at least the 5th injection (Table 3).

In separate experiments, the time course of the development of cross-tolerance to oxotremorine (156 μg/kg, IP) following repeated administration of soman (70 μg/kg, SC) was determined. The dose of oxotremorine that was chosen was found to be approximately 70% of the maximum as determined from the oxotremorine dose-response curve (10). The results in Fig. 1 illustrate that there was cross-tolerance to oxotremorine-induced (156 μg/kg) hypothermia after the 3rd soman injection which appeared to reach a maximum by the 5th soman injection; the 5th and 10th injection results were not significantly different from one another. Following cessation of the soman injections, the oxotremorine hypothermia approached control levels within 12 days, indicating that the cross-tolerance to oxotremorine was slowly reversible.

The effect of repeated soman administration on the soman hypothermia dose-response curve was also investigated. In order to keep the mortality to a minimum (Table 2), mice were injected repeatedly with soman (70 μg/kg, SC) as described in the Method section. This particular injection schedule resulted in extensive inhibition of brain acetylcholinesterase (Table 2). Two days after the 5th soman injection, mice were administered an additional dose of soman (either 40 or 60 μg/kg, SC) and the core temperature monitored. The doses of soman (40 and 60 μg/kg, SC) were on the lower portion of the soman hypothermia dose-response curve (in naive mice) and would thus be more sensitive in demonstrating a shift in the soman hypothermia dose-response curve. Soman pretreatment (5 injections of 70 μg/kg dose) did not appear to shift significantly the soman-induced hypothermia dose-response curve. The mean minimum temperature data for the 40 and 60 μg/kg doses of soman in control and following repeated administration of soman were as follows: Control, $40=35.62\pm0.5$ (N = 7), $60=32.30\pm3.09$; after repeated administration, $40=35.63\pm0.30$, $60=30.16\pm1.2$. The AUC data for these groups were as follows: Control, $40=27837\pm397$, $60=26397\pm1227$; after repeated administration, $40=27470\pm260$, $60=26140\pm660$.

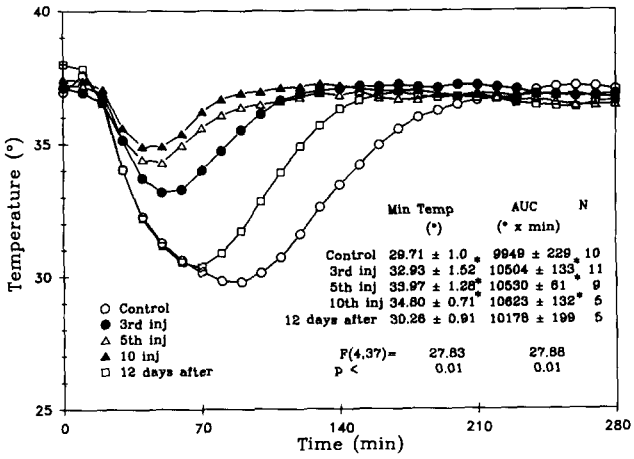

FIG. 1. Effect of repeated administration of soman (70 μg/kg) on the development of tolerance to oxotremorine-induced hypothermia. In separate experiments, oxotremorine (156 μg/kg, IP) was administered 2 days after the 3rd, 5th and 10th injections of soman (70 μg/kg, SC). To determine the disappearance of the cross-tolerance to oxotremorine, 12 days after the 10th injection of soman, oxotremorine (156 μg/kg, IP) was injected. Each point represents the mean of 7–10 observations. Values in the table are the mean ± SD. The asterisk (*) indicates that the value was significantly different ($p<0.05$) from the control group.

Repeated Administration of DFP

In experiments similar to those for soman, another organophosphorus anticholinesterase, DFP, was administered repeatedly to mice. There was no mortality after the first injection of DFP and 58.3% mortality after the second injection, with no further mortality following the additional doses of DFP. The results following repeated administration of DFP were similar to those following repeated administration of soman, namely, there was no tolerance development to the organophosphate-induced hypothermia (Table 4), but there did appear to be cross-tolerance to oxotremorine-induced (625 μg/kg) hypothermia. The oxotremorine-induced hypothermia measured 2 days after the 5th injection of DFP was reduced significantly when compared to the control [mean minimum temperature: 30.13 ± 1.59 (N = 5) vs. 28.78 ± 1.04 (N = 24), $p<0.05$; AUC, 9968 ± 214 vs. 9553 ± 307, $p<0.01$, respectively].

TABLE 4

REPEATED ADMINISTRATION OF DFP: MEAN MINIMUM TEMPERATURE AND AUC*

Injection Number	Min. Temperature (°C)	AUC (°C × min)
1	30.66 ± 1.62†	24498 ± 1027
2	29.56 ± 1.39	24629 ± 1127
3	29.54 ± 1.15	25090 ± 615
4	29.85 ± 0.88	25586 ± 408
5	29.80 ± 1.33	25569 ± 596
ANOVA	$F(4,20)=0.62$ NS	$F(4,20)=1.97$ NS

*Mice received 5 injections of DFP (1.0 mg/kg, SC).

†Mean ± SD. N = 5.

DISCUSSION

Based on the pharmacology, anticholinesterase-induced hypothermia is presumed to be cholinergic in nature. The increased concentrations of acetylcholine (41,47) may activate muscarinic receptors in the anterior hypothalamus (29) leading to a lowering of the set point for heat release (35). The tolerance to the repeated administration of sublethal doses of soman took greater than 3 injections to occur, and the degree and development of tolerance did not appear to be as great as the cross-tolerance to oxotremorine. This result suggests that perhaps, in the case of soman-induced hypothermia, there is an additional component which contributes to the hypothermia. The neurotransmitter acetylcholine will activate muscarinic and nicotinic receptors. Since the hypothalamus is rich in nicotinic receptors (14), perhaps the nicotonic receptors play a role in soman-induced hypothermia. Cross-tolerance to nicotinic agonists, either carbachol (36) or nicotine (11), was not apparent after soman poisoning. If there was no downregulation of the nicotinic receptors, then there would not be as great a decrease in the soman-induced hypothermia, as the nicotinic component would still be contributing to the overall effect. However, this argument is incompatible with other information from this laboratory which indicates that the soman-induced hypothermia is primarily a muscarinic-receptor-related event as muscarinic antagonists, such as atropine, are effective in antagonizing, although not completely, soman hypothermia, whereas nicotinic antagonists, such as mecamylamine, are not. Thus the apparently smaller degree of tolerance to soman-induced hypothermia could be due to the fact that the dose of soman is near the maximum following repeated administration or else that there is some other component involved in organophosphate-induced hypothermia. The latter is not without precedence, as monoamines have been implicated in soman poisoning (15).

Other investigators have demonstrated tolerance to the organophosphate-induced hypothermia following repeated administration. The number of injections required before tolerance became evident varied from as few as 1 (37), to 5 (25) and as many as 10 or more (29,43) for DFP or 7 for soman (39). Also, there appeared to be differences in the degrees of tolerance between the various studies. Tolerance to anticholinesterase-induced hypothermia developed in the rat (25, 30, 37–39) but not in the mouse (12,43). There also appears to be strain differences. The DBA (12,43) mouse strain did not develop tolerance, whereas the C_3H (12) and the CD-1 (this study) strains did develop tolerance to anticholinesterase-induced hypothermia. From an examination of the details of the various studies, perhaps the major factor in the differences observed among the various studies is the species and, in the case of mice, the strain used.

The degree of tolerance could also be moderated by factors unrelated to synaptic events but due to dispositional factors. Plasma carboxylesterase has been shown to be an important detoxification route for organophosphates such as soman (8,34). Anticholinesterase administration was reported to stimulate protein synthesis (7, 18, 19). Upon repeated administration of the organophosphate, the synthesis of carboxylesterase may be stimulated. The concentration of plasma carboxylesterase could be increased to a degree such that following administration of the organophosphate anticholinesterase increasing amounts would be bound to the carboxylesterase and, as a result, the characteristic signs of poisoning and hypothermia would decrease in intensity. Following repeated administration of soman (100 μg/kg, SC), there was a slight decrease in the inhibition of plasma carboxylesterase (Table 2) over the duration of the experiment which would suggest that there may be an increase in the concentration of plasma carboxylesterase over that which was present at the initiation of the experiment. However, in this study, it was not enough to alter significantly the inhibition of the acetylcholinesterase in the various brain regions or to affect the anticholinesterase-induced hypothermia. Similar results were found following repeated administration of DFP (24) and soman (39). Perhaps the rate of recovery and even the isoenzyme pattern of carboxylesterase in the plasma of rats and mice might be different and contribute to the species difference in response to repeated organophosphate administration discussed above.

The results of the present study show that, following repeated administration of either soman or DFP, cross-tolerance developed to oxotremorine-induced hypothermia. There are a number of possibilities to account for this cross-tolerance to the oxotremorine. The decrease in the oxotremorine response may be metabolic in nature and could be explained if soman altered the metabolism of oxotremorine. However, oxotremorine-induced hypothermia does not appear to be influenced by the activity of hepatic mixed function oxidases [this study; (26)], and repeated organophosphate exposure does not appear to affect the activity of the hepatic mixed function oxidases, making this argument untenable. In survivors of soman poisoning, a distinct neuropathology was evident, characterized by the loss of neuronal tissue in certain areas of the brain (31,32). The cross-tolerance to oxotremorine could be due to the loss of muscarinic-receptor-containing neurons which participate in thermoregulation. However, in order for this neuropathology to become evident following soman administration, the animals must display sustained convulsive activity. The animals in this study exhibited tremors but not overt convulsions. Also, soman (130 μg/kg, SC) failed to produce the characteristic neuropathology in mice [(J. G. Clement, unpublished observations)] viewed by others (31,32) in rats. Thus it is unlikely that the loss of muscarinic-receptor-containing neurons could account for the cross-tolerance to oxotremorine-induced hypothermia.

Tolerance to the symptoms following repeated administration of organophosphate anticholinesterases is frequently paralleled by downregulation of muscarinic receptors. Using receptor binding studies, numerous laboratories have consistently demonstrated a decrease in the number, but not affinity, of muscarinic receptors in various brain regions, except the hypothalamus, after repeated administration of either soman (5) or DFP (4, 33, 43). Similarly, the physiological response (hypothermia) following administration of a cholinergic agonist such as pilocarpine or carbachol was reduced following subchronic administration of DFP (37). Thus it was assumed that the two events, i.e., receptor downregulation and the decreased response to the cholinergic agonist, were directly related. The anatomical site of the downregulation should be the hypothalamus, based on the proposed site of action of oxotremorine (29). However, it has been reported that there was no change in the muscarinic receptor number in the hypothalamus following repeated administration of soman or DFP (4,5). Similarly, others have reported that there is a poor correlation between receptor binding in the hypothalamus and the development of cross-tolerance (12,43). It may be that the radioligand used, QNB, labeled all receptors and not just the functional receptors (3,22). Desensitization, following exposure to a cholinergic agonist or an anticholinesterase inhibitor, has been associated with the loss of N-methyl scopolamine (NMS) binding sites (6, 30, 22). This downregulation of NMS binding sites was associated with a selective loss of the agonist low-affinity and pirenzepine high-affinity binding sites (6). These NMS binding sites could represent the receptor population in-

volved in the oxotremorine-induced hypothermia. The cross-tolerance to the oxotremorine-induced hypothermia may be indicative of the functionality of the receptor coupling, i.e., downregulation (of the functional receptors), a reduction in the intracellular signal transduction, or both. This relationship will be investigated in a separate study.

REFERENCES

1. Aronstam, R. S.; Smith, M. D.; Buccafusco, J. L. Clonidine prevents the short-term down regulation of muscarinic receptors in the mouse brain induced by the acetylcholinesterase inhibitor soman. Neurosci. Lett. 78:107–112; 1987.
2. Baumgold, J.; Cooperman, B. B.; White, T. M. Relationship between desensitization and sequestration of muscarinic receptors in two neuronal cell lines. Neuropharmacology 28:1253–1261; 1989.
3. Brown, J. H.; Goldstein, D. Analysis of cardiac muscarinic receptors recognized selectively by nonquaternary but not quaternary ligands. J. Pharmacol. Exp. Ther. 238:580–586; 1986.
4. Churchill, L.; Pazdernik, T. L.; Samson, F.; Nelson, S. R. Topographical distribution of down-regulated muscarinic receptors in rat brains after repeated exposure to diisopropylphosphorofluoridate. Neuroscience 11:463–472; 1984.
5. Churchill, L.; Pazdernik, T. L.; Jackson, J. L.; Nelson, S. R.; Samson, F. E.; McDonough, J. H. Topographical distribution of decrements and brain recovery in muscarinic receptors from rat brains repeatedly exposed to sublethal doses of soman. J. Neurosci. 4:2069–2079; 1984.
6. Cioffi, C. L.; El-Fakahany, E. E. Short-term desensitization of muscarinic cholinergic receptors in mouse neuroblastoma cells: Selective loss of agonist low-affinity and pirenzepine high-affinity binding sites. J. Pharmacol. Exp. Ther. 238:916–923; 1986.
7. Cisson, C. M.; Wilson, B. W. Paraoxon increases the rate of synthesis of acetylcholinesterase in cultured muscle. Toxicol. Lett. 9:131–135; 1981.
8. Clement, J. G. Role of aliesterase in organophosphate poisoning. Fund. Appl. Toxicol. 4:s96–s105; 1984.
9. Clement, J. G. Hormonal consequences of organophosphate poisoning. Fundam. Appl. Toxicol. 5:s61–s77; 1985.
10. Clement, J. G.; Mills, P.; Brockway, B. Use of telemetry to record body temperature and activity in mice. J. Pharmacol. Methods 21: 129–140; 1989.
11. Clement, J. G. Pharmacological nature of soman-induced hypothermia. In preparation.
12. Collins, A. C.; Smolen, T. N.; Smolen, A.; Medhurst, L. J. Cross-tolerance between muscarinic agonists: Role of muscarinic receptors. Pharmacol. Biochem. Behav. 26:173–182; 1987.
13. Costa, L. G.; Schwab, B. W.; Murphy, S. D. Tolerance to anticholinesterase compounds in mammals. Toxicology 25:79–97; 1982.
14. Costa, L. G.; Murphy, S. D. [^{3}H]Nicotine binding in rat brain: Alteration after chronic acetylcholinesterase inhibition. J. Pharmacol. Exp. Ther. 226:392–397; 1983.
15. Coudray-Lucas, C.; Prioux-Guyonneau, M.; Sentenac, H.; Cohen, Y.; Wepierre, J. Brain catecholamine metabolism changes and hypothermia in intoxication by anticholinesterase agents. Acta Pharmacol. Toxicol. 52:224–229; 1983.
16. Coult, D. B.; Marsh, D. J.; Read, G. Dealkylation studies on inhibited acetylcholinesterase. Biochem. J. 98:869–873; 1966.
17. Dilsaver, S. C.; Alessi, N. E. Temperature as a variable in the study of cholinergic mechanisms. Prog. Neuropsychopharmacol. Biol. Psychiatry 12:1–32; 1988.
18. Domschke, W.; Domagk, G. F.; Domschke, S.; Erdmann, W. D. Inhibition of soman-induced enzyme biosynthesis in rat liver by ethionine. Arch. Toxicol. 26:142–148; 1970.
19. Domschke, W.; Domagk, G. F.; Domschke, S.; Erdmann, W. D. Effects of soman and diisopropylfluorophosphate upon the enzyme biosynthesis of the rat liver. Arch. Toxicol. 26:76–83; 1970.
20. Ehlert, F. J.; Kokka, N.; Fairhurst, A. S. Altered ^{3}[H]quinuclidinyl benzoate binding in the striatum of rats following chronic administration with diisopropylfluorophosphate. Mol. Pharmacol. 17:24–30; 1980.
21. Feigenbaum, P.; El-Fakahany, E. E. Short-term regulation of muscarinic acetylcholine receptor binding in cultured nerve cells. Res. Commun. Chem. Pathol. Pharmacol. 43:519–522; 1984.
22. Feigenbaum, P.; El-Fakahany, E. E. Regulation of muscarinic cholinergic receptor density in neuroblastoma cells by brief exposure to agonist: Possible involvement in desensitization of receptor function. J. Pharmacol. Exp. Ther. 233:134–140; 1985.
23. Ferguson, G. A. Statistics in psychology and education. 3rd ed. New York: McGraw-Hill; 1971:270.
24. Gupta, R. C.; Patterson, G. T.; Dettbarn, W-D. Mechanisms involved in the development of tolerance to DFP toxicity. Fundam. Appl. Toxicol. 5:s17–s28; 1985.
25. Gupta, R. C.; Dettbarn, W-D. Role of uptake of [^{14}C]Valine into protein in the development of tolerance to diisopropylphosphorofluoridate (DFP) toxicity. Toxicol. Appl. Pharmacol. 84:551–560; 1986.
26. Hammer, W.; Karlen, B.; Rane, A.; Sjoqvist, F. Rate of metabolism of tremorine and oxotremorine in rats and mice. Life Sci. 7:197–204; 1968.
27. Harris, L. W.; Yamamura, H. I.; Fleisher, J. H. De novo synthesis of acetylcholinesterase in guinea pig retina after inhibition by pinacolyl methylphosphonofluoridate. Biochem. Pharmacol. 20:2927–2930; 1971.
28. Ho, I. K.; Hoskins, B. Variation of the commercial diisopropyl fluorophosphate preparations in toxicological studies. Drug Chem. Toxicol. 6:421–427; 1983.
29. Lomax, P.; Jenden, D. J. Hypothermia following systematic and intracerebral injection of oxotremorine in the rat. Int. J. Neuropharmacol. 5:353–359; 1966.
30. Lomax, P.; Kokka, N.; Lee, R. J. Acetylcholinesterase inhibitors and thermoregulation. Homeostasis and thermal stress. Experimental and therapeutic advances. Cooper, K.; Lomax, P.; Schonbaum, E.; Veale, W., eds. Basel: Karger; 1986:108–112.
31. McDonough, J. H.; Jaax, N. K.; Crowley, R. A.; Mays, M. Z.; Modrow, H. E. Atropine and/or diazepam therapy protects against soman-induced pathology. Fundam. Appl. Toxicol. 13:256–276; 1989.
32. McLeod, C. G. Pathology of nerve agents: Perspectives on medical management. Fundam. Appl. Toxicol. 5:s10–s16; 1985.
33. Marks, M. J.; Artman, L. D.; Patinkin, D. M.; Collins, A. C. Cholinergic adaptations to chronic oxotremorine infusion. J. Pharmacol. Exp. Ther. 218:337–343; 1981.
34. Maxwell, D. M.; Brecht, K. M.; O'Neill, B. L. The effect of carboxylesterase inhibition on interspecies differences in soman toxicity. Toxicol. Lett. 39:35–42; 1987.
35. Meeter, E.; Wolthuis, O. L.; Van Benthem, R. M. J. The anticholinesterase hypothermia in the rat: Its practical application in the study of the central effectiveness of oximes. Bull. WHO 44:251–257; 1971.
36. Meeter, E. Investigation of the rapid recovery of rat thermoregulation from soman poisoning. Eur. J. Pharmacol. 24:105–107; 1973.
37. Overstreet, D. H.; Kozar, M. D.; Lynch, G. S. Reduced hypothermic effects of cholinomimetic agents following chronic anticholinesterase treatment. Neuropharmacology 12:1017–1032; 1973.
38. Overstreet, D. H.; Schiller, G. D.; Day, T. A. Failure of cycloheximide to alter the rate of recovery of temperature following acute DFP treatment. Eur. J. Pharmacol. 44:187–190; 1977.
39. Russell, R. W.; Booth, R. A.; Lauretz, S. D.; Smith, C. A.; Jenden, D. J. Behavioral, neurochemical and physiological effects of repeated exposures to subsymptomatic levels of the anticholinesterase, soman. Neurobehav. Toxicol. Teratol. 8:675–685; 1986.
40. Schiller, G. D. Reduced binding of 3[H]quinuclidinyl benzilate associated with chronically low acetylcholinesterase activity. Life Sci. 24:1159–1164; 1979.
41. Shih, T-M. Time course of the effects of soman on acetylcholine and choline levels in six discrete areas of the rat brain. Psychopharmacology (Berlin) 78:170–175; 1982.
42. Siakotos, A. N.; Filbert, M.; Hester, R. A specific radioisotopic assay for acetylcholinesterase and pseudocholinesterase in brain and plasma. Biomed. Med. 3:1–12; 1969.
43. Smolen, T. N.; Smolen, A.; Collins, A. C. Dissociation of the de-

creased numbers of muscarinic receptors from tolerance to DFP. Pharmacol. Biochem. Behav. 25:1293–1301; 1986.
44. Uchida, S.; Takeyasu, K.; Matsuda, T.; Yoshida, H. Changes in muscarinic acetylcholine receptors of mice by chronic administration of diisopropylfluorophosphate and papaverine. Life Sci. 24: 1805–1812; 1979.
45. Yamada, S.; Isogai, M.; Okudaira, H.; Hayashi, E. Regional adaptation of muscarinic receptors and choline uptake in brain following repeated administration of diisopropylfluorophosphate and atropine. Brain Res. 268:315–320; 1983.
46. Yamada, S.; Isogai, M.; Okudaira, H.; Hayashi, E. Correlation between cholinesterase inhibition and reduction in muscarinic receptors and choline uptake by repeated diisopropylfluorophosphate administration: antagonism by physostigmine and atropine. J. Pharmacol. Exp. Ther. 226:519–525; 1983.
47. Zhang, X.; Qin, B-Y. Relationship between cholinesterase activity and acetylcholine of the brains of mice acutely intoxicated with soman. Acta Pharmacol. Sin. 6:16–19, 1985.