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Pharmacological Nature of Soman-Induced Hypothermia in Mice

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CLEMENT, J. G. *Pharmacological nature of soman-induced hypothermia in mice.* PHARMACOL BIOCHEM BEHAV 44(3) 689–702, 1993. – The object of the study was to determine the pharmacological nature of pinacolyl methylphosphonofluoridate (soman)-induced hypothermia in mice. This was accomplished by examining the soman hypothermia dose response and the effect of various pharmacological antagonists in comparison to the hypothermia responses of muscarinic and nicotinic cholinergic agonists such as oxotremorine and nicotine and another anticholinesterase, physostigmine. Core temperature in mice was monitored by telemetry. In general, atropine antagonized oxotremorine, physostigmine, and soman hypothermia but not nicotine hypothermia whereas mecamylamine antagonized nicotine hypothermia but not that produced by the other agonists. Soman hypothermia was not affected significantly by various pharmacological antagonists, suggesting that other neurotransmitters were not involved in the expression of soman hypothermia. Soman hypothermia appears to be due to muscarinic receptor stimulation and can be effectively antagonized, but not completely, by the use of atropine. Acetylcholinesterase oxime reactivators, such as HI-6 and toxogonin, were ineffective in antagonizing soman-induced hypothermia and reactivating hypothalamic acetylcholinesterase, whereas HI-6 was effective in reactivating soman-inhibited diaphragm acetylcholinesterase when administered up to 10 min after soman, indicating that aging of the soman-inhibited acetylcholinesterase had not occurred. Soman hypothermia appears to be primarily a muscarinic receptor-related event.

Soman Organophosphate Anticholinesterase Reactivation Acetylcholinesterase inhibition Oxime HI-6 Toxogonin Oxotremorine Nicotine Physostigmine Thermoregulation Telemetry

FOLLOWING administration of a sublethal dose of an organophosphate anticholinesterase, such as diisopropylphosphonofluoridate (DFP) or pinacolyl methylphosphonofluoridate (soman) to rats, a severe, transient hypothermia developed (15,26–28) that disappeared within 24 h. A similar syndrome has also been reported following soman administration to mice (5–7). Meeter et al. (29) concluded that organophosphate-induced hypothermia was the result of excitation of cholinergic synapses in the anterior hypothalamus that led to a) a lowering of the set point for heat release of the hypothalamic thermostat and b) a reduced heat production, probably caused by a decrease in metabolism in the liver.

In vivo, the primary toxic action of an organophosphate anticholinesterases, such as soman, is phosphorylation of the enzyme acetylcholinesterase, which results in an increase in the synaptic concentration of acetylcholine and leads to overstimulation of the postsynaptic receptors. Postsynaptic cholinergic receptor stimulation by acetylcholine is characterized pharmacologically as either muscarinic or nicotinic in nature. The purpose of this investigation was to examine the pharmacological nature of soman-induced hypothermia in mice and compare it to that of muscarinic and nicotinic cholinergic agonists such as oxotremorine and nicotine, respectively, and a reversible carbamate acetylcholinesterase inhibitor, physostigmine.

METHOD

Animals

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

Recording of Core Temperature

Core temperature was monitored using a telemetry system (Data Sciences, Inc., Roseville, MN) as described in Clement et al. (10). Mice were anesthetized with sodium pentobarbital (75 mg/kg, IP). An abdominal incision was made and the telemetry transmitter was implanted into the peritoneal cavity. The abdominal muscle incision was closed using sutures (000 plain gut) and sprinkled with an antibiotic powder. The skin incision was closed using wound clips (9-mm Michel clips). The mouse was then allowed to recover for at least 1 week (10) prior to use in an experimental situation.

In experiments using physostigmine, oxotremorine, nicotine, or soman, core temperature was monitored at 5-, 10-, 10-, and 30-min intervals, respectively. The first three data

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points established the control core temperature; then, the various drugs were administered. The entire observation period (200 min for physostigmine, 280 min for oxotremorine, 240 min for nicotine, and 720 min for soman) was used in determination of the area under the curve (AUC) and the mean minimum temperature.

The data were collected and the mean minimum temperature and AUC calculated (10). When examining the data from the table and the temperature time course curve, the mean minimum temperature may appear to be different. The minimum temperature reported in the table or plotted in the mean minimum temperature graph, for example, Fig. 1B, is the mean of the lowest temperature 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 temperature time course graphs (e.g., Fig. 1A) are the mean core temperatures at a particular time. If individual animals reach the minimum temperature at different times, then this is reflected in the apparent differences in the values between the time course graph and the mean minimum temperature curve.

Acetylcholinesterase Activity

Mice were sacrificed by decapitation and exsanguination. The tissue (hypothalamus or diaphragm) was removed and rinsed in 0.9% saline, blotted dry on filter paper, and weighed. The tissue was homogenized (10–20 strokes in a glass-Teflon homogenizer for brain or using a Polytron for diaphragm) in a buffer (4°C) containing 1 M NaCl, 0.05 M MgCl2, 0.01 M Tris, and 1% Triton X-100, pH 7.4. The tissue concentration of the final homogenate was 10 or 100 mg wet weight tissue/ml buffer for the hypothalamus or diaphragm, respectively. The homogenates were then centrifuged at 20,000× *g* for 20 min at 4°C. Fresh, unfrozen tissue was used in all experiments. Acetylcholinesterase activity was determined, at room temperature, in a microplate assay using the method of Ellman et al. (19). Each fraction contained

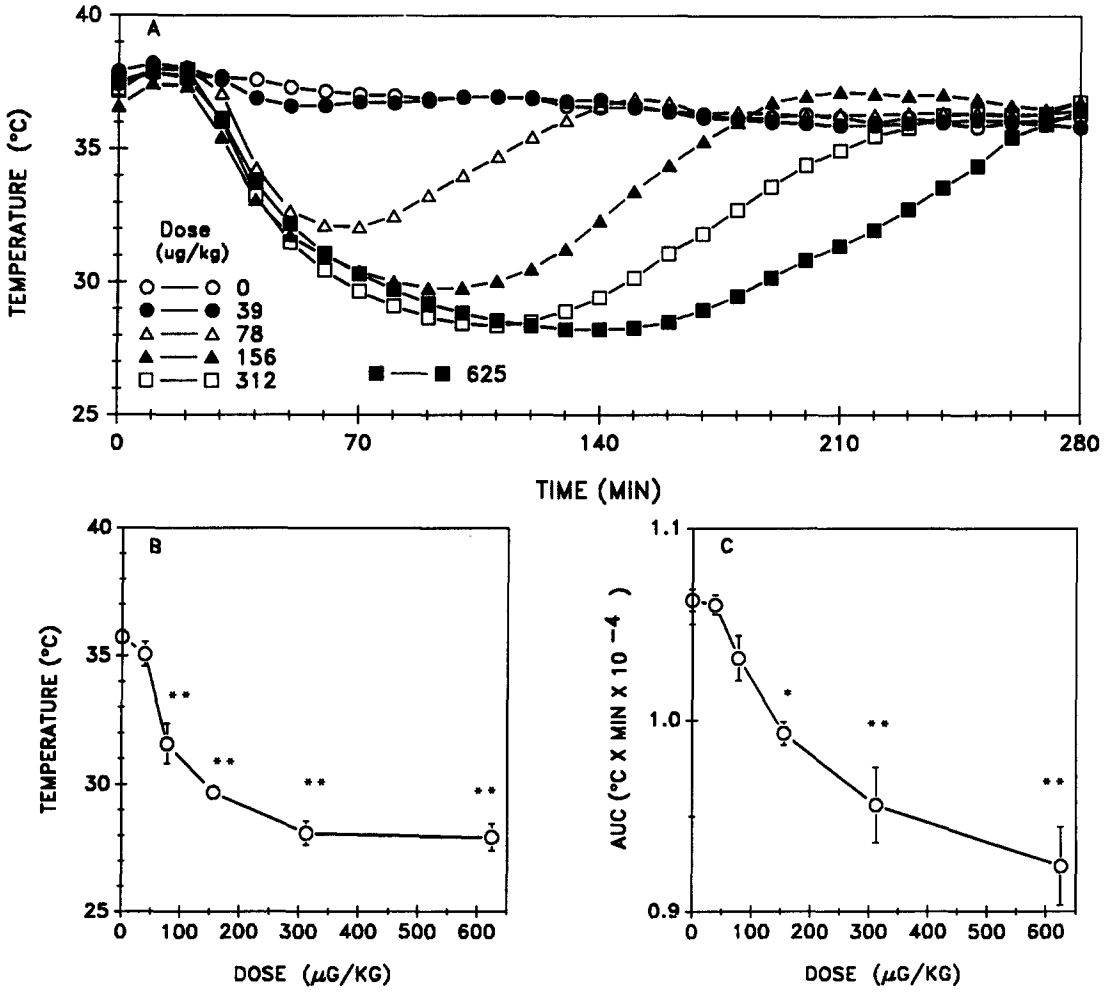

FIG. 1. (A). Temporal response of various doses of oxotremorine on core temperature. Mice were administered various doses of oxotremorine (μg/kg, IP) immediately after the collection of the third data point and the core temperature monitored. $n = 4$. For clarity, the error bars were omitted from this figure and others to follow. (B) Mean minimum temperature and (C) area under the curve (AUC) were determined from the data in Fig. 1a. Each point represents the mean ± SE. If the error bar is absent, it is because the SE was small and did not appear outside the symbol. *The value was significantly different (*$p < 0.05$; **$p < 0.01$) from the saline-injected (0) control.

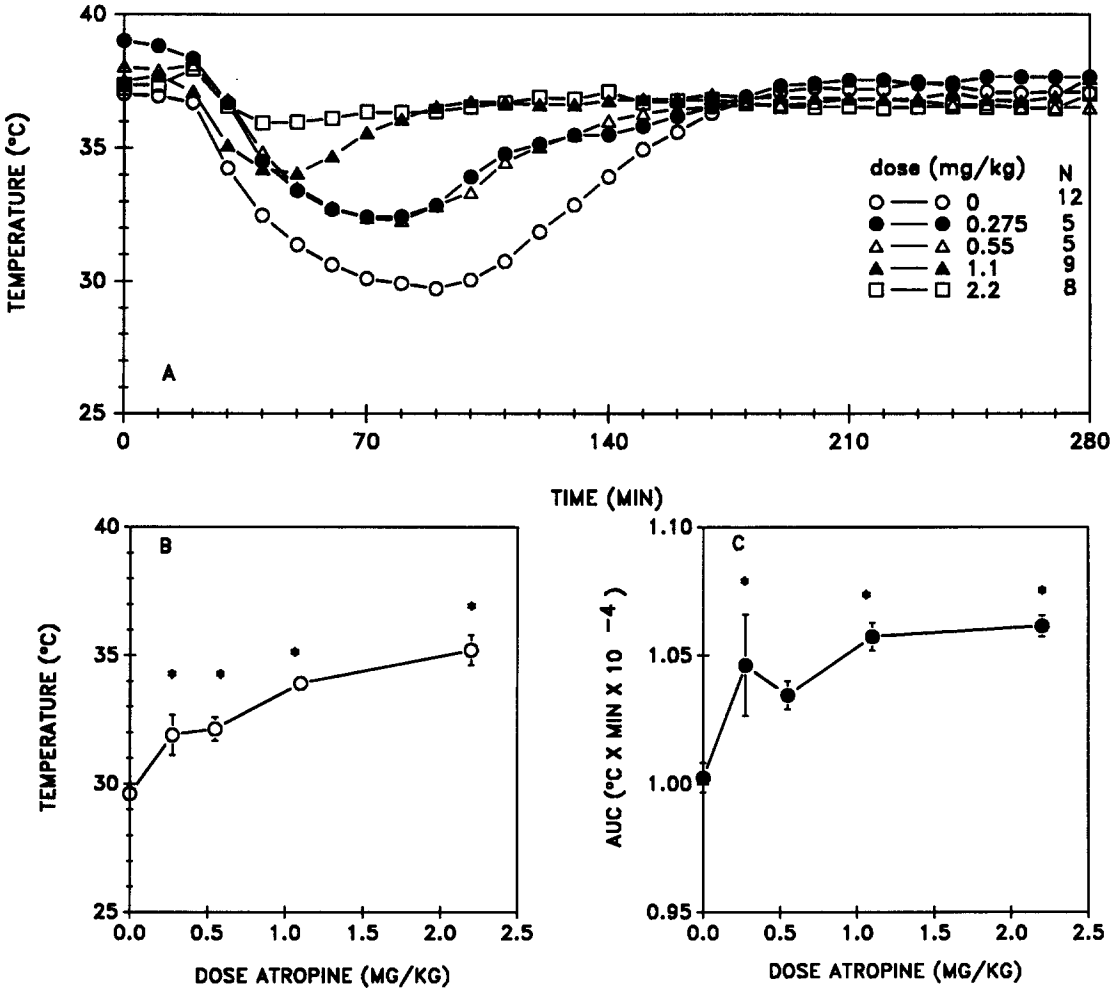

FIG. 2. (A). Atropine antagonism of oxotremorine-induced hypothermia. Mice were injected with various doses of atropine (mg/kg, IP) 5 min before oxotremorine 156 μg/kg, IP. N, number of observations. (B) Mean minimum temperature and (C) area under the curve (AUC) were determined from the atropine data in Fig. 2a. Each point represents the mean ± SE. If the error bar is absent, it is because the SE was small and did not appear outside the symbol. *The value was significantly different ($p < 0.05$) from the saline-injected (0) control.

ISO-OMPA (10 μM) to selectively inhibit pseudocholinesterase activity.

Data Analysis

The data were analyzed by one-way analysis of variance (ANOVA). If a significant overall effect was found, the group means were compared using the Scheffe multiple-comparison test (20). A p value of < 0.05 was considered statistically significant.

Materials

Soman, HI-6, and toxogonin, prepared at the Defence Research Establishment Suffield, was greater than 98% pure. The following drugs were obtained from various commercial sources: oxotremorine (Aldrich Chemical Co., Milwaukee, WI); physostigmine hemisulfate, nicotine hydrogen bitartrate, aminophylline, haloperidol HCl, and mecamylamine HCl (Sigma Chemical Co., St. Louis, MO); atropine sulfate (BDH Pharmaceuticals Ltd., Toronto, Ontario); naloxone HCl (Endo Laboratories, Garden City, NY); atropine methylnitrate (McFarlan, Smith Ltd.); prazosin HCl (Pfizer Co., Ltd., New York, NY); yohimbine (Research Biochemicals, Inc., Natick, MA); and methysergide (Sandoz, Inc., East Hanover, NJ). The drugs were dissolved in saline with the following exceptions: Prazosin and yohimbine were dissolved in distilled water with stirring and gentle heating; haloperidol was dissolved in 0.3% tartaric acid w/v. The volume of injection was 1% body weight in all cases. The weight of the telemetry transmitter was tared so that the mouse was injected with the dose based upon tissue weight of the animal.

RESULTS

Oxotremorine

The effect of various doses (39–625 μg/kg) of oxotremorine, a centrally active muscarinic receptor agonist, on core temperature was investigated. Depending upon the dose, hypothermia reached a maximum within 40–120 min following

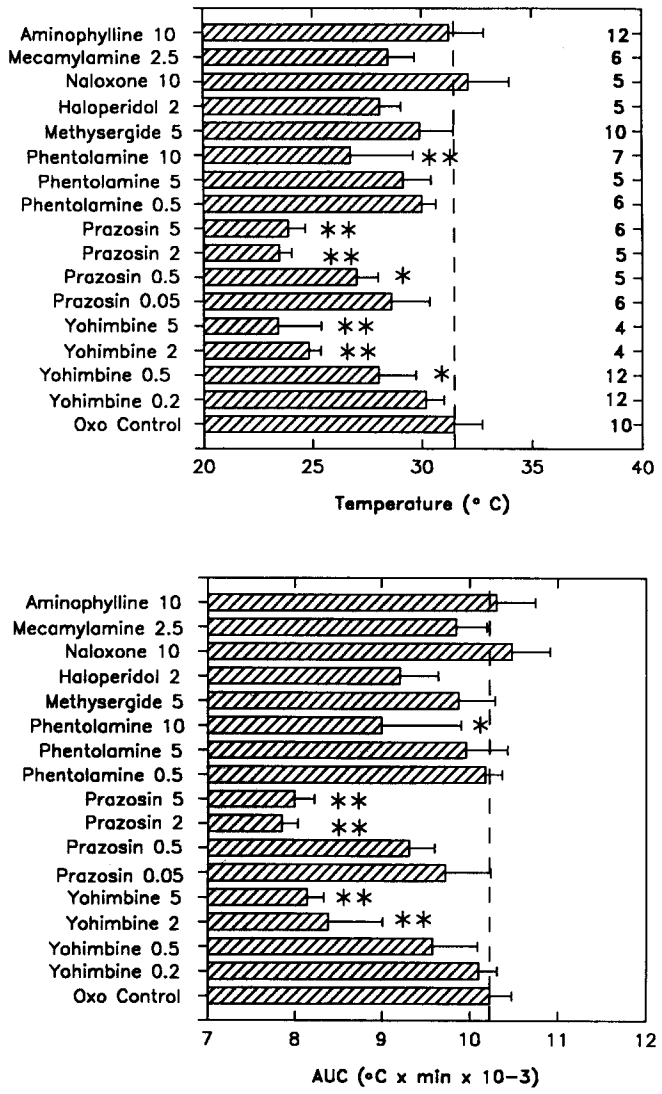

FIG. 3. Effect of various antagonists on oxotremorine-induced hypothermia. (A). Mean minimum temperature. (B) Area under the curve (AUC). The various antagonists were administered IP 5 min prior to oxotremorine (156 μg/kg, SC). The number following the name of the drug represents the dose in mg/kg, while the numbers on the right side of the figure indicate the number of observations. Values are the mean ± SD. Significantly different from oxotremorine control (*$p < 0.05$, **$p < 0.01$).

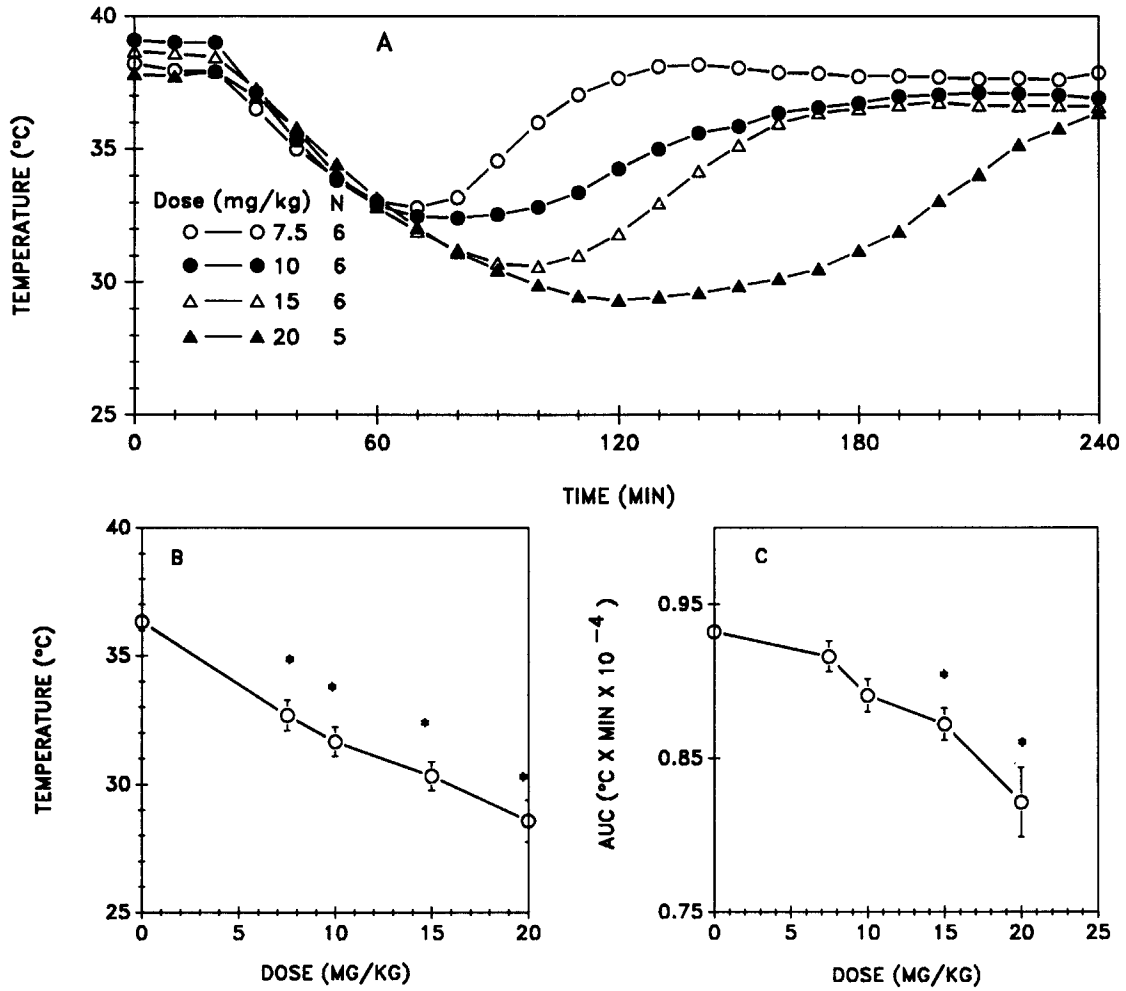

FIG. 4. (A). Temporal response of various doses of nicotine on core temperature. Mice were injected with various doses of nicotine (mg/kg, SC) immediately after the collection of the third data point and core temperature monitored. N, number of observations. (B). Mean minimum temperature and (C) area under the curve (AUC) were determined from the data in A. Each point represents the mean ± SE. If the error bar is absent, it is because the SE was small and did not appear outside the symbol. *The value was significantly different ($p < 0.05$) from the saline-injected (0) control.

oxotremorine administration and recovered to control temperature within 120–240 min following administration (Fig. 1A). Oxotremorine was a potent agonist with doses in the range of 0.19–3.03 μmol/kg evoking a hypothermic response. There was a definite dose-response relationship with regard to the mean minimum temperature [Fig. 1B; $F(5, 18) = 47.75$, $p < 0.01$] and AUC [Fig. 1C; $F(5, 18) = 18.72$, $p < 0.01$] following oxotremorine administration.

From the oxotremorine dose-response study, a dose of 156 μg/kg (0.76 μmol/kg), a submaximal dose of oxotremorine on the linear portion of the dose-response curve, was chosen as the standard oxotremorine dose for the antagonism studies. Oxotremorine hypothermia was antagonized effectively by the muscarinic receptor antagonist atropine (Figs. 2A–C) in a dose-related fashion [minimum temperature, $F(4, 34) = 28.83$, $p < 0.01$; AUC, $F(3, 34) = 12.41$, $p < 0.01$]. The effect of various pharmacological antagonists on oxotremorine hypothermia were also evaluated [Fig. 3; minimum temperature, $F(16, 103) = 22.06$, $p < 0.01$; AUC, $F(16, 103) = 23.06$, $p < 0.01$]. The nicotinic receptor antagonist mecamylamine (Fig. 3) did not antagonize the oxotremorine-induced hypothermia; in fact, it appeared to increase the oxotremorine hypothermia. The various α-adrenergic blockers—phentolamine, prazosin, and yohimbine—increased significantly, in a dose-related manner, oxotremorine hypothermia. Neither naloxone, methysergide, aminophylline, nor haloperidol had any significant effect on oxotremorine hypothermia.

Nicotine

Nicotine is a centrally active agonist of the nicotinic cholinergic receptor. The effect of various doses (7.5–20 mg/kg) of nicotine on core temperature in mice was investigated (Fig. 4). Depending upon the dose of nicotine, maximum hypothermia was reached within 50–100 min and returned to control levels within 80–220 min following administration. The mean minimum temperature [Fig. 4B; $F(4, 30) = 48.35$, $p < 0.01$] and AUC [Fig. 4C; $F(4, 30) = 17.43$, $p < 0.01$] data for nicotine demonstrated a definite dose-response relationship in the range of 16.25–43.4 μmol/kg, which was much less potent than the muscarinic receptor agonist oxotremorine. Based upon this data, a dose of nicotine of 15 mg/kg was chosen as being submaximal and on the linear portion of the nicotine dose-response curve for use in further experiments examining the effect of antagonists on nicotine-induced hypothermia. Nicotine hypothermia was antagonized by the centrally active nicotinic receptor antagonist mecamylamine ($p < 0.01$) but not the muscarinic receptor antagonist atropine (Fig. 5; $p > 0.05$).

Soman

Various sublethal doses of soman (40–110 μg/kg, SC) were administered to mice and the temporal effect on core temperature monitored (Fig. 6A). Depending upon the dose of soman, the maximum hypothermia was seen within 2–4 h following administration. Core temperature recovered toward control values over the next 8–10 h, and 24 h after soman poisoning the core temperature had completely recovered to control levels (data not shown). The mean minimum temperature [Fig. 6B; $F(7, 41) = 23.76$, $p < 0.01$] and AUC [Fig. 6C; $F(7, 41) = 21.86$, $p < 0.01$] following administration of various doses of soman demonstrated a definite dose-response relationship. There was no mortality at the doses examined; however, at doses above 60 μg/kg mice typically displayed signs of acetylcholinesterase inhibition, such as tremors and salivation, which appeared to increase in intensity and duration (subjective observation) as the dose of soman increased.

From the dose-response data, soman (70 μg/kg, SC; 0.38 μmol/kg) was chosen as the standard dose for examining the

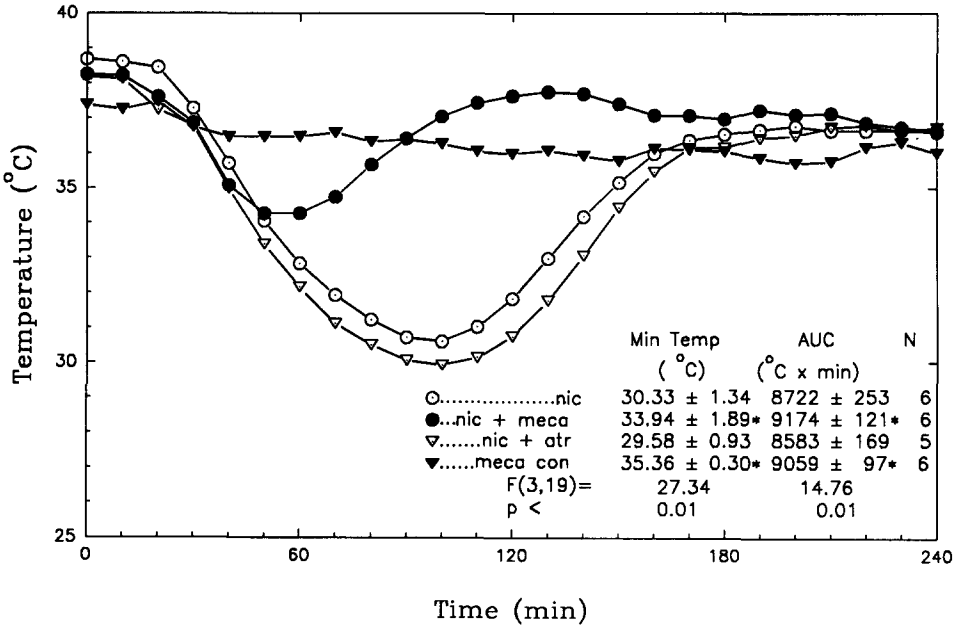

FIG. 5. Antagonism of nicotine-induced hypothermia. The antagonist, either mecamylamine (meca; 2.5 mg/kg, IP) or atropine (atr; 2.2 mg/kg, IP), was administered 5 min prior to injection of nicotine (nic; 15 mg/kg, SC). Mecamylamine (meca-con; 2.5 mg/kg, IP) was administered in the absence of nicotine. *Indicates that the value was significantly different ($p < 0.001$) from the nicotine control.

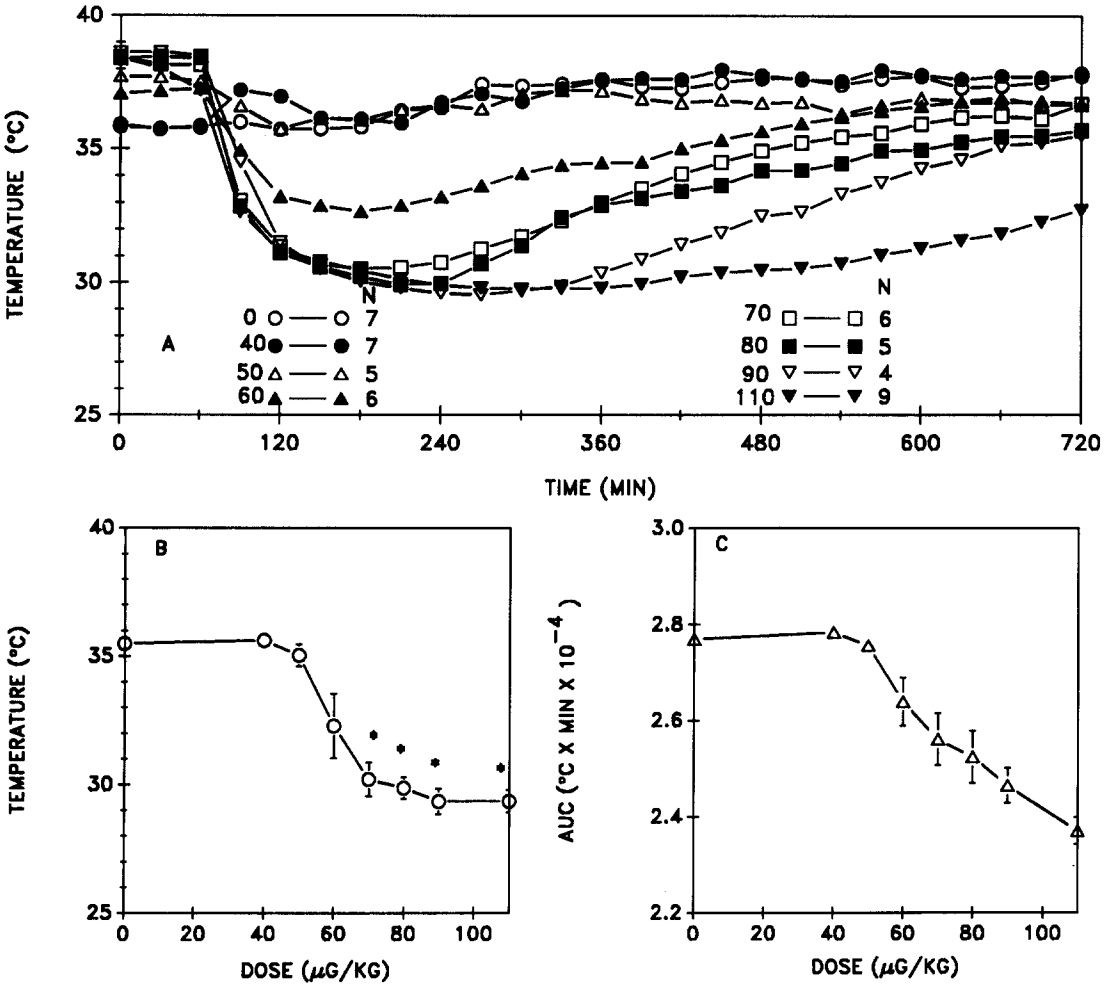

FIG. 6. (A). Temporal response of various doses of soman on core temperature. Mice were administered soman by SC injection immediately after the collection of the third data point at 60 min. The legend on the figure indicates the dose of soman administered expressed in μg/kg and the number of observations (N) for each dose. (B) Mean minimum temperature and (C) area under the curve (AUC) were determined from the data in Fig. 6A. For both the mean minimum temperature and AUC in this figure, each point represents the mean ± SE. If the error bars are absent, it is because the SE was small and did not appear outside the symbol. *The value was significantly different ($p < 0.01$) from the saline-injected (0) control.

effect of various pharmacological antagonists. The basic pharmacology of anticholinesterases suggests that acetylcholine is the probable agent for nerve agent-induced hypothermia. Thus, the effects of classic centrally acting cholinergic antagonists, such as atropine and mecamylamine, on soman-induced hypothermia were examined (Fig. 7). The muscarinic receptor antagonist atropine sulfate administered IP 5 min before was effective in reducing soman-induced hypothermia, whereas the nicotinic receptor antagonist mecamylamine was ineffective.

To determine if there were other neurotransmitters involved in the expression of soman hypothermia, additional experiments were performed examining the effect of a number of pharmacological antagonists on soman (70 μg/kg, SC) hypothermia [Fig. 8; Minimum temperature, $F(15, 137) = 5.70$, $p < 0.01$; AUC, $F(15, 137) = 7.70$, $p < 0.01$]. Methysergide, a serotonin antagonist, appeared to antagonize soman hypothermia in a dose-related fashion; however, the results were not statistically significant. α-Adrenergic antagonists such as yohimbine (0.05–5 mg/kg) and phentolamine (5–10 mg/kg) did not antagonize soman hypothermia, whereas prazosin (2 mg/kg, IP) tended to increase soman hypothermia. A higher dose of prazosin (5 mg/kg, IP) produced equivocal results. Prazosin (5 mg/kg) produced more variable results than other drugs in that there were some animals where soman hypothermia was increased and others where either no effect or antagonism of soman hypothermia was evident. This experiment was repeated two times with the large variability prominent in both studies. The results of both studies were combined. Atropine methylnitrate (5 mg/kg), a peripherally acting muscarinic antagonist, aminophylline (10 mg/kg), a centrally active adenosine antagonist, haloperidol (2 mg/kg), a centrally active dopamine antagonist, and naloxone (10 mg/kg), an endogenous opioid antagonist, did not antagonize signifi-

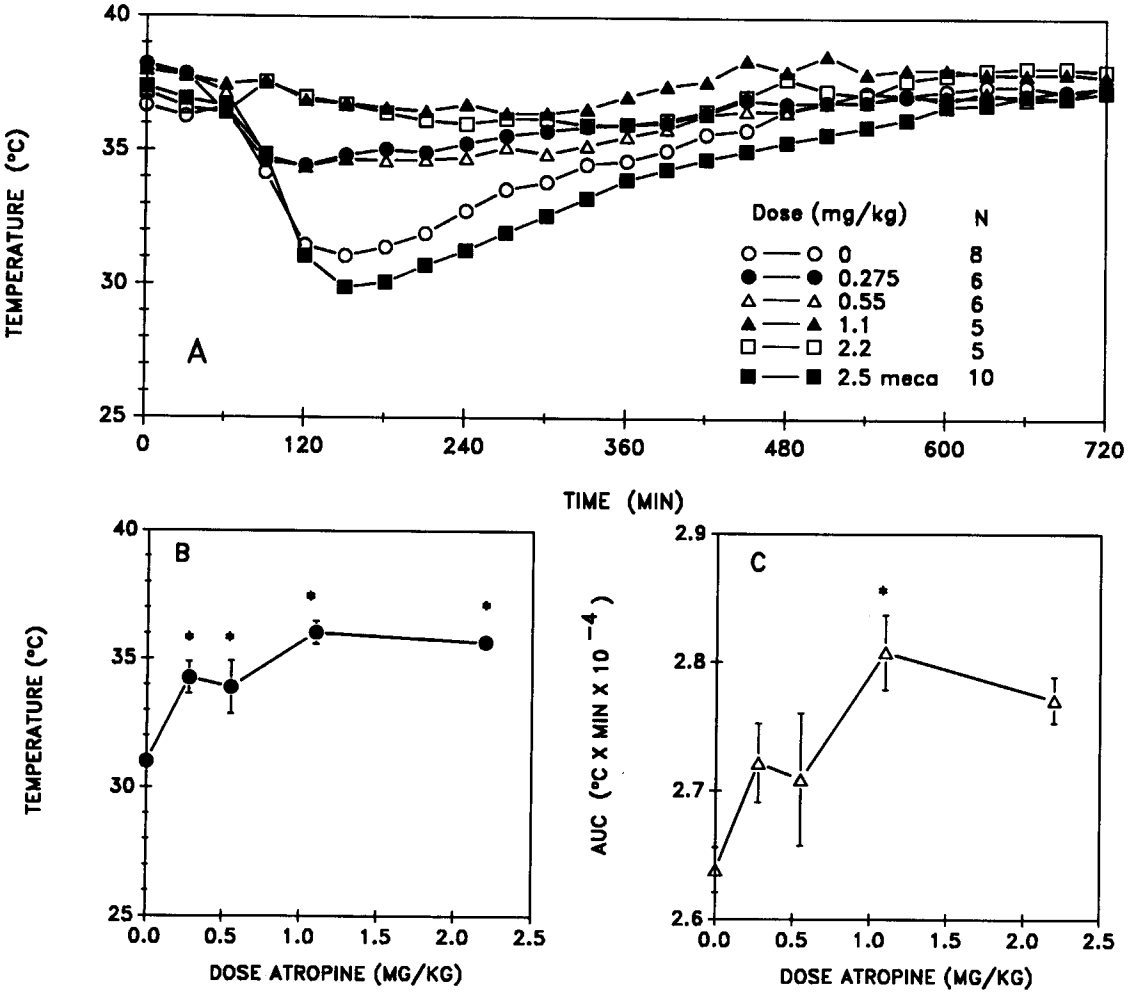

FIG. 7. (A). Atropine antagonism of soman-induced hypothermia. Mice were administered various doses of atropine (IP) 5 min prior to soman (70 μg/kg, SC). N, the number of observations. The antagonism produced by mecamylamine (meca) 2.5 mg/kg IP was also evaluated. (B) Mean minimum temperature and (C) area under the curve (AUC) were determined from the atropine data in Fig. 7A. Each point represents the mean ± SE. If the error bar is absent, it is because the SE was small and did not appear outside the symbol. *The value was significantly different ($p < 0.05$) from the saline-injected (0) control.

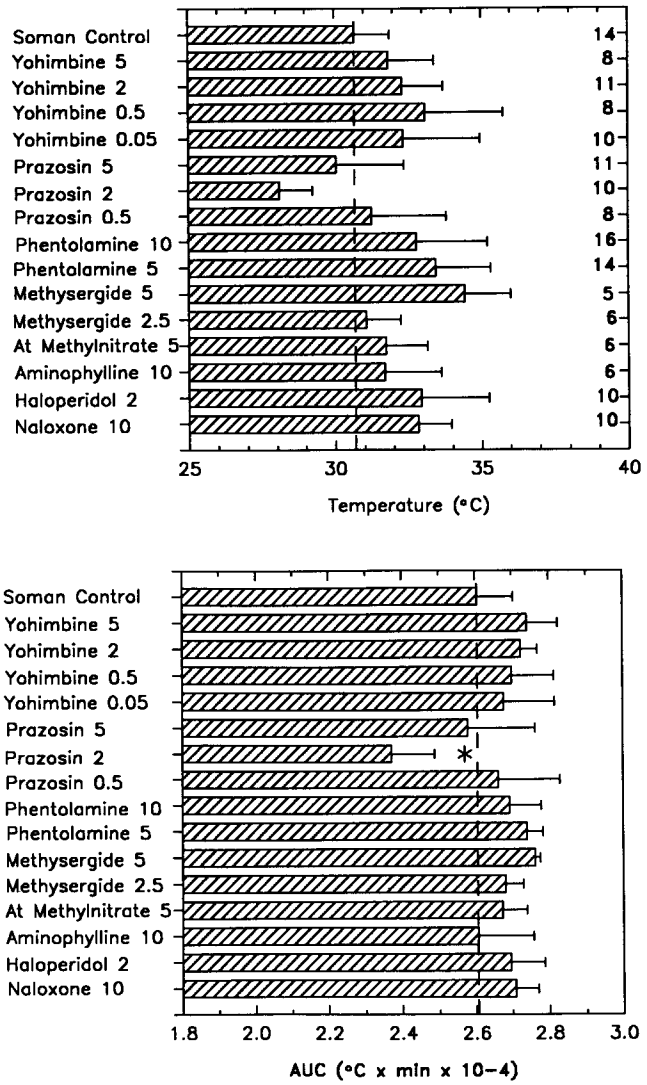

FIG. 8. Effect of various antagonists on soman-induced hypothermia. (A). Mean minimum temperature. (B) Area under the curve (AUC). The various antagonists were injected IP 5 min prior to administration of soman (70 μg/kg, SC). The number following the name of the drug represents the dose in mg/kg, while the numbers on the right side of the figure indicate the number of observations. Values are the mean ± SD. *Significantly different from the soman control ($p < 0.05$).

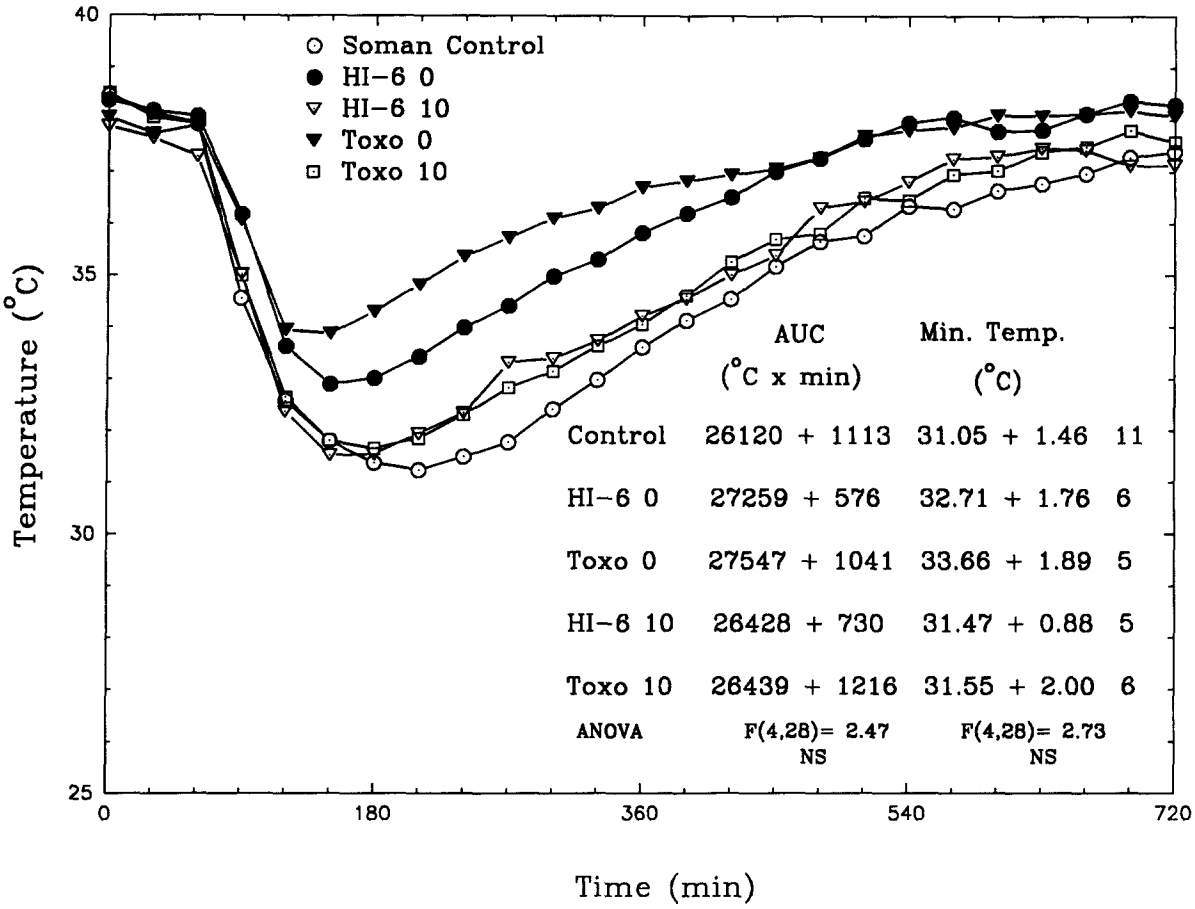

FIG. 9. Effect of acetylcholinesterase oxime reactivators on soman-induced hypothermia. Soman (70 μg/kg, SC) was administered followed by either saline or equimolar doses of HI-6 (50 mg/kg, IP) or toxogonin (47.4 mg/kg, IP) either immediately (0) or 10 min (10) after soman.

cantly soman hypothermia. The doses of the various antagonists used in this study did not produce hypothermia when administered alone (data not shown).

The effect of various acetylcholinesterase oxime reactivators, HI-6 and toxogonin, on soman-induced hypothermia and peripheral and central acetylcholinesterase activity was determined. The oximes were administered either immediately (0 min) or 10 min after soman (Fig. 9). Neither HI-6 nor toxogonin effected a significant reversal of soman-induced hypothermia although when the oxime was administered at the same time as soman there appeared to be some antagonism of soman-induced hypothermia. Because the sarin-inhibited hypothalamic acetylcholinesterase showed the greatest reactivation following HI-6 administration and was correlated to the recovery from sarin-induced hypothermia (9), this region was examined for reactivation of acetylcholinesterase activity in soman-treated mice. Neither HI-6 nor toxogonin reactivated soman-inhibited hypothalamic acetylcholinesterase at any of the time periods examined, whereas in separate experiments (Table 1) soman-inhibited diaphragm acetylcholinesterase was effectively reactivated when HI-6 was administered up to 10 min after soman (Table 2).

Physostigmine

Physostigmine is a potent, centrally active carbamate anticholinesterase. In general, the inhibition of acetylcholinesterase by physostigmine is readily reversible and relatively short lived in vivo (21). The results in Fig. 10 illustrate the hypothermia produced following administration of various doses of physostigmine. The parameters of mean minimum temperature [Fig. 10B; $F(3, 32) = 80.93$, $p < 0.01$] and AUC [Fig. 10C; $F(3, 32) = 41.15$, $p < 0.01$] demonstrated a definite dose-response relationship. At the two lower doses of physostigmine (125 and 250 μg/kg, SC), signs of toxicity were minimal. At the 500-μg/kg dose, salivation was apparent, along with agitation, tremors, ataxia, and Straub tail. Also, at the 500-μg/kg dose there was 33% mortality. This dose (500 μg/kg, SC; 1.54 μmol/kg) was chosen for the experiments with the various pharmacological antagonists.

Physostigmine (500 μg/kg, SC) hypothermia was effectively antagonized by various doses of atropine [minimum temperature, [$F(7, 59) = 11.67$, $p < 0.01$; AUC, $F(7, 59) = 41.60$, $p < 0.01$], whereas mecamylamine was ineffective (Fig. 11). In addition, neither naloxone, haloperidol, aminophylline, atropine methylnitrate, methysergide, nor phentolamine antagonized physostigmine hypothermia [Fig. 12; minimum temperature, $F(8, 46) = 25.03$, $p < 0.01$; AUC, $F(8, 46) = 16.65$, $p < 0.01$]. Prazosin (5 mg/kg) increased significantly ($p < 0.01$) physostigmine hypothermia, whereas the lower dose (2 mg/kg) was ineffective (Fig. 12). Haloperidol increased significantly ($p < 0.05$) the minimum temperature.

TABLE 1

EFFECT OF HI-6 AND TOXOGONIN ON HYPOTHALAMIC ACETYLCHOLINESTERASE FOLLOWING ADMINISTRATION OF SOMAN

Treatment Group	Acetylcholinesterase Activity (nmoles/ml/min)								
	0 min			10 min			60 min		
		% Con	% Reac*		% Con	% Reac		% Con	% Reac
Untreated control	150.1 ± 11.8†	100‡	–	150.1 ± 11.8	100	–	140.1 ± 10.0†	100	–
Soman + Saline	46.4 ± 10.9	31	–	44.5 ± 11.5	30	–	47.3 ± 7.3	34	–
Soman + HI-6	47.0 ± 17.4	31	1	45.9 ± 10.1	31	1	42.4 ± 15.6	30	0
Soman + toxogonin	47.9 ± 13.0	32	1	35.2 ± 4.6	23	0	45.3 ± 7.2	32	0
ANOVA§	$F(3, 16) = 72.40, p < 0.01$			$F(3, 16) = 149.90, p < 0.01$			$F(3, 16) = 100.87, p < 0.01$		

Mice were inject with soman (70 μg/kg, SC) and then either immediately (0 min), 10 min, or 60 min later injected IP with either saline, HI-6 (132 μmoles/kg), or toxogonin (132 μmoles/kg). Mice were then sacrificed 30 min after administration of saline or oxime.

*Reactivation is determined from the acetylcholinesterase activity = [(oxime0 − (saline)]/[(control) − (saline)] × 100.

†Mean ± SD. $n = 5$. The same control was used for the 0- and 10-min groups as these 2 groups were done on the same day.

‡% Control.

§All treated groups were significantly different ($p < 0.01$) from the untreated control group; however, the HI-6 and toxogonin treatment groups were not significantly different from the some control group.

TABLE 2

EFFECT OF HI-6 AND TOXOGONIN ON DIAPHRAGM ACETYLCHOLINESTERASE FOLLOWING SOMAN ADMINISTRATION TO MICE

Experimental Group	Acetylcholinesterase Activity (nmoles/ml/min)	% Control	% Reactivation†	n
Untreated control	104.1 ± 3.4	100	–	5
Soman control	31.5 ± 2.5	30	–	5
HI-6	70.0 ± 7.8*	67	53	5
Toxogonin	41.8 ± 6.4	40	22.6	5
HI-6 10 min after	74.4 ± 3.3*	71	59	4
ANOVA‡ $F(4, 18) = 35.46, p < 0.01$				

Mice were treated as in Table 1.

*Mean ± SEM.

†% Reactivation is determined from the acetylcholinesterase activity = [(oxime) − (soman control)]/[(untreated control) − (soman control)] × 100.

‡All treatment groups were significantly different ($p < 0.01$) from the soman control group.

DISCUSSION

The results of this study indicated that anticholinesterase (soman and physostigmine)-induced hypothermia was due to muscarinic receptor stimulation because the hypothermia was effectively reversed by muscarinic but not nicotinic receptor antagonists. The primary role of muscarinic receptors in cholinergic-induced hypothermia in mice was also emphasized by the fact that a muscarinic receptor agonist, oxotremorine, was more potent than a nicotinic receptor agonist, nicotine, in the production of hypothermia. Oxotremorine stimulates both M_1 and M_2 receptors but oxotremorine-induced hypothermia appears to be moderated by M_2 muscarinic receptors (30) because atropine (this study) and scopolamine (30) but not pirenzepine, a specific M_1 antagonist, (2) antagonized oxotremorine hypothermia.

The anterior hypothalamus is the site of action in oxotremorine-induced hypothermia (23). From the present systemic studies, we can surmise that this is the same area important in soman-induced hypothermia. The rat hypothalamus was reported to contain a relatively large concentration of nicotinic receptors (12). However, in mice the low potency of nicotine in producing hypothermia suggests that there are few nicotinic receptors located on thermoregulatory neurons in the hypothalamus, a view supported by the results of Pauley et al. (33) with regard to the binding and distribution of nicotine binding in the mouse hypothalamus.

In mice, other neurotransmitters and receptors do not appear to play a major role in the expression of soman- , physostigmine-, or oxotremorine-induced hypothermia because various pharmacological antagonists were ineffective in preventing hypothermia. The increase in hypothermia by various α-adrenergic antagonists (prazocin, phentolamine, yohimbine) is most likely due to the peripheral vasodilation.

There were differences in potency–toxicity relationships among the various hypothermia-producing agents examined. For instance, hypothermia-producing doses of oxotremorine were well below the doses that were lethal whereas hypothermia-producing doses of soman, nicotine, physostigmine (this study), and sarin (8,9) were at doses close to their LD_{50}. This can be rationalized by the fact that in the cases of soman, sarin, physostigmine, and nicotine there is a nicotinic cholinergic component affecting the respiratory musculature that may be responsible for the toxicity.

If soman-induced hypothermia is strictly a central event, its duration may be a reflection of the recovery of acetylcholinesterase activity to normal, a decrease in the sensitivity of the postsynaptic cholinergic receptors, a change in the synaptic concentration of acetylcholine, or the presence of another neurotransmitter involved in the expression of hypothermia.

Following soman poisoning, acetylcholinesterase activity recovers over the 12-h time period but not to control levels (6,7,11); however, the animal fully recovers from soman-induced hypothermia. It appears that acetylcholinesterase activity can be inhibited to a considerable degree (>50%) before there is any affect on thermoregulation (8) and complete recovery of acetylcholinesterase activity is not required for normal activity to be restored to the cholinergic system involved in thermoregulation (6,7), suggesting that a new equilibrium has been established. Others have reported no correlation between acetylcholinesterase recovery and thermoregulation. Overstreet (32) found that the use of cycloheximide did not interfere with the recovery of core temperature to normal; similar results were found in my laboratory (Clement, unpublished observations). Coudray-Lucas et al. (14) found no correlation between the activity of acetylcholinesterase activity in a particular brain region and the incidence or degree of organophosphate-induced hypothermia. Acetylcholinesterase is a polymorphic enzyme composed of several molecular forms. In the rodent brain, two molecular forms of acetylcholinesterase predominate with sedimentation coefficients of 4S and 10S. Perhaps recovery of activity of a particular molecular form of acetylcholinesterase is responsible for the recovery from hypothermia. The 4S molecular form of acetylcholinesterase recovered relatively quickly compared to the 10S molecular form in the hypothalamus following soman poisoning (11), suggesting that the 4S form may participate in the recovery of the core temperature to normal. This is unlikely because the 4S enzyme is normally sequestered within the neuron (1,11) and thus does not play a direct role in the synaptic events. However, there is still the possibility that the recovery of the acetylcholinesterase activity, insensitive to cycloheximide (i.e., that which has already been synthesized but has not been assembled or translocated), in a critical area of the

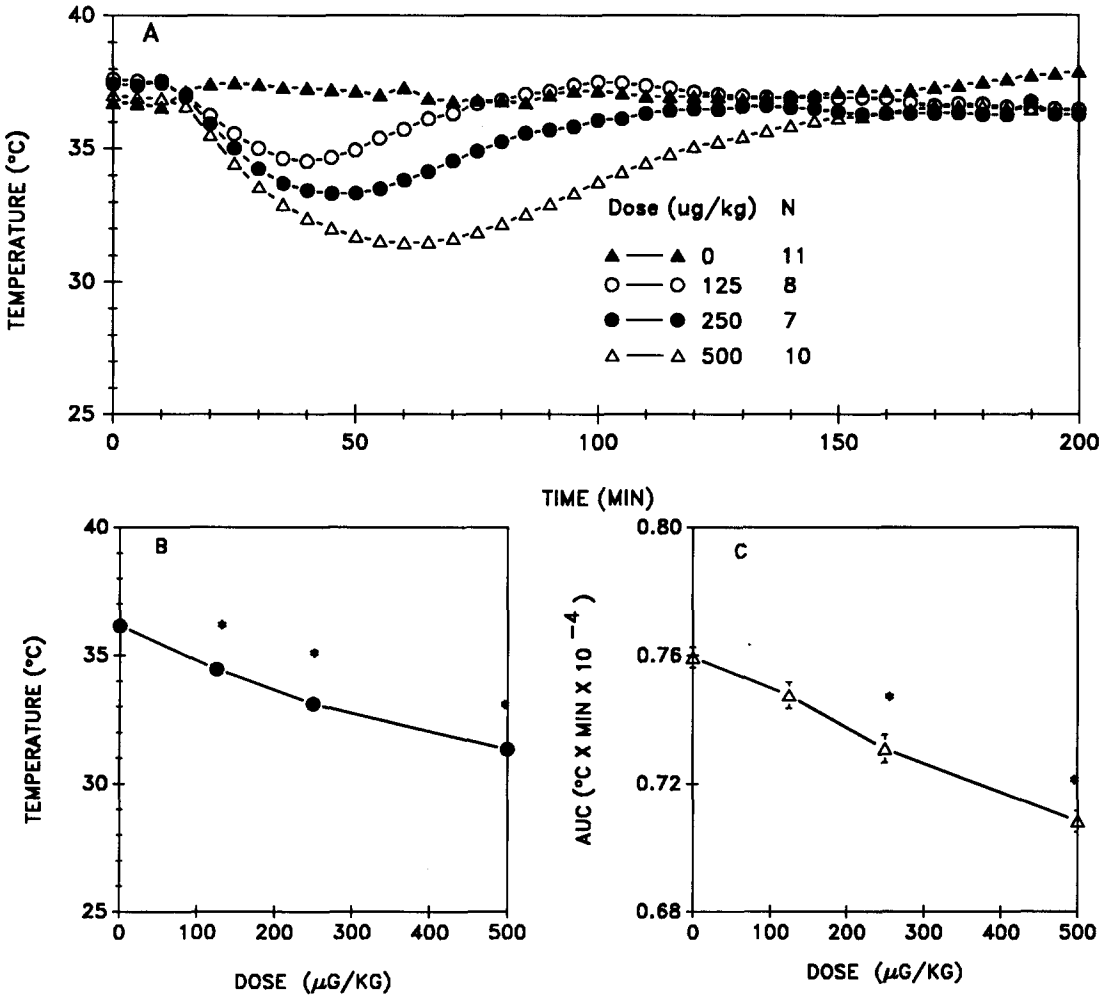

FIG. 10. (A). Temporal response of various doses of physostigmine on core temperature. Various doses of physostigmine (μg/kg, SC) were administered immediately after the collection of the third data point and the core temperature monitored. N, number of observations. (B) Mean minimum temperature and (C) area under the curve (AUC) were determined from the data in Fig. 10A. Each point represents the mean ± SE. If the error bar is absent, it is because the SE was small and did not appear outside the symbol. *The value was significantly different ($p < 0.05$) from the saline-injected (0) control.

hypothalamus is responsible for the recovery from hypothermia. The importance of acetylcholinesterase in recovery from organophosphate-induced hypothermia is supported by data that shows a strong correlation between the recovery from sarin-induced hypothermia and the reactivation of hypothalamic acetylcholinesterase by the various oximes (9).

A change in receptor sensitivity may be a reason for the recovery from soman-induced hypothermia. Meeter (28) found no change in the carbachol response following soman poisoning and so concluded that there was no change in postsynaptic receptor sensitivity. But, as the results of the present study have shown, soman-induced hypothermia is primarily a muscarinic receptor-related event and the nicotinic receptor is not a major component of cholinergic-induced hypothermia. The use of carbachol, a nicotinic receptor agonist, would not be the agonist of choice to demonstrate receptor desensitization. The recovery from hypothermia may be due to changes in muscarinic receptor coupling sensitivity. A decrease in oxotremorine-induced hypothermia at various times after a 100- but not 70-μg/kg dose of soman suggests that there was either a downregulation of muscarinic receptors or a change in the secondary messenger system or both (6). The response took a long time to recover to the control level (7), suggestive of resynthesis. Similarly, Overstreet et al. (31) found that pilocarpine-induced hypothermia decreased after organophosphate poisoning, suggestive of a downregulation of cholinergic receptors. There are a number of reports indicating a decrease in the postsynaptic response following exposure to a sublethal dose of organophosphate (3,4). However, the temporal response did not coincide with the recovery from hypothermia. At 12 h after DFP, there were no changes in receptor binding (4) yet mice had fully recovered from DFP hypothermia [(7); Clement, unpublished observations]. Cioffi and El-Fakahany (4) suggested that a physiological desensitization does not necessarily accompany acute downregulation of muscarinic receptors. Dilsaver and Alessi (18) stated that binding data are nothing but adynamic measures that convey nothing about the function of the system. In the case of soman, recovery

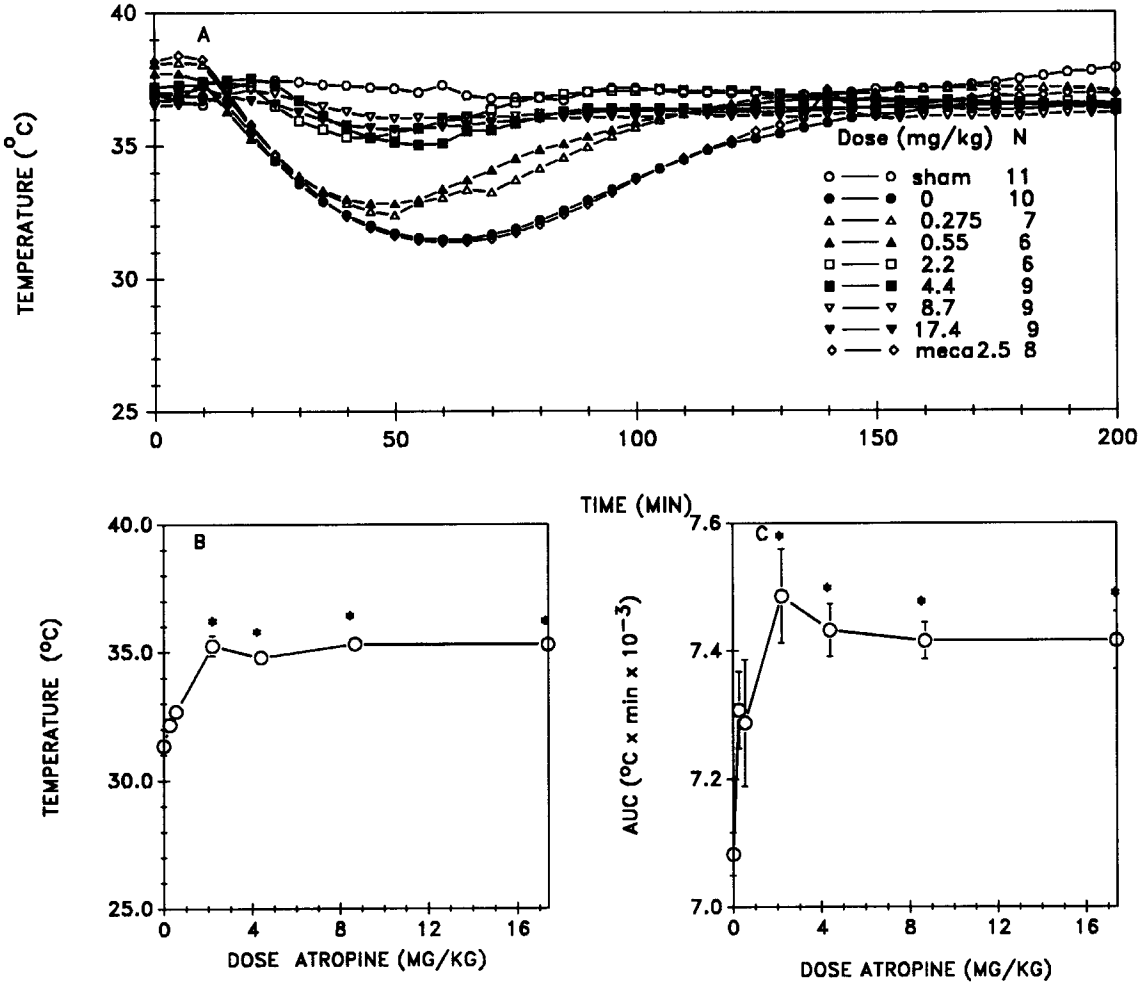

FIG. 11. (A). Atropine antagonism of physostigmine-induced hypothermia. Various doses of atropine (mg/kg, IP) were administered 5 min prior to physostigmine 500 μg/kg SC. The antagonism of mecamylamine (meca, 2.5 mg/kg, IP) was also evaluated. N, number of observations. (B) Minimum temperature and (C) area under the curve (AUC) were determined from the data in Fig. 11A. Each point represents the mean ± SE. If the error bar is absent, it is because the SE was small and did not appear outside the symbol. *The value was significantly different ($p < 0.05$) from the saline-injected (0) control.

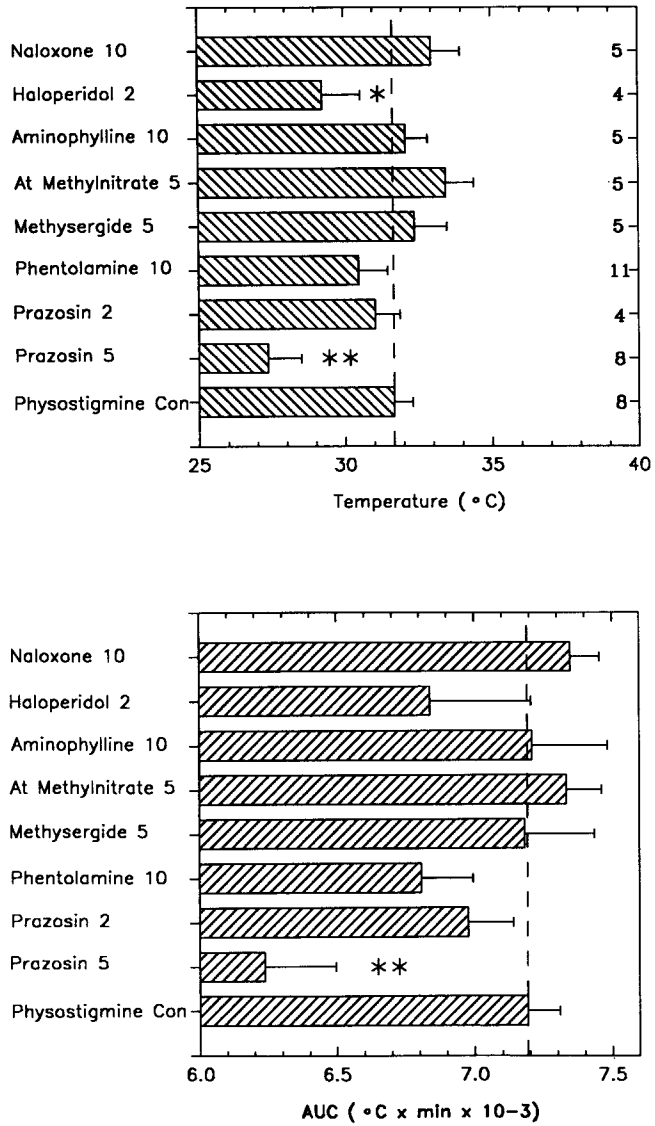

FIG. 12. Effect of various antagonists on physostigmine-induced hypothermia in mice. (A). Mean minimum temperature. (B) Area under the curve (AUC). The various antagonists were administered IP 5 min prior to administration of physostigmine (500 μg/kg, SC). The number following the name of the drug represents the dose in mg/kg, while the numbers on the right side of the figure indicate the number of observations. Values are the mean ± SD. Significantly different from physostigmine control (*$p < 0.05$, **$p < 0.01$).

from hypothermia could be due to desensitization at some point in the receptor coupling mechanism. However, because oxotremorine hypothermia was not necessarily affected following the lower doses of soman this would not be a phenomenon that would be in general applicable and thus is unlikely to play a part in the recovery from soman hypothermia.

With physostigmine, there is a temporal correlation among the brain concentration of physostigmine, acetylcholinesterase inhibition, acetylcholine concentrations, and the hypothermia [(21,25); this study]. Oxotremorine-induced hypothermia was directly related to the brain concentrations of this centrally active agonist (22). Following administration of an organophosphate such as soman, there is an increase in the concentration of acetylcholine due to a decrease in the hydrolysis (35,37). The elevated concentrations have a similar temporal relationship to that of soman-induced hypothermia. For soman hypothermia, recovery may be due to the normalization of the acetylcholine concentration by a decrease in the release of neurotransmitter and a slight recovery of acetylcholinesterase activity with the establishment of a new equilibrium at the cholinergic synapses.

Soman-induced hypothermia could be mediated by another neurotransmitter. Neurotransmitters such as dopamine, noradrenaline, and serotonin and (13,15,34) may be involved in soman hypothermia. [GABA did not appear to be changed following soman poisoning (16)]. However, this is doubtful because none of the antagonists except atropine were effective in reversing soman-induced hypothermia. The data from experiments using the α-adrenergic antagonists, especially prazocin, a peripheral-acting α-1 antagonist, indicate the importance of peripheral input on thermoregulation in a small animal such as a mouse.

Oximes were ineffective in reversing soman-induced hypothermia and reactivating hypothalamic acetylcholinesterase in contrast to the results found with sarin-induced hypothermia (9). The lack of reactivation of soman-inhibited acetylcholinesterase was not due to aging of the inhibited enzyme because diaphragm acetylcholinesterase could still be reactivated when administered 10 min after soman. Even though HI-6 is the most potent reactivator of soman-inhibited acetylcholinesterase (17), it could be that the concentration of HI-6 in the brain was not high enough to effect reactivation of the phosphorylated enzyme. This point is supported by data on ICV administration of HI-6 where 25 μg ICV HI-6 produced no reactivation (24,36) whereas higher doses of 75 and 200 μg ICV produced 9 and 14% reactivation, respectively (36). It is doubtful that the concentrations of HI-6 in the brain were even close to these amounts. Even though HI-6 passes the blood–brain barrier in physiologically and biochemically significant quantities, in the case of sarin poisoning (9) this does not preclude that these same concentrations would be effective against other nerve agents, such as was found in this study. Again, this data suggests that the major site of action of HI-6 is in the periphery.

A number of investigations indicated that there are neurotransmitter changes following poisoning by soman and other anticholinesterases. In light of the hypothermia produced by organophosphates, the question that has not been addressed is "Are the neurotransmitter changes observed after organophosphate poisoning due to the treatment or are they a normal response to hypothermia?" Only the use of appropriate control groups would address this question (5). Thus, if the anticholinesterase dose was high enough and hypothermia was present neurotransmitter changes after anticholinesterase poisoning are equivocal and should be examined with this argument in mind.

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