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Effect of a Single Dose of an Acetylcholinesterase Inhibitor on Oxotremorine- and Nicotine-Induced Hypothermia in Mice

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CLEMENT, J. G. *Effect of a single dose of an acetylcholinesterase inhibitor on oxotremorine- and nicotine-induced hypothermia in mice.* PHARMACOL BIOCHEM BEHAV **39**(4) 929–934, 1991.—Downregulation of cholinergic receptors is a consequence of subchronic exposure to an organophosphate anticholinesterase. The purpose of this investigation was to determine if there was a downregulation of the cholinergic receptors in mice following administration of a single dose of soman (pinacolyl methylphosphonofluoridate) or physostigmine. The change in the temporal response (mean minimum temperature and area under the curve) of core temperature following administration of either a muscarinic or nicotinic agonist such as oxotremorine (156 μg/kg, IP) or nicotine hydrogen tartrate (15 mg/kg, SC) was used as an indicator of downregulation of muscarinic or nicotinic receptors, respectively. Twenty-four h following soman (100 μg/kg, SC) administration, there was a significant decrease ($p<0.05$) in oxotremorine- but not nicotine-induced hypothermia. The significant differences in the mean minimum temperature and AUC were still present 4 days after exposure to the soman. Neither lower doses of the organophosphate anticholinesterase, soman (50 and 70 μg/kg), nor the carbamate anticholinesterase, physostigmine (500 μg/kg), produced a significant change in either oxotremorine- or nicotine-induced hypothermia. The results of this study suggest that receptor downregulation observed after subchronic administration of soman is also evident following administration of a single, sublethal dose of an organophosphate anticholinesterase, soman, but not after administration of a carbamate anticholinesterase, physostigmine. The in vivo assessment of the muscarinic receptor using oxotremorine hypothermia may be a sensitive indicator of the functionality of the drug-receptor coupling and indicate a physiological consequence of receptor downregulation.

Core temperature Thermoregulation Hypothermia Acetylcholinesterase inhibition Organophosphate Carbamate Physostigmine Soman Oxotremorine Nicotine

FOLLOWING soman (pinacolyl methylphosphonofluoridate) poisoning, symptoms such as miosis, salivation, lacrimation, diarrhea and tremors are evident. These symptoms are due to inhibition of the enzyme acetylcholinesterase which results in an increase in the concentration of the neurotransmitter, acetylcholine (33,42), and an overstimulation of the postsynaptic cholinergic receptors. The recovery of acetylcholinesterase to control levels, after inhibition by soman, is by resynthesis of new enzyme (19), a process that may take weeks to occur depending upon the anatomical location of the tissue (9). Chronic or subchronic exposure to anticholinesterase agents results in the development of tolerance. This is characterized by the disappearance or decrease of symptoms upon subsequent exposure to either the anticholinesterase (12, 23, 29) or a cholinergic agonist (12, 28, 29, 37) and a downregulation (decrease) in the number of cholinergic receptors (1, 3, 4, 12, 15, 31, 36, 41).

The downregulation of cholinergic receptors observed in drug receptor binding assays does not indicate if the decrease in receptor number was of a functional, physiological significance. Oxotremorine-induced hypothermia apparently results from activation of muscarinic cholinergic receptors in the anterior hypothalamus (22). In the present experiments, measurement of core temperature in mice was accomplished using a telemetry system (10). By measuring the temporal response of core temperature following the administration of either oxotremorine or nicotine, the mean minimum temperature and area under the curve (AUC) could be determined. There was a dose-response relationship for mean minimum temperature and AUC following the administration of either oxotremorine (10) or nicotine (J. G. Clement, unpublished observations) to mice. In this study, it was assumed that, if there were a downregulation of muscarinic or nicotinic receptors, there should be a parallel change in the oxotremorine- or nicotine-induced hypothermia, respectively. Thus mean minimum temperature and AUC following administration of standard doses of either oxotremorine or nicotine were used as indicators of receptor downregulation following exposure to a single dose of an acetylcholinesterase inhibitor such as soman or physostigmine.

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TABLE 1

EFFECT OF ANTICHOLINESTERASE POISONING ON THE MEAN MINIMUM TEMPERATURE AND AUC PRODUCED BY EITHER OXOTREMORINE OR NICOTINE 24 HOURS LATER*

	Oxotremorine			Nicotine		
Treatment	Minimum Temperature (°C)	AUC (°C × Min)	N	Minimum Temperature (°C)	AUC (°C × Min)	N
Control	30.37 ± 1.52†	10023 ± 265	11	30.58 ± 1.62	8729 ± 249	12
Soman (50)‡	29.87 ± 0.84	10079 ± 207	5	—	—	
Soman (70)	31.10 ± 0.96	10364 ± 300	5	30.41 ± 2.05	8785 ± 331	6
Soman (100)	33.29 ± 1.03§	10855 ± 235§	6	31.20 ± 0.94	8853 ± 165	10
Physostigmine (500)	29.86 ± 1.12	10123 ± 255	8	—	—	
ANOVA	$F(4,30) = 10.39$ $p<0.01$	$F(4,30) = 14.18$ $p<0.01$		$F(2,25) = 0.66$ ns	$F(2,25) = 0.71$ ns	

*Mice were administered either soman (50, 70 or 100 μg/kg, SC) or physostigmine (500 μg/kg, SC), then 24 h later were injected with either oxotremorine (156 μg/kg, IP) or nicotine (15 mg/kg, SC) and the hypothermia monitored.

†Mean ± SD with N = number of observations.

‡Drug pretreatment with the value in parentheses representing the dose administered in μg/kg, SC.

§Significantly different ($p<0.01$) from control as determined by the Scheffe test; ns = not significant.

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

Tolerance Assessment

Initially, mice were poisoned with soman (50, 70 or 100 μg/kg) or physostigmine (500 μg/kg) by subcutaneous (SC) injection. Twenty-four h after the injection of soman, mice were administered either oxotremorine (156 μg/kg, IP) or nicotine (15 mg/kg, SC) by intraperitoneal (IP) injection, and the temporal core temperature response was monitored. The doses of oxotremorine and nicotine used in the evaluation were on the linear portion (approximately 70%) of the dose-response curve for each of the agonists (J. G. Clement, unpublished observations).

In another series of experiments, the duration of tolerance following a single injection of soman was investigated. On day 0, mice were injected with either saline or soman (100 μg/kg, SC). Daily, beginning on the day after soman administration (Day 1), mice were injected with either oxotremorine (156 μg/kg, IP) or nicotine (15 mg/kg, SC) and the core temperature monitored.

Core Temperature Measurement

Core temperature was monitored using a telemetry system (10). For implantation of the telemetry transmitters, 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 peritoneal incision was closed using sutures (000 plain gut), and the skin was closed using wound clips (9-mm Michel clips). The mice were allowed to recover for 1 week prior to use in an experimental situation (10). The telemetry transmitter was activated by bringing a magnet close to the abdomen, which turned on the battery power. The weight of the telemetry transmitter was tared prior to recording the body weight of the mouse so that the animal was injected with the proper dose of the drug based on tissue weight of the animal. Mice were placed in individual cages, and the core temperature was monitored by telemetry. Typically, the first 3 data points established a control baseline. The drug was administered immediately after the acquisition and storage of the 3rd data point. The data were acquired at 10-min intervals, for a total of 240 and 280 min for nicotine and oxotremorine, respectively. The entire time period, including the control interval, was then used in the calculation of the mean minimum temperature and AUC.

Data Analysis

The data were analyzed by one-way analysis of variance (ANOVA) and, when appropriate, significant differences of the means were determined using the multiple comparison Scheffe test. Significant differences of the means in the presence and absence of soman were determined by the Student *t*-test. A value of $p<0.05$ was considered statistically significant.

Acetylcholinesterase Assay

The degree of acetylcholinesterase inhibition and the time course recovery in various brain regions following administration of soman (100 μg/kg) were investigated. Acetylcholinesterase activity was determined at 37°C using a radiometric procedure (34) 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 of each of these tissues 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.

Materials

Soman (pinacolyl methylphosphonofluoridate), prepared at Defence Research Establishment Suffield, was greater than 99% pure. The 24-h LD_{50} of the soman used in this study was between 130–140 μg/kg (SC). The following drugs were obtained

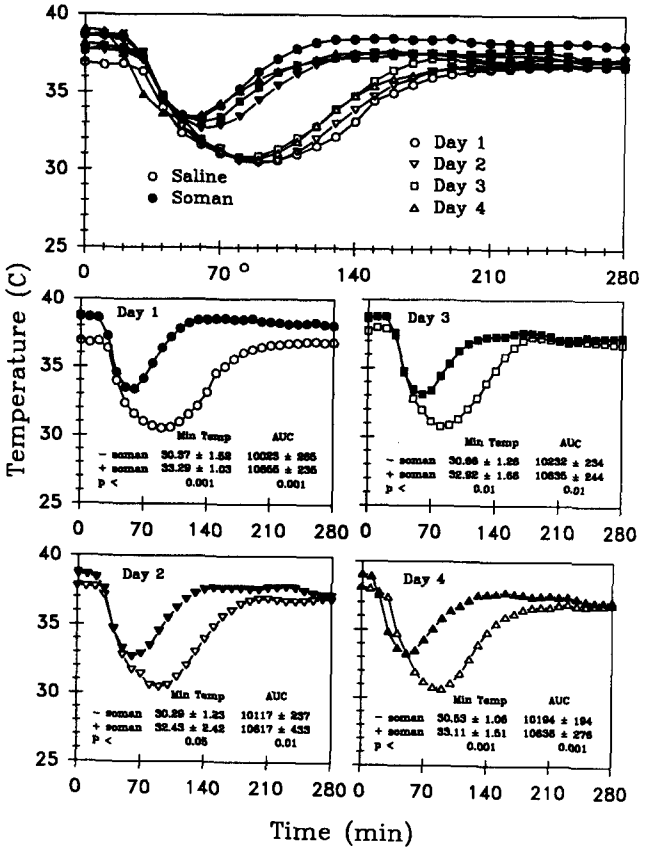

FIG. 1. Effect of soman pretreatment on tolerance development to oxotremorine-induced hypothermia. Mice were injected on day 0 with either saline or soman (100 μg/kg) and then injected 1 day after and daily for 4 days with oxotremorine (156 μg/kg, IP). The standard error bars were omitted for clarity. The additional panels below the top graph are the individual curves (extracted from the combined graph) for that particular day. Values in the table portion of the figures represent the mean ± SD. Mean minimum temperature (Min Temp) = °C while AUC = °C × min. Statistical differences were determined using the Student *t*-test. N = 11 for − soman and 8 for + soman.

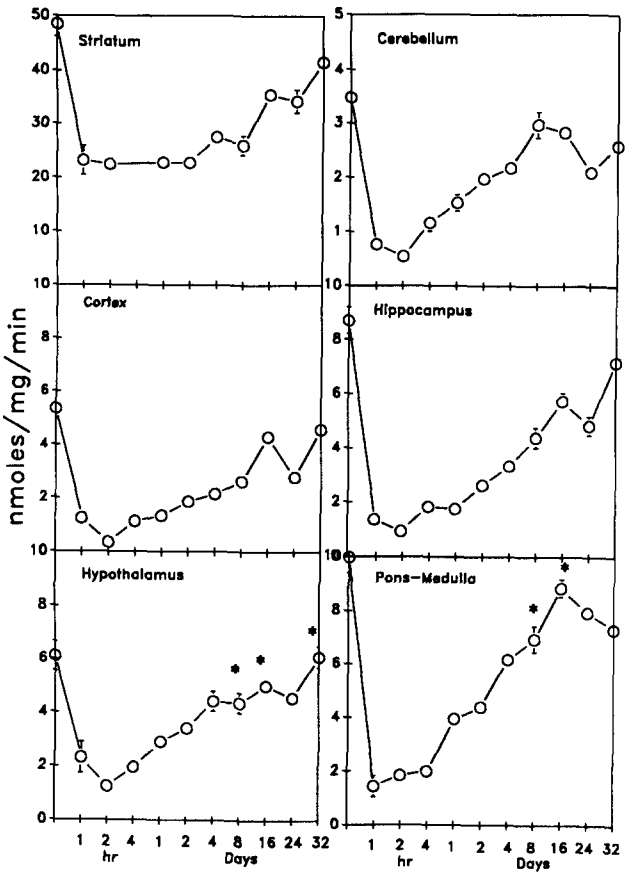

FIG. 2. Degree of inhibition of acetylcholinesterase by soman and the time course of the recovery. Mice were sacrificed at the various time periods after injection of soman (100 μg/kg, SC) and the acetylcholinesterase activity determined. Each point represents the mean ± SEM of at least 5 observations. A nonlinear abscissa was used so that the data at the early time points could be seen more clearly. If the error bars are not visible, then the SEM was not greater than the radius of the point. Those time points marked with an asterisk (*) were not significantly different from control, whereas all the other points were significantly different from the control group (day 0) to at least the 5% level.

from various commercial sources: oxotremorine (Aldrich), nicotine hydrogen tartrate and physostigmine hemisulfate (Sigma). All drugs were dissolved in saline prior to injection. The volume of injection was 1% of body weight.

RESULTS

Following administration of soman (70 and 100 μg/kg, SC), the core temperature decreased to 29–31°C within 4–6 h and gradually returned to normal within 12 h [(10); J. G. Clement, unpublished observations]. There was no apparent hypothermia following administration of the 50-μg/kg dose of soman. The administration of oxotremorine or nicotine was delayed until 24 h after soman pretreatment so that the measurements were made in a system where core temperature had returned to control levels.

The temporal response of oxotremorine- and nicotine-induced hypothermia was assessed (Table 1) following administration of soman. At the lower doses of soman (50 and 70 μg/kg), there was no significant effect on the muscarinic-induced hypothermia. Only following the 100-μg/kg dose of soman were the changes in oxotremorine-induced hypothermia statistically significant (Table 1). There was no significant effect on the nicotine-induced hypothermia following soman poisoning (70- or 100-μg/kg dose). The tolerance to oxotremorine hypothermia seen following soman (100 μg/kg) was not apparent following administration of the carbamate anticholinesterase, physostigmine, as there were no significant changes in the oxotremorine-induced mean minimum temperature and AUC 24 h following physostigmine poisoning. This particular dose of physostigmine was on the upper portion of the physostigmine hypothermia dose-response curve (J. G. Clement, unpublished observations) and was a dose that produced marked signs of cholinergic overstimulation and a modest degree (10%) of mortality. It was assumed that the dose of physostigmine produced a marked inhibition of brain acetylcholinesterase (17).

The duration of tolerance following a single soman injection was investigated by recording the temporal response of core temperature to oxotremorine administration at various times after soman and determining the mean minimum temperature and AUC. The results in Fig. 1 illustrate the time course of hypothermia produced following daily administration of oxotremorine

(156 μg/kg, IP) in mice that received either saline or soman. The mean minimum temperature and AUC for oxotremorine hypothermia were calculated for each day and analyzed by one-way ANOVA (Fig. 1). There were no significant differences in the mean minimum temperature, $F(3,40)=0.18$, $p>0.05$, and the AUC, $F(3,40)=1.71$, $p>0.05$, in the response to daily administration of oxotremorine (156 μg/kg) in the absence of soman poisoning suggesting that daily administration of oxotremorine (156 μg/kg) did not induce tolerance to its own response. Similarly, in the presence of soman poisoning, the mean minimum temperature, $F(3,28)=0.37$, $p>0.05$, and AUC, $F(3,28)=1.08$, $p>0.05$, were not significantly different over the 4-day observation period. However, in the soman-treated group, there were significant differences in the oxotremorine-induced hypothermia (mean minimum temperature and AUC) when compared to the saline group on successive days after soman poisoning. The oxotremorine mean minimum temperature and AUC were significantly higher on days 1, 2, 3, and 4 after soman pretreatment; e.g., minimum temperature on day 1 after saline pretreatment was 30.37°C compared to 33.29°C day 1 after soman poisoning (Fig. 1). Generally, there was a decrease in the oxotremorine-induced hypothermia following soman (100 μg/kg, SC), suggesting that tolerance had developed, and it was still present 4 days later.

Soman (100 μg/kg, SC) produced a profound inhibition of acetylcholinesterase in all regions of the brain (Fig. 2). The cortex acetylcholinesterase was inhibited to the greatest degree, and acetylcholinesterase in the striatum was inhibited the least. In all brain regions, it took a prolonged time (16 to >32 days) for the acetylcholinesterase activity to recover to control levels. Interestingly, the soman hypothermia disappeared within 12 h at a time when the brain acetylcholinesterase activity was still severely inhibited.

DISCUSSION

Various investigators, utilizing drug receptor binding assays, have reported a downregulation of the muscarinic cholinergic receptors, without any change in the affinity constant, following acute (1, 5, 16, 28) and subchronic anticholinesterase administration (3, 4, 11, 15, 16, 32, 40, 41). However, measurement of a decrease in the number of cholinergic receptors in a drug receptor binding assay does not give any indication of the functional significance of the downregulation. Dilsaver and Alessi (14) stated that "binding data are adynamic measures which convey nothing about the function of the system." This argument was supported by the fact that downregulation of muscarinic receptors following repeated injections of DFP was not accompanied by a "physiological desensitization" in the muscarinic-receptor-mediated phosphoinositide hydrolysis (6). In the present study, it was hypothesized that, if there were a downregulation of cholinergic receptors due to increased concentrations of acetylcholine (the result of extensive and prolonged acetylcholinesterase inhibition) following the administration of a single dose of soman, there should be a decrease in the oxotremorine- and/or nicotine-induced hypothermia. Following administration of a single dose of soman (100 μg/kg), tolerance developed within 24 h due to effects at the muscarinic but not the nicotinic receptors. This was evidenced by an increase in the mean minimum temperature and AUC following oxotremorine- but not nicotine-induced hypothermia 24 h after soman poisoning. The lower doses of soman tended to produce a decrease in the oxotremorine hypothermia. However, only after administration of the 100-μg/kg dose of soman was there a significant ($p<0.05$) decrease in the oxotremorine hypothermia measured 24 h later. These results are similar to those of Overstreet et al. (28) who reported a decrease in the pilocarpine hypothermia 24 h following a single dose of DFP.

Tolerance was not a property common to all anticholinesterases. Tolerance was not evident 24 h following administration of the carbamate anticholinesterase, physostigmine (Table 1). In the present study, the acetylcholinesterase inhibition and resulting increase in acetylcholine concentrations produced by physostigmine were probably not long enough (17,26) to produce downregulation of the muscarinic receptor. Acetylcholinesterase inhibitors, such as soman or physostigmine, result in the same biochemical event, namely inhibition of the enzyme acetylcholinesterase which produces an increase in the synaptic concentration of acetylcholine and an overstimulation of cholinergic receptors. Both produced hypothermia in mice (J. G. Clement, unpublished observations, this study); however, the physostigmine hypothermia was short-lived due to the fact that the acetylcholinesterase inhibition is readily reversible, and the synaptic situation is normalized. The reversibility of the inhibition and the effect on cholinergic receptors were emphasized in the study where physostigmine was administered twice daily for 7 days and did not produced downregulation of muscarinic receptors in the rat brain (13). In the case of soman poisoning, the acetylcholinesterase inhibition is irreversible, but still normal core temperature is restored within 12–24 h. This indicates that, after soman poisoning, there is a change in the synaptic function which brings about a new equilibrium, most likely the result of a change in muscarinic cholinergic receptor coupling.

The time course of tolerance development following a single exposure to soman indicated that the recovery of the normal response took longer than 4 days (Fig. 1). Following repeated administration of soman, the half-time for recovery of muscarinic receptors varied from 14 days for the superior colliculus to 19 days for the hippocampus (4). This prolonged recovery time is not unusual, as it takes at least 15–30 days, depending upon the area of the brain, for acetylcholinesterase to recover to control levels following soman poisoning [(9), Fig. 2]. It would not be unreasonable to assume that it would take a similar time frame for the resynthesis and/or externalization of the receptors to occur and that different areas of the brain are affected to different degrees (3) and recover at different rates (4). Thus it is apparent that, even though the animal has established a new equilibrium (as evidenced by the return of the body temperature to control levels in the presence of extensive acetylcholinesterase inhibition), it may take a prolonged time for the animal to recover to control levels from the toxic insult even though the animal may respond toxicologically as though it were completely recovered (9). Thus, as Russell et al. (30) stated, physiological variables are "capable of normal activity when less than the total population of functional m ACh receptors was available."

The number of nicotinic receptors in the central nervous system is small in relation to the number of muscarinic receptors; however, the largest concentration of nicotinic receptors is in the hypothalamus (11). Following soman administration, there was no effect on the nicotine-induced hypothermia, suggesting that there was no downregulation of the nicotinic receptors. These results agree with those of others (20,38) who found that repeated soman administration produced a downregulation of the muscarinic but not nicotinic receptors, and there was no desensitization to the nicotinic agonist, carbachol at various times after soman poisoning (27).

The decrease in the oxotremorine hypothermia 24 h following soman pretreatment suggested a downregulation of muscarinic receptors. Alternatively, this change in the oxotremorine response could be accounted for by a change in the metabolism of oxotremorine. However, this is unlikely, since the mouse

metabolizes oxotremorine relatively slowly, and pretreatment of the animal with a potent enzyme inducer, such as phenobarbital, does not alter the metabolism of oxotremorine significantly (8,18). Generally, soman did not alter the activity of hepatic mixed-function oxidases (7). Thus the decrease in oxotremorine hypothermia following soman exposure is most likely representative of a muscarinic-receptor-related phenomenon.

Oxotremorine hypothermia is purported to result from the stimulation of muscarinic receptors in the thermoregulatory center in the preoptic anterior hypothalamus (22). Administration of either radioactive soman, sarin or DFP resulted in much higher concentrations of radioactivity in the hypothalamus compared to other brain areas (21) and was found to produce profound inhibition of acetylcholinesterase in this region [(9), this study]. Thus it is curious as to why, following subchronic administration of either soman (4) or DFP (3, 35, 41), there was a down-regulation of muscarinic receptors (i.e., a decrease in the number but not the affinity of the receptor) in all brain regions examined except the hypothalamus. Perhaps there was a methodological reason. Labeling of muscarinic receptors using radioactive quinuclidinyl benzilate (QNB) (3, 4, 35, 41) gives an estimate of the total number of receptors, whereas the use of N-methyl-scopolamine (NMS) may provide a better estimation of the number of available, *functional* muscarinic receptors contributing to a cellular response (2). Alternatively, the decrease in the QNB binding noted in various brain areas (3, 4, 35, 41) could be due to the disappearance of cholinergic neurons, whereas in the hypothalamus, there may be no loss of cholinergic neurons, and thus there would be no apparent decrease in QNB binding as QNB would label receptors on the surface and those sequestered in the membrane. In the present study, it is doubtful that the decrease in the oxotremorine response following subacute soman exposure is due to a loss of muscarinic receptors as a result of neuropathology. The dose of soman (100 μg/kg, SC) used in the present study did not produce convulsions, which appear to be a prerequisite for observing the neuropathology (24,25). It could also point to the fact that the locus of the action of oxotremorine downregulation is not in the hypothalamus but at another neuronal connection in the thermoregulatory control circuit and/or that the downregulation in the hypothalamus was occurring at a point in the system after the initial binding of the agonist, i.e., G proteins, second messengers, etc.

The in vivo assessment of muscarinic receptor function using oxotremorine-induced hypothermia may be a more sensitive indicator of a change in the response of the drug-receptor coupling than measurement of drug-receptor binding. Perhaps the oxotremorine hypothermia may be representative of an interaction with the "functional" NMS binding sites. The muscarinic receptor downregulation following exposure to organophosphate anticholinesterases may serve as a protective mechanism for the organism which allows it to adapt to the changing conditions of the internal milieu.

REFERENCES

1. Aronstam, R. S.; Smith, M. D.; Buccafusco, J. J. Clonidine prevents the short term down regulation of muscarinic receptors in mouse brain induced by the acetylcholinesterase inhibitor soman. Neurosci. Lett. 78:107–112; 1987.
2. Brown, J. H.; Goldstein, D. Analysis of cardiac muscarinic receptors recognized selectively by nonquaternary but not by quaternary ligands. J. Pharmacol. Exp. Ther. 238:580–586; 1986.
3. Churchill, L.; Pazdernik, T. L.; Samson, F.; Nelson, S. R. Topographical distribution of down-regulated muscarinic receptors in rat brains after repeated exposure to diisopropyl phosphonofluoridate. Neuroscience 11:463–472; 1984.
4. Churchill, L.; Pazdernik, T. L.; Jackson, J. L.; Nelson, S. R.; Samson, F. E.; McDonough, J. H. Topographical distribution of decrements and recovery in muscarinic receptors from rat brains repeatedly exposed to sublethal doses of soman. J. Neurosci. 4:2069–2079; 1984.
5. Cioffi, C. L.; El-Fakahany, E. E. Decreased binding of the muscarinic antagonist [3H]-methylscopolamine in mouse brain following acute treatment with an organophosphate. Eur. J. Pharmacol. 132: 147–154; 1986.
6. Cioffi, C. L.; El-Fakahany, E. E. Lack of alterations in muscarinic receptor subtypes and phosphoinositide hydrolysis upon acute DFP treatment. Eur. J. Pharmacol. 156:35–45; 1988.
7. Clement, J. G. Hormonal consequences of organophosphate poisoning. Fundam. Appl. Toxicol. 5:s61–s77; 1985.
8. Clement, J. G. Hypothermia: Limited tolerance to repeated soman administration and cross-tolerance to oxotremorine. Pharmacol. Biochem. Behav., in review; 1990.
9. Clement, J. G. Survivors of soman poisoning: Recovery of the soman LD50 to control value in the presence of extensive acetylcholinesterase inhibition. Arch. Toxicol. 63:150–154; 1989.
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. Costa, L. G.; Murphy, S. D. [3H]Nicotine binding in rat brain; alteration after chronic acetylcholinesterase inhibition. J. Pharmacol. Exp. Ther. 226:392–397; 1983.
12. Costa, L. G.; Schwab, B. W.; Murphy, S. D. Tolerance to anticholinesterase compounds in mammals. Toxicology 25:79–97; 1982.
13. DeSarno, P.; Giacobini, E. Modulation of acetylcholine release by nicotinic receptors in the rat brain. J. Neurosci. Res. 22:194–200; 1989.
14. Dilsaver, S. C.; Alessi, N. E. Temperature as a dependent variable in the study of cholinergic mechanisms. Prog. Neuropsychopharmacol. Biol. Psychiatry 12:1–32; 1988.
15. Ehlert, F. J.; Kokka, N.; Fairhurst, A. S. Altered [3H] quinuclidinyl benzoate binding in the striatum of rats following chronic administration with diisopropylfluorophosphate. Mol. Pharmacol. 17: 24–30; 1980.
16. Gazit, H.; Silman, I.; Dudai, Y. Administration of an organophosphate causes a decrease in muscarinic receptors in the rat brain. Brain Res. 174:351–356; 1979.
17. Hallak, M.; Giacobini, E. Relation of brain regional physostigmine concentration to cholinesterase activity and acetylcholine and choline levels in rat. Neurochem. Res. 11:1037–1048; 1986.
18. 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.
19. 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.
20. Lichtblau, L.; Park, E. H.; Takemori, A. E.; Miller, J. W. Differential down-regulation of cholinergic receptors in brain and heart by soman and DFP. Pharmacologist 28:130; 1986.
21. Little, P. J.; Scimeca, J. A.; Martin, B. R. Distribution of [3H]diisopropylfluorophosphate, [3H]soman, [3H]sarin and their metabolites in mouse brain. Drug Metab. Dispos. 16:515–520; 1988.
22. Lomax, P.; Jenden, D. J. Hypothermia following systematic and intracerebral injection of oxotremorine in the rat. Int. J. Neuropharmacol. 5:353–359; 1966.
23. Lomax, P.; Kokka, N.; Lee, R. J. Acetylcholinesterase inhibitors and thermoregulation. In: Cooper, K.; Lomax, P.; Schonbaum, G., eds. Homeostasis thermal stress. 6th International Symposium. Basel: Karger; 1986:108–112.
24. McDonough, J. H.; Jaax, N. K.; Crowley, R. A.; Mays, M. Z.; Modrow, H. E. Atropine and/or diazepam therapy protects against soman-induced neural and cardiac pathology. Fundam. Appl. Toxicol. 13:256–276; 1989.
25. McLeod, C. G. Pathology of nerve agents: Perspectives on medi-

cal management. Fundam. Appl. Toxicol. 5:s10–s16; 1985.
26. Maayani, S.; Egozi, Y.; Pinchasi, I.; Sokolovsky, M. On the interaction of drugs with the cholinergic nervous system—V. characterization of some effects induced by physostigmine in mice: in vivo and in vitro studies. Biochem. Pharmacol. 27:203–211; 1978.
27. Meeter, E. Investigation of the rapid recovery of rat thermoregulation from soman poisoning. Eur. J. Pharmacol. 24:105–107; 1973.
28. Overstreet, D. H.; Helps, S. C.; Prescott, A. M.; Schiller, G. D. Development and disappearance of subsensitivity to pilocarpine following a single administration of the irreversible anticholinesterase agent, DFP. Psychopharmacology (Berlin) 52:263–269; 1977.
29. Overstreet, D. H.; Kozar, M. D.; Lynch, G. S. Reduced hypothermic effects of cholinomimetic agents following chronic anticholinesterase treatment. Neuropharmacology 12:1017–1032; 1973.
30. Russell, R. W.; Booth, R. A.; Smith, C. A.; Jenden, D. J.; Roch, M.; Rice, K. M.; Lauretz, S. D. Roles of neurotransmitter receptors in behavior: Recovery of function following decreases in muscarinic receptor density induced by cholinesterase inhibition. Behav. Neurosci. 103:881–892; 1989.
31. Schiller, G. D. Reduced binding of [3H] quinuclidinyl benzilate associated with chronically low acetylcholinesterase activity. Life Sci. 24:1159–1164; 1979.
32. Schwartz, R. D.; Kellar, K. J. In vivo regulation of [3H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs. J. Neurochem. 45:427–433; 1985.
33. Shih, T. M. Time course effects of soman on acetylcholine and choline levels in six discrete areas of the rat brain. Psychopharmacology (Berlin) 78:170–175; 1982.
34. Siakotos, A. N.; Filbert, M.; Hester, R. A. Specific radioisotopic assay for acetylcholinesterase and pseudocholinesterase in brain and plasma. Biochem. Med. 3:1–12; 1969.
35. Smolen, T. N.; Smolen, A.; Collins, A. C. Dissociation of the decreased numbers of muscarinic receptors from tolerance to DFP. Pharmacol. Biochem. Behav. 25:1293–1301; 1986.
36. Uchida, S.; Takeyasu, K.; Matsuda, T.; Yoshida, H. Changes in muscarinic receptors of mice by chronic administration of diisopropylfluorophosphate and papaverine. Life Sci. 24:1805–1812; 1979.
37. Ukai, Y.; Taniguchi, N.; Ishima, T.; Kimura, K. Muscarinic supersensitivity and subsensitivity induced by chronic treatment with atropine and diisopropylfluorophosphate in rat submaxillary glands. Arch. Int. Pharmacodyn. Ther. 297:148–157; 1989.
38. VanDongen, C. J.; Wolthuis, O. L. On the development of tolerance in rats following repeated injections of sublethal doses of two organophosphates. Pharmacol. Biochem. Behav. 34:473–481; 1989.
39. Viana, G. B.; Davis, L. H.; Kauffman, F. C. Effects of organophosphates and nerve growth factor on muscarinic receptor binding number in rat pheochromocytoma pc12 cells. Toxicol. Appl. Pharmacol. 93:257–266; 1988.
40. 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.
41. Yamada, S.; Isogai, M.; Okudaira, H.; Hayashi, E. Regional adaptation of muscarinic receptors and choline uptake in brain following repeated administration of diisoproplyfluorophosphate and atropine. Brain Res. 268:315–320; 1983.
42. Zhang, X.; Qin, B.-Y. Relationship between cholinesterase activity acetylcholine of the brain of mice acutely intoxicated with soman. Acta Pharmacol. Sin. 6:16–19; 1985.