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Behavioral and Biochemical Effects of the Carbamate Insecticide, Mobam[1,2,3]

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KURTZ, P. J. *Behavioral and biochemical effects of the carbamate insecticide, MOBAM.* PHARMAC. BIOCHEM. BEHAV. 6(3) 303–310, 1977. – Decreases in rat plasma, erythrocyte and brain cholinesterase levels after intraperitoneal injection of 1 to 5 mg/kg of 4-benzothienyl-N-methylcarbamate (MOBAM) were compared with decrements in both spontaneous motor activity and conditioned avoidance performance produced by this compound. Significant effects were observed with all five measured phenomena at dosages producing no obvious clinical signs. In albino rats, a dosage of 2 mg/kg significantly depressed plasma and erythrocyte cholinesterase activity, and decreased motor activity 15 min after injection but only higher dosages (3 and 5 mg/kg) significantly depressed brain cholinesterase activity and avoidance performance. In Long-Evans rats, both brain cholinesterase activity and avoidance performance were significantly reduced by the lower (2 mg/kg) dosage. The avoidance impairments observed after 3 mg/kg could be prevented by prior injection with atropine sulfate. It is suggested that both central and peripheral cholinesterase changes are important in determining the nature of the behavioral effects observed after exposure to this compound.

Behavioral toxicology Avoidance Cholinesterase MOBAM Pesticide
Carbamate Insecticide 4-Benzothienyl-N-methylcarbamate

A NUMBER of pharmacological agents which inhibit cholinesterase (ChE) activity have been shown to disrupt the performance of learned behavior [1, 2, 3, 17, 22]. Organophosphorous and carbamate insecticides also possess anticholinesterase properties and behavioral changes have been reported following human [6, 7, 20, 23] and animal [4, 10, 11, 13, 14, 15, 16, 18] exposure to some of these compounds as well. However, the nature of the relationship between the behavioral and biochemical effects of anti-cholinesterase pesticides is not clearly established. While there is some evidence to suggest that the behavioral toxicity of these substances is directly related to the inhibition of peripheral and/or central [2,11] ChE activity, there are also reports indicating little correlation between these behavioral and biochemical effects [10, 14, 15]. One investigator, for example, has offered the possibility that some behavioral changes may reflect sympathomimetic effects [18]. This issue is of interest from a toxicological standpoint because it is often assumed that if clinical measures of ChE activity are within normal ranges after human pesticide exposure then no serious threat to health or safety exists. If ChE activity and behavioral effects are not directly related, however, this assumption may be misleading.

The research reported here explored some of the behavioral and biochemical effects of the experimental contact insecticide, 4-benzothienyl-N-methylcarbamate (MOBAM) [21]. The purpose was to examine further the relationship between ChE activity and behavioral changes after exposure to a carbamate insecticide.

EXPERIMENT 1

The first experiment examined the dose- and time-dependent effects of MOBAM on conditioned avoidance behavior and on plasma and erythrocyte ChE activity.

METHOD

Animals

Two hundred male Sprague-Dawley albino rats (Wistar-derived strain), approximately 10 weeks of age (mean body weight 296 ± 32 g) were supplied from the colonies of the US Army Environmental Hygiene Agency. The rats were

[1] Registered trade name of the Mobil Chemical Company, New York, NY. Use of trademarked names does not imply endorsement by the US Army, but is used only to assist in identifying a specific product.

[2] Technicon and AutoAnalyzer are registered trademarks of the Technicon Instruments Corporation, Tarrytown, NY.

[3] The experiments reported here were conducted according to the Guide for Care and Use of Laboratory Animals (1972) as prepared by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council, DHEW Publication No. (NIH) 74-23.

[4] The opinions or assertions contained herein are the private views of the author and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

housed individually with free access to food and water in animal facilities accredited by the American Association for Accreditation of Laboratory Animal Care.

Apparatus and Procedure

The behavioral training procedure was adapted from one described by Clark [5] for the rapid acquisition of avoidance performance in the rat. The apparatus consisted of a small translucent plexiglass start box (9 cm × 12 cm × 21 cm) which opened into a larger (28 cm × 26 cm × 24 cm) cardboard-lined safe compartment by means of an 8 cm × 8 cm port. Training took place the day before injection and consisted of four shock-escape trials followed by two avoidance trials, each separated by 5 min intervals. A 1000 Hz 80 dB tone sounded from the beginning of each trial until an avoidance or escape response occurred. During the escape phase of the training, the hinged top of the start box was lifted and the animal placed inside facing a side wall. This was followed immediately by tone onset and the simultaneous delivery of 1.75 mA scrambled foot shock to the grid floor of the start box. When the animal had passed over the threshold to the safe compartment it was removed and returned to its cage. The avoidance trials which followed escape training were similar except that the animal was allowed 20 sec to leave the start box before foot shock onset.

On the next day, animals were given a preliminary avoidance trial. Rats failing to avoid shock during this trial were rejected from the study and replaced. The remaining rats were injected with the test compound, and then tested for avoidance at a preselected interval after injection. After this test, approximately 1 ml of blood was withdrawn by intracardiac puncture for the ChE analyses.

Cholinesterase activity was measured using the method described by Levine, Scheidt, and Nelson [12] except that 10 mM acetylthiocholine was used as a substrate. This micromethod employs a Technicon Auto Analyzer® in which the substrate is hydrolyzed and the released thiocholine measured colorimetrically after reaction with the sulfhydryl reagent 5,5'dithiobis(2-nitrobenzoic acid). The results were expressed in Garry and Routh [9] units. This procedure does not differentiate between true acetylcholinesterase and pseudocholinesterase, but Fowler and McKenzie [8] have shown that it is capable of detecting ChE inhibition by both organophosphate and carbamate pesticides.

Exposure

Technical grade MOBAM, with a stated purity of 99.6 percent, was obtained from Mobil Oil Co., New York, NY, and solutions were prepared using a commercial corn oil diluent. All injections were intraperitoneal (IP) and adjusted to 1 ml/kg volume. In the first experiment, rats received 1, 2, 3 or 5 mg/kg MOBAM or the corn oil vehicle (0 mg/kg) and were tested 15 min, 1 hr, 4 hr or 24 hr later. The animals were run in squads of five, one from each dosage group. Injections were staggered at one minute intervals to allow for subsequent testing. Ten animals from each dosage were tested at each interval and all injections, behavioral tests and biochemical analyses were performed blind.

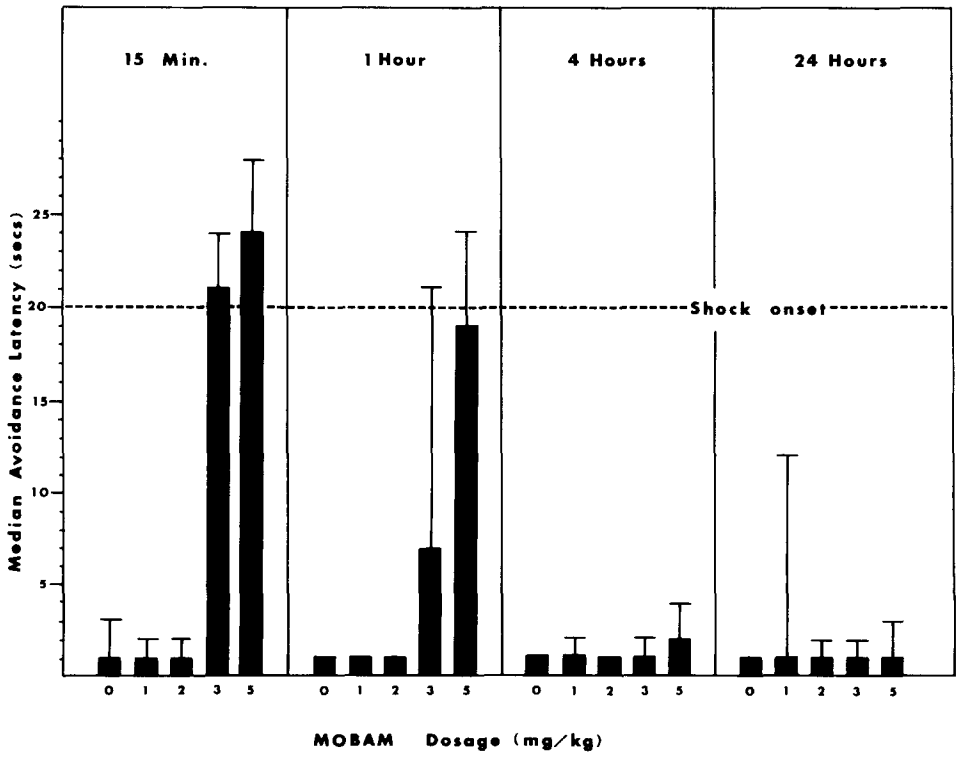

FIG. 1. Median avoidance latencies at various intervals following MOBAM injection. The range between the median and the third quartile is shown for groups in which the upper limit of this range exceeded 1.0.

RESULTS

Four rats failed to avoid shock during the preinjection avoidance test and were replaced. The overall median preinjection avoidance latency was 0.7 sec (intraquartile range 0.6–1.1 sec). The median postinjection latencies are illustrated in Fig. 1. Kruskal-Wallis Analyses of Variance [19] indicated significant effects at 15 min, $H(4) = 32.78$, $p<0.001$ and 1 hr, $H(4) = 20.88$, $p<0.001$, but not at 4 and 24 hr, $H(4) = 5.84$, $p>0.05$ and $H(4) = 4.22$, $p>0.05$, respectively. Comparisons within time intervals using Mann-Whitney U tests [19] showed that the avoidance latencies of both the 3 and 5 mg/kg groups were significantly greater than that of the 0 mg/kg control 15 min, $U = 7.5$, $p<0.002$ and $U = 0.0$, $p<0.002$, respectively, and 60 min, $U = 19.0$, $p<0.02$ and $U = 3.5$, $p<0.002$, respectively, following injection.

Two way analyses of variance indicated significant interactions between the dose and time interval factors for both plasma, $F(12,180) = 2.30$, $p<0.01$ and erythrocyte, $F(12,180) = 4.86$, $p<0.01$ ChE activity. The results of individual group comparisons are shown in Table 1. Statistically significant decreases in both plasma and erythrocyte ChE were observed from 15 min through 4 hr after injection at dose levels lower than those which produced avoidance decrements.

Clinical signs of toxicity (mild tremor, chewing movements) were observed in four of the ten animals treated with 5 mg/kg 15 min after injection but the appearance of rats treated with lower dosages was not distinguishable from that of normal animals.

In summary, plasma and erythrocyte ChE activity appeared to be most sensitive to MOBAM effects. Significant decreases in both were observed at 2 mg/kg. Avoidance performance was normal at this dosage although well-defined avoidance decrements were observed after 3 and 5 mg/kg. Finally, clinical signs of toxicity appeared at 5 mg/kg, the highest dose level studied.

EXPERIMENT 2

The second experiment examined two additional aspects of MOBAM toxicity: effects on spontaneous motor activity and brain ChE activity.

METHOD

Procedure

Five groups of eight naive male rats, similar to those used in Experiment 1, were injected with corn oil or 1, 2, 3 or 5 mg/kg MOBAM. Fifteen minutes later, the time interval corresponding to the peak effects observed in the first experiment, the rats were placed in an actophotometer (Lehigh Valley Electronics Model 1497). Activity counts were comprised of the total number of photo-beam interruptions accumulated by each animal during individual 5 minute test sessions. Immediately after the test, animals were decapitated, the brains removed and frozen. Later, 0.5 g samples were taken from the cerebral cortex and, using a 1:50 homogenization with 0.9 percent saline, assessed for ChE activity as described above.

RESULTS

Figure 2 illustrates the effects of MOBAM on spontaneous motor activity. The data are expressed as percentage of the mean control group score. A one-way analysis of variance conducted on the activity totals indicated a significant dose response effect $F(4,35) = 29.36$, $p<0.001$. In comparison with the corn oil control (0 mg/kg), the activity scores of the 2, 3 and 5 mg/kg groups were significantly depressed, $t(14) = 2.85$, $p<0.02$; $t(14) = 7.15$, $p<0.002$; $t(14) = 18.15$, $p<0.002$, respectively. The effects of MOBAM on brain ChE are shown in Table 2. A one-way analysis of variance on these data also indicated a significant dose response-effect $F(4,35) = 9.27$, $p<0.001$. Significant decreases were observed after treatment with 3 and 5 mg/kg, $t = 3.38$, $p<0.01$; $t = 4.54$, $p<0.002$, respectively, but not with 1 or 2 mg/kg.

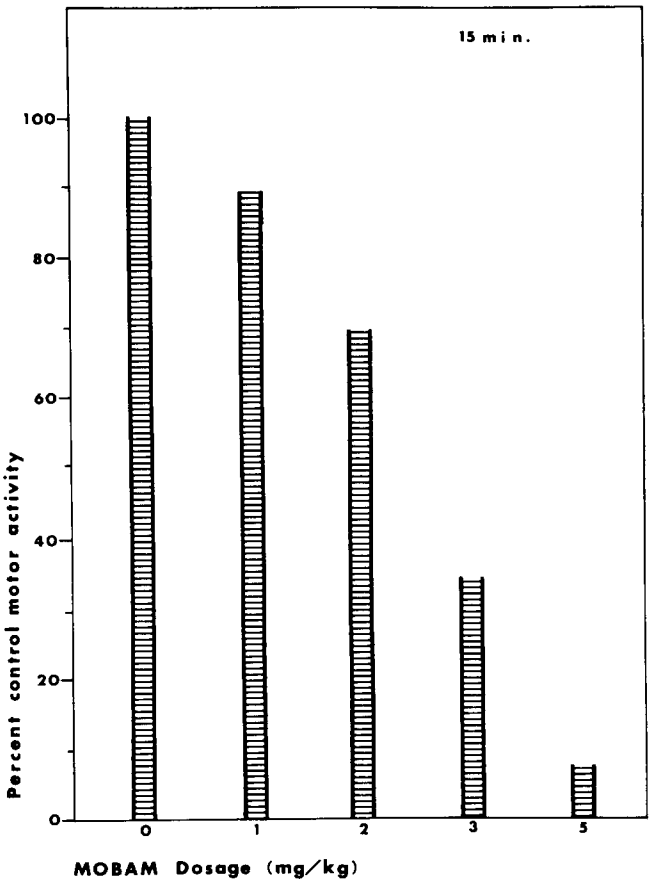

FIG. 2. Spontaneous motor activity as percentage of the mean control group score after MOBAM injection.

While the avoidance performance of animals given 2 mg/kg was identical to that of control animals in the first experiment, the results of the second experiment indicate that this dosage significantly depressed motor activity. Thus, the two behavioral measures appear to exhibit differential sensitivity to MOBAM effects. It also appears that plasma and erythrocyte ChE activity are more sensitive than brain ChE activity to MOBAM effects.

It is possible that in the latter case, however, the difference in MOBAM sensitivity may reflect the fact that blood ChE values were taken from animals which had previously received avoidance training while the animals assessed for brain ChE had not. The dose response effects of MOBAM on ChE activity may vary depending upon the nature of previous experience, particularly exposure to foot shock.

TABLE 1

ALBINO RAT ERYTHROCYTE AND PLASMA CHOLINESTERASE ACTIVITY (G & R UNITS) FOLLOWING MOBAM INJECTION

	MOBAM Dosage (mg/kg)*				
	0	1	2	3	5
			15 MIN		
Erythrocyte ChE Activity					
Mean ± SD	10.1 ± 1.2	7.8 ± 1.6	5.4 ± 1.6	4.2 ± 1.5	3.1 ± 0.7
% Control		77%	53%	42%	31%
p vs control†		$p<0.002$	$p<0.002$	$p<0.002$	$p<0.002$
Plasma ChE Activity					
Mean ± SD	3.6 ± 0.5	3.3 ± 1.2	2.6 ± 0.9	2.3 ± 1.2	1.4 ± 0.4
% Control		90%	70%	63%	38%
p vs control		NS	$p<0.01$	$p<0.01$	$p<0.002$
			1 HR		
Erythrocyte ChE Activity					
Mean ± SD	9.6 ± 1.9	8.8 ± 2.3	8.3 ± 2.2	7.5 ± 3.1	5.1 ± 1.8
% Control		92%	87%	78%	54%
p vs control		NS	NS	NS	$p<0.002$
Plasma ChE Activity					
Mean ± SD	3.6 ± 0.8	3.4 ± 1.2	3.0 ± 1.0	2.6 ± 0.7	2.4 ± 1.4
% Control		95%	82%	73%	68%
p vs control		NS	NS	$p<0.02$	$p<0.05$
			4 HR		
Erythrocyte ChE Activity					
Mean ± SD	10.0 ± 2.0	9.8 ± 2.0	9.4 ± 1.5	9.7 ± 1.6	7.9 ± 1.5
% Control		98%	94%	97%	79%
p vs control		NS	NS	NS	$p<0.02$
Plasma ChE Activity					
Mean ± SD	4.0 ± 0.8	4.1 ± 1.4	4.0 ± 0.7	3.7 ± 0.6	3.3 ± 0.4
% Control		103%	100%	93%	83%
p vs control		NS	NS	NS	$p<0.05$
			24 HR		
Erythrocyte ChE Activity					
Mean ± SD	10.0 ± 1.8	9.4 ± 1.3	9.3 ± 2.0	9.5 ± 1.5	9.1 ± 1.4
% Control		94%	93%	95%	91%
p vs control		NS	NS	NS	NS
Plasma ChE Activity					
Mean ± SD	4.3 ± 0.6	4.2 ± 1.1	4.0 ± 0.6	4.1 ± 0.4	4.0 ± 0.5
% Control		98%	95%	96%	93%
p vs control		NS	NS	NS	NS

*n = 10 per group.
†Two-tailed probability values associated with Student's t-statistics.
NS (not significant) is given where $p>0.05$.

EXPERIMENT 3

If the avoidance impairments produced by MOBAM are related to the inhibition of ChE activity, it may be possible to attenuate the performance deficits by administering the cholinergic antagonist, atropine sulfate, prior to MOBAM injection. The third experiment explored this possibility. In addition, the effects of MOBAM on brain ChE were reexamined using animals which had received avoidance training.

TABLE 2

ALBINO RAT BRAIN CHOLINESTERASE ACTIVITY (G & R UNITS) 15 MIN AFTER MOBAM INJECTION

	Dosage MOBAM (mg/kg)				
	0	1	2	3	5
Experiment 2*					
Mean ± SD	61 ± 16	60 ± 14	51 ± 10	40 ± 7	34 ± 6
Experiment 3†					
Mean ± SD	53 ± 18	‡	48 ± 11	41 ± 15	‡
Experiments 2 & 3 Combined					
Mean ± SD	56 ± 17	60 ± 14	49 ± 10	41 ± 12	34 ± 6
% Control		107%	88%	73%	61%
p vs -control§		NS	NS	$p<0.01$	$p<0.002$

*n=8 per group.
†n=10 per group.
‡No animals run in this dosage group.
§Two-tailed probability values associated with Student's t-statistics.
NS (not significant) is given where $p>0.05$.

METHOD

Six groups of ten male rats were given avoidance training as before. Following the preliminary avoidance test on the day after training, two groups received IP injections of 5 mg/kg atropine sulfate while the remaining three were injected with the 0.9 percent saline vehicle. Forty-five minutes later, the two groups pretreated with atropine were injected with either corn oil or 3 mg/kg MOBAM. The remaining three saline pre-injected groups received corn oil, 2 mg/kg or 3 mg/kg MOBAM. All were tested for avoidance 15 min after the second injection. Following the avoidance test, the animals were sacrificed and brain samples taken for cholinesterase analysis as in Experiment 2.

RESULTS

The overall median preinjection avoidance latency was 0.8 sec (intraquartile range 0.7–1.2). The postinjection avoidance latencies are shown in Fig. 3. For comparison, the avoidance scores of the 15 min groups from the first experiment are also shown in this figure. In the groups receiving the saline preinjection, the effects of corn oil (0 mg/kg), 2, and 3 mg/kg were highly similar to those found in the first experiment. As with that experiment, the avoidance latencies of the 3 mg/kg group were significantly greater than those of the 0 mg/kg control, U = 0.0, $p<0.002$, while the latencies of the 2 mg/kg group were not, U = 31.5, $p>0.05$. However, if administration of 3 mg/kg MOBAM was preceded by 5 mg/kg atropine, avoidance performance was similar to that of the control group, U = 28, $p>0.05$. Animals receiving atropine followed by saline also showed normal performance, U = 41, $p>0.05$. Thus, atropine pretreatment effectively blocked the effects of MOBAM on avoidance performance.

The effects of 0, 2 and 3 mg/kg MOBAM on brain ChE were compared with the respective values obtained in Experiment 1 using t-tests and no significant differences were observed. Consequently the data from the two experiments were combined and the results with appropriate statistical comparisons are shown in Table 2. As

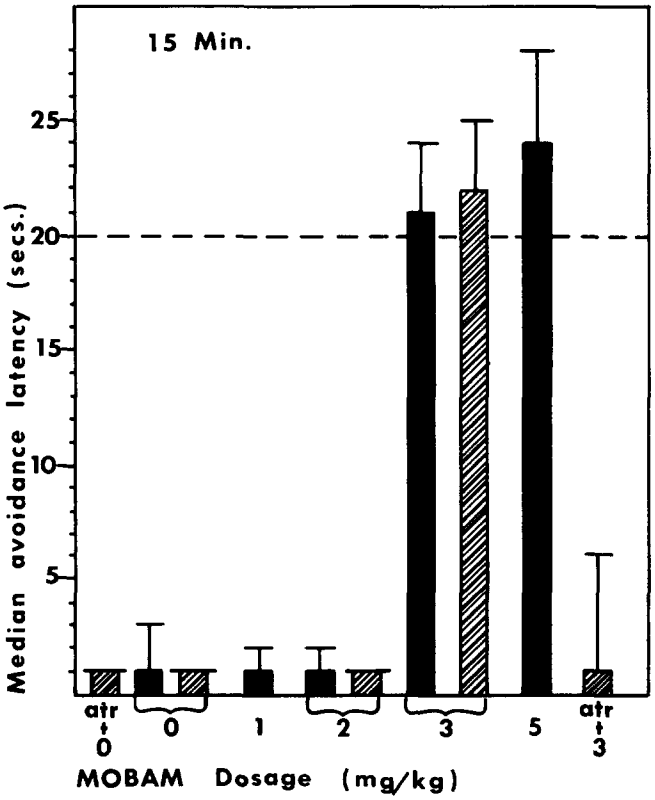

FIG. 3. Median avoidance latencies 15 min following MOBAM injection. The range between the median and the third quartile is shown for each group. Solid bars refer to the scores of animals in Experiment 1, cross-hatched to those in Experiment 3. Two groups (atr + 0 and atr + 3) received atropine sulfate 45 min prior to MOBAM injection. The dashed horizontal line indicates shock onset.

before, significant changes were observed only with the 3 and 5 mg/kg dosages.

The results of this experiment replicated the effects of MOBAM on avoidance performance and brain ChE activity

TABLE 3

HOODED RAT AVOIDANCE PERFORMANCE AND BRAIN, PLASMA, AND ERYTHROCYTE ChE ACTIVITY 15 MIN AFTER INJECTION

	MOBAM Dosage (mg/kg)*			
	0	2	3	5
Avoidance				
Median latency	1.0	2.6	22.4	23.6
IQ Range	0.4–1.2	1.2–19.4	16.4–22.9	21.4–25.8
p vs control†		$p<0.02$	$p<0.002$	$p<0.002$
Brain ChE Activity				
Mean ± SD	80 ± 18	57 ± 11	49 ± 10	33 ± 6
% Control		71%	62%	42%
p vs control		$p<0.01$	$p<0.002$	$p<0.002$
Erythrocyte ChE Activity				
Mean ± SD	10.2 ± 1.4	5.3 ± 1.4	4.1 ± 2.0	2.3 ± 0.3
% control		52%	40%	22%
p vs control		$p<0.002$	$p<0.002$	$p<0.002$
Plasma ChE Activity				
Mean ± SD	3.2 ± 1.0	2.4 ± 1.0	2.2 ± 0.6	1.6 ± 0.4
% control		75%	68%	32%
p vs control		NS	$p<0.02$	$p<0.002$

*n=10 per group.
†Two-tailed probability values associated with statistical comparisons.
NS is given where $p>0.05$.

found in the previous two experiments. Again, it appears that both brain ChE activity and avoidance behavior remain at or near normal levels after 2 mg/kg MOBAM, a dosage which significantly reduces motor activity and blood ChE activity. When the dosage is increased to 3 mg/kg however, brain ChE levels and avoidance performance are also significantly reduced. Of course, the correspondence between the onset of significant brain ChE inhibition together with avoidance impairment at the 3 mg/kg dosage may simply be coincidental. On the other hand, the finding that avoidance deficits could be prevented by atropine preinjection suggests, at least, that the behavioral effect is in some way related to cholinergic activity. The correspondence between the effects of MOBAM on avoidance and brain ChE observed at 3 mg/kg may reflect the possibility that central ChE changes play an important role in the effects of the compound on avoidance behavior.

EXPERIMENT 4

Preliminary work in our laboratory suggested that rats of the Long-Evans ("hooded") strain may be more sensitive to MOBAM than the albino strain used in the previous experiments. Specifically, the MOBAM IP LD_{50} for albino rats of the same description as those used here was 88 mg/kg (95 percent confidence limits 63–122) while for the hooded strain it was 44 mg/kg (95 percent confidence limits 35–54). This difference in MOBAM sensitivity offered an opportunity to extend the investigation of the relationship between the behavioral and biochemical effects of this compound in the rat. We wished to know whether or not the threshold dosage for avoidance impairments and significant brain ChE inhibition would be the same in this strain as they were for the albino rats.

METHOD

Forty male hooded rats (mean body weight 319 ± 24 g) were given avoidance training as described above. Following the preinjection test trial, groups of ten received IP injections of either corn oil or 2, 3, or 5 mg/kg MOBAM. All were tested for avoidance 15 min later. Following this, 1 ml blood samples were taken for plasma and erythrocyte ChE activity analysis. The rats were then decapitated and the brains removed and analyzed for brain ChE activity as in Experiments 2 and 3.

RESULTS

Clinical signs of toxicity were observed in all of the hooded rats injected with 5 mg/kg 15 min after injection. There were no clinical signs in the other groups.

The overall median preinjection avoidance latency was 0.7 sec (intraquartile range, 0.5–0.9 sec), which is similar to that of the albino rats. The median postinjection latencies are shown in Table 3. A Kruskal-Wallis Analysis of Variance indicated a significant dose response effect, $H(3) = 27.63$, $p<0.001$. The results of the individual group comparisons shown in the table indicated that, in contrast with the albino rat data, the avoidance latencies of the 2 mg/kg group were significantly greater than those of the control, as were the scores of the 3 and 5 mg/kg group.

Analyses of variance indicated significant MOBAM effects on brain, erythrocyte, and plasma ChE activity,

$F(3,36) = 25.78, 53.90$, respectively, all $p<0.001$. The results of individual comparisons shown in the table indicated significant brain ChE activity decreases in the 2 mg/kg group, which also contrasts with the albino rat data. Decreases in erythrocyte but not plasma ChE activity were also statistically significant at this dosage. The 3 and 5 mg/kg dosages produced significant decreases in all three ChE measures.

If one compares erythrocyte and plasma ChE activity 15 min after injection in the hooded and albino rats (Tables 1 and 3), it appears that the two strains show similar levels of blood ChE activity in both control and MOBAM-exposed groups. Control levels of brain ChE activity, however, appear appreciably higher in the hooded strain (Tables 2 and 3). When the effect of MOBAM injection is measured as percentage of control group brain ChE activity, it can be seen that the response of the hooded rats to 2 mg/kg MOBAM (71 percent of control) is more like that of the albino rats to 3 mg/kg (73 percent of control). In both cases, this extent of brain ChE inhibition coincided with significant avoidance impairments. These results support the possibility, suggested earlier, that the effects of MOBAM on avoidance performance are related to the inhibition of brain ChE activity.

GENERAL DISCUSSION

The preceding experiments examined the effects of the carbamate insecticide, MOBAM, on one-way avoidance performance, spontaneous motor activity, and plasma, erythrocyte and brain ChE activity. Significant effects on all measures were observed at dosages below those necessary to produce obvious clinical signs of toxicity. In albino rats, a dosage of 2 mg/kg significantly depressed plasma and erythrocyte ChE activity and decreased motor activity 15 min after injection. Higher dosages, 3 and 5 mg/kg, significantly depressed brain ChE activity and avoidance performance as well. The avoidance impairments observed after 3 mg/kg could be prevented by prior injection with atropine sulfate. Hooded rats appeared to be slightly more sensitive to the effects of MOBAM. In this strain, a lower dosage, 2 mg/kg, produced a small but statistically significant decrement in avoidance performance together with a significant decrease in brain ChE activity.

One might speculate that the avoidance deficits reported here are simply a consequence of the more severe decrements in spontaneous motor activity observed at the higher dosages. However, the fact that in the albino rats avoidance performance was normal at a dosage which significantly decreased spontaneous motor activity (2 mg/kg) suggests that other factors may be involved. The correspondence between the onset of significant decreases in both blood ChE and motor activity at one dosage and the onset of significant decrements in both brain ChE activity and avoidance performance at a higher dosage suggests a second possible interpretation. This is that reductions in spontaneous motor activity may be related to peripheral, perhaps neuromuscular ChE inhibition while avoidance impairments may reflect the additional effects of significant ChE inhibition in the brain.

The assumption that different mechanisms underlie the two behavioral effects observed here would help to explain the apparent difference in their dose-response functions: Avoidance performance rapidly deteriorates between 2 and 3 mg/kg while motor activity declines more gradually throughout the dose range explored.

More specific behavioral tests could help to elucidate the issue but the data presented here suggest that the behavioral toxicity of anticholinesterase agents such as the carbamate pesticides may be more clearly understood by considering the possibility of multiple behavioral effects mediated to different degrees by both peripheral and central cholinesterase inhibition.

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