Separation of the Effects of Magnesium Pemoline on Avoidance Learning and Memory from Its Central Nervous System Stimulant Properties by Chlordiazepoxide (35948)

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Since Plotnikoff (1) reported that magnesium pemoline (PMH) enhanced the acquisition and retention of a jump out response in rats, numerous attempts to both substantiate and disprove his findings have been undertaken. There are those who take the PMH effect to reflect an actual facilitation of the processes underlying the mechanisms of learning and memory (2–7) such as stimulation of true RNA polymerase (8–9), while opposing views attribute the PMH phenomenon to be the result of nonspecific hyperactivity which may lead to an improvement in general performance (10–13).

To exclude the latter possibility, namely, that the increase in learning and memory following the administration of PMH was due to nondirected central nervous system (CNS) stimulation, I studied the effects of PMH on the acquisition and retention of a maze task in mice that had been pretreated with chlordiazepoxide (CDP). It was hoped that in this way the stimulant property inherent in the activity of PMH would be antagonized so that the effects on learning and memory could be more clearly evaluated.

Chlordiazepoxide was chosen for this purpose because it has been shown to act more specifically at the midbrain and limbic structures (14, 15), rather than as a general CNS depressant.

To determine a dose of CDP that would block the increase in motor activity following PMH, 25 male Swiss-Webster mice were treated with 20 mg/kg, ip, of PMH, placed in a photocell activity cage (16), and observed for 30 min (Fig. 1). These animals showed a marked increase in spontaneous activity when compared with saline-injected controls.

To antagonize the stimulant effects of PMH as seen in the activity parameter, increasing doses of CDP (5, 10, 15, and 20 mg/kg) were administered subcutaneously 5 min prior to intraperitoneal PMH.[1] To duplicate the dosing schedule employed in the learning situation, 30 min were allowed to pass before testing. It was found that 15 mg/kg of CDP inhibited the heightened motor activity produced by PMH so that mice treated with both agents were indistinguishable from controls in the photocell cage.

The next objective was to evaluate PMH in the "sedated" mouse to determine whether the inhibition of motor activity (general CNS action) could be separated from the reported effects on learning and memory.

To achieve this end, a double-T maze was employed after the method of Barondes and Cohen (17). Briefly, this apparatus consists of a dull grey wooden chamber constructed in a double-T configuration, with a grid of 3/16 in. stainless steel rods separated by 1/4 in. A constant current of 1.5 mA at 0.75 kV, dc was delivered in 1 sec shocks to the grid which was wired in parallel through a power transformer. The correct path was the left arm of the first T and the right arm of the second T. A. clear plastic guillotine door closed after the mouse left the start and after it reached the goal. Each training period proceeded as follows: 30 min prior to being placed in the maze, the animal received a subcutaneous injection and 5 min later an

[1] Since it has been found that the maximal brain concentration of PMH is reached 30 min following its intraperitoneal administration (7, 8), I waited this time before testing the animals both in the activity device as well as in the maze. CDP was given subcutaneously to take advantage of the more prolonged onflow to cover the 30 min delay and the additional 45 min trial.

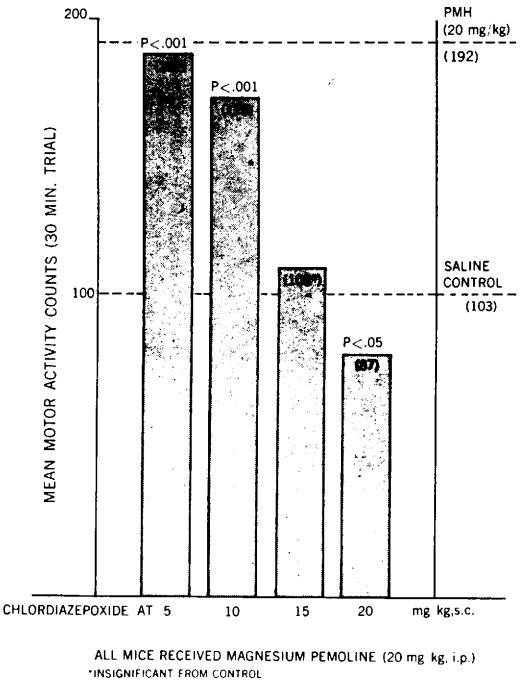

FIG. 1. The effect of magnesium pemoline (20 mg/kg, ip) and chlordiazepoxide on random motor activity in mice during a 30 min trial. Injections are 5 min apart, starting 30 min *before* placement in the activity cage. Data are the means of a minimum of 10 male mice. The standard error is less than 2.14 for all groups. The control is saline & saline. The combination of PMH and CDP was compared to the saline control using Student's t test; $p<.05$ was taken to indicate a significant difference.

intraperitoneal injection according to its group designation. Group 1: saline (0.2 ml, the mean injected dose), saline; Group 2: saline, tragacanth (PMH was suspended in 0.3% tragacanth[2]); Group 3: CDP (15 mg/kg sc), saline; Group 4: saline, PMH (20 mg/kg, ip); and Group 5: CDP (15 mg/kg, sc), PMH (20 mg/kg, ip).

The animals were then placed in the maze and allowed 1 min of free exploration before being put into the starting compartment. If the animal remained in the start box for 15 sec, a shock was delivered until the mouse proceeded from this area. Animals were shocked subsequently each time the wrong alley was entered. The subject was allowed to retrace its path, but was only shocked for entering an incorrect alley.[3] Trials were continued during the training and retesting sessions until 9 out of 10 consecutive error-free trials were achieved. Twenty-four hours following the training period, the mice were retested; however, incorrect alley choices were only punished during the first 2 trials in which they occurred.[4] Mean scores with standard errors for the total number of errors, as well as number of trials to criterion for both training and retesting periods (during pretrial testing) are shown in Table I.

The data are divided into three control groups (1, 2, and 3) and two experimental groups (4 and 5), each composed of a minimum of 10 randomly chosen male Swiss mice. The Student's t test was used to determine the significance of all data.

These data show that control animals took an average of 36.1 trials before achieving the criterion score during the initial training period and when retested 24 hr later, required 34.3 trials. This represents an insignificant improvement in the actual number of trials. However, these control animals are significantly ($p<.01$) more efficient during retesting as evidenced by the errors parameter. Whereas animals made an average of 33.0 mistakes during training, when retested 24 hr later, they improved to 17.7 errors. This reflects a 46.4% increase in efficiency/trial.

It is of interest that mice injected with CDP showed no alterations either in the acquisition or the retention of the maze task compared with the control groups. This emphasizes the fact that CDP does not interfere with higher cortical functions, making this drug ideally suited for these experiments.

On the other hand, mice treated with PMH 30 min before training showed a marked improvement in both acquisition and retention of the maze. The data show that

[2] PMH was suspended in tragacanth, an inert gum, to facilitate even administration of the insoluble agent. Once I determined that it was inert in my system, I substituted saline in its place in the remaining groups.

[3] This method involves avoidance behavior and may not provide the ideal approach to my problem, if only the pretrial situation is considered.

[4] There were no differences among the five drug groups as to when the two shock trials occurred.

TABLE I. Pretrial Testing: The Effect of Magnesium Pemoline (20 mg/kg, ip, suspended in 0.3% tragacanth) and Chlordiazepoxide (15 mg/kg, sc) on Acquisition and Retention of a Double-T Maze Task in Mice.

Data are the means of a minimum of 10 mice ± standard error of the mean. Retesting is 24 hr after training, and criterion is 9 out of 10 consecutive error-free trials. Trials and errors are total to criterion. Injections are 5 min apart, starting 30 min *before* training.

Drug treatment	Group	Training		Retesting	
		Errors	Trials	Errors	Trials
Saline + saline	1	33.0 ± 0.61	36.1 ± 0.78	17.7 ± 0.63	34.3 ± 0.76
Saline + tragacanth	2	31.8 ± 1.15	34.1 ± 1.07	17.4 ± 0.65	34.0 ± 0.87
Chlordiazepoxide + saline	3	31.7 ± 0.99	36.0 ± 0.67	16.3 ± 0.73	33.3 ± 0.60
Saline + magnesium pemoline[a]	4	14.7 ± 0.56	21.8 ± 0.55	5.3 ± 0.46	17.2 ± 0.36
Chlordiazepoxide + magnesium pemoline[a]	5	15.5 ± 0.69	23.6 ± 0.54	5.4 ± 0.30	17.5 ± 0.54

[a] Significant differences ($p < .01$ by Student's t test) from saline control (Group 1) in all columns.

PMH animals required only 21.8 trials (control, 36.1) to achieve criterion during training and 17.2 trials (control, 34.3) during retesting. Even more striking was the efficiency score of 14.7 errors (control, 33.0) for training and 5.3 errors (control, 17.7) for retesting. Animals receiving both PMH and CDP were not significantly different from those animals treated with PMH alone in the maze paradigm. The data for this group show 23.6 trials with 15.5 errors to criterion during training and 17.5 trials with 5.4 errors during retesting. Thus, although CDP completely reversed the nonspecific stimulation produced by PMH, the same dose has no apparent effect on the increased acquisition and retention produced by PMH on the maze response.

Certain shortcomings are inherent in all methods utilized in studies of this kind. Because my procedure involves an avoidance task, the question arises as to what effects the drugs may have upon fear, sensitivity to shock, and other extraneous factors that may lead to erroneous conclusions. Therefore, to further support the pretrial data, posttrial injections were employed. In this part of the study, the animals were treated exactly as before except that the series of injections was given from 1 min following termination of the last trial to 240 min thereafter. Thus, the maze task was learned without drug treatment, eliminating the possible interfering factors. Table II presents the data from this experiment.

It is clear that animals injected immediately posttrial showed improvement quite similar to that observed in the pretrial groups. Control mice injected either with saline or CDP after the last training trial show a significant improvement in errors but not trials to criterion. Animals injected with PMH at the same time after training and retested 24 hr later showed a marked improvement in errors to criterion (6.2 as compared to controls, 21.6).

Unlike the control groups, PMH-treated mice also showed a significant improvement in trials to criterion (controls, 35.2; PMH, 20.2). When drugs were administered 15 and 30 min posttrial, no differences are seen compared to the 1 min group. Significant differences do emerge between 30 and 60 min and again between 120 and 180 min. When PMH is administered 180 min posttrial, no further improvement is seen. These data suggest that PMH may augment the early phase of consolidation, although to comment on the mechanism of this facilitation would be speculative.

When both CDP and PMH were administered posttrial, the mice behaved exactly as animals treated with PMH alone with regard to performance in the maze. Thus, as demonstrated earlier, although CDP completely suppresses the increased motor activity seen following the administration of PMH, it does not alter its facilitative actions upon the ac-

TABLE II. Posttrial Testing: The Effect of Magnesium Pemoline (20 mg/kg, ip, suspended in 0.3% tragacanth) and Chlordiazepoxide (15 mg/kg, sc) on Acquisition and Retention of a Double-T Maze Task in Mice.

Data are the means of a minimum of 10 mice ± standard error of the mean. Retesting is 1 and 7 days after training, and criterion is 9 out of 10 consecutive error-free trials. Trials and errors are total to criterion. Injections are 5 min apart, starting at various times *after* the last training trial (called "injection time"). Group 2 (saline and tragacanth control) was not included in this experiment.[2]

Drug treatment	Group	Injection time (min)	Training[a]		Retesting			
					1 day		7 days	
			Errors	Trial	Errors	Trials	Errors	Trials
Saline + saline	1	1	36.0 ± 1.12	36.2 ± 0.40	21.2 ± 0.72	35.2 ± 1.97	27.0 ± 1.91	37.2 ± 1.22
		30	34.9 ± 0.51	36.1 ± 0.41	21.1 ± 0.81	36.1 ± 1.18	26.6 ± 0.82	36.1 ± 1.14
		60	37.2 ± 0.62	38.0 ± 0.66	19.1 ± 1.47	37.4 ± 0.79	28.1 ± 0.99	38.1 ± 0.81
Chlordiazepoxide + saline	3	1	32.8 ± 0.51	37.0 ± 0.38	21.6 ± 0.87	36.1 ± 2.20	26.6 ± 1.66	38.3 ± 2.41
		30	35.1 ± 1.12	36.6 ± 0.91	23.0 ± 0.68	33.9 ± 2.11	24.4 ± 1.24	34.2 ± 0.36
		60	33.4 ± 0.72	34.3 ± 0.76	22.1 ± 1.41	35.0 ± 1.01	27.6 ± 1.29	36.4 ± 1.51
Saline + magnesium pemoline[b]	4	1	35.1 ± 0.68	37.2 ± 0.45	6.9 ± 1.09	20.2 ± 1.42	7.2 ± 0.67	23.2 ± 1.60
		15	33.6 ± 0.67	38.1 ± 1.20	5.8 ± 0.81	19.0 ± 0.96	9.0 ± 0.81	21.8 ± 0.91
		30	32.8 ± 1.12	37.8 ± 1.16	7.1 ± 0.62	18.2 ± 1.35	8.3 ± 0.48	23.1 ± 1.21
		60	34.3 ± 1.26	39.1 ± 1.64	12.3 ± 0.51	25.3 ± 1.58	15.1 ± 0.71	29.4 ± 0.82
		120	34.9 ± 0.81	38.7 ± 0.98	11.8 ± 0.40	28.8 ± 1.12	16.0 ± 0.99	30.7 ± 0.87
		180	32.0 ± 0.62	39.5 ± 0.91	19.4 ± 0.37	37.2 ± 1.62	24.1 ± 1.46	38.1 ± 1.61
		240	35.0 ± 1.40	37.2 ± 0.79	18.6 ± 0.82	38.5 ± 2.44	25.2 ± 1.60	39.0 ± 2.01
Chlordiazepoxide + magnesium pemoline[b]	5	1	33.4 ± 1.69	36.9 ± 1.38	6.2 ± 0.71	19.1 ± 0.99	7.4 ± 0.80	21.8 ± 1.61
		15	35.1 ± 2.02	39.2 ± 2.07	6.8 ± 0.44	20.3 ± 0.84	8.3 ± 0.91	23.5 ± 1.80
		30	32.4 ± 1.61	36.4 ± 1.62	8.2 ± 0.38	18.7 ± 0.88	9.0 ± 0.86	22.8 ± 1.92
		60	34.3 ± 0.79	38.1 ± 0.66	11.4 ± 0.84	24.3 ± 1.25	16.2 ± 1.22	30.1 ± 1.42
		120	35.8 ± 0.33	37.1 ± 0.62	12.9 ± 0.88	27.9 ± 1.41	15.5 ± 1.48	31.2 ± 1.51
		180	32.9 ± 0.44	38.4 ± 0.81	20.3 ± 0.62	36.2 ± 1.92	26.1 ± 1.76	37.8 ± 1.68
		240	31.8 ± 1.01	37.0 ± 0.71	21.0 ± 0.41	37.5 ± 2.01	27.9 ± 1.32	38.9 ± 1.21

[a] No significant differences among groups in training.

[b] In Groups 4 and 5 during retesting the values at injection times 1 through 120 are significantly different ($p < .01$) from Group 1.

quisition and retention of the maze task. Furthermore, PMH shows this action both when given 25 min prior to testing, as well as 1 to 120 min following the final trial. With regard to the posttrial sequence, PMH animals tested 7 days following the training session still demonstrated a marked improvement in the retention of the task.

One issue of concern is the lack of marked improvement in trials to criterion both in the control mice, as well as in the PMH-treated mice (during pretrial testing). It is quite common for two parameters to measure the same learning situation differently. Some insight into this question may be obtained from the experiment employing posttrial injections. Here it can be seen that control animals again do not improve in the trials to criterion parameter. Animals receiving either PMH or PMH and CDP show almost a 50% improvement in trials to criterion. It should be realized, however, that in the pretrial study, trials to criterion was significantly lower in the PMH groups compared to controls, although no further improvement was noted upon retesting. The base line for trials was 23.6, and during retesting this dropped slightly to 17.5. This may indicate that a limit has been reached in this species, and little if any further improvement is possible. In the posttrial experiment, the training sessions for PMH- and saline-treated animals result in the same number of trials to criterion, since the injections are given after training. This lack of difference among over 200 mice points to the consistency of the results. The base line for trials is therefore at the control level of 36.2, allowing a large measure of improvement towards the limiting level of about 17 trials. This is borne out by trials to criterion during posttrial testing in both the PMH (20.2) and CDP and PMH (19.1) groups. This explanation may help clarify a commonly encountered issue.

The combined administration of CDP, a minor tranquilizer of the benzodiazepam group, and PMH, assumed for many years to be one of the nonspecific CNS stimulants, provides a suitable model for examining the proposed property of PMH on learning and memory processes. I have succeeded in separating the nonspecific stimulant effect as demonstrated by photocell activity measurements and have further clearly shown a remaining facilitatory influence on maze acquisition and retention.

Although this study was not designed to disclose the mechanism by which PMH acts, it does point to some specific action upon brain circuits which underlie information storage and/or retrieval. Further studies should be undertaken using other testing procedures to more fully elucidate the precise effect of PMH on various learning parameters. The present report simply emphasizes that PMH is acting by augmenting the handling of learned input, rather than by nonselective CNS stimulation.

Summary. Magnesium pemoline (20 mg/kg, ip) was found to enhance the acquisition and retention of a double-T maze in male Swiss-Webster mice when administered 25 min prior to training or from 1 to 180 min posttrial. The general stimulant action of magnesium pemoline was completely abolished by pretreatment with chlordiazepoxide (15 mg/kg, sc), although the mice still showed enhanced memory of the avoidance task. These data suggest that the nonspecific arousal associated with magnesium pemoline is not responsible for the demonstrated effects upon avoidance learning and memory.

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