Title
MEMORY FACILITATING AND ANTI-AMNESIC EFFECTS OF CORTICOSTEROIDS

Author
Flood, J.F.

Publication Date
1977-07-01

Submitted to Pharmacology, Biochemistry and Behavior

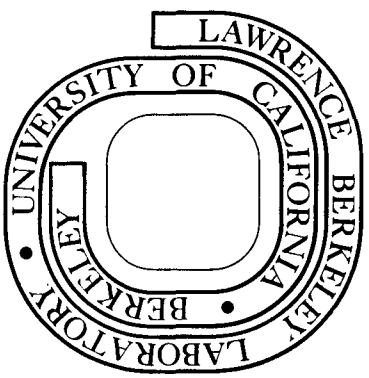
LBL-6709
Preprint

MEMORY FACILITATING AND ANTI-AMNESIC EFFECTS OF CORTICOSTEROIDS

James F. Flood, Daniel Vidal, Edward L. Bennett, Ann E. Orme, Sergio Vasquez, and Murray E. Jarvik

July 21, 1977

Prepared for the U. S. Energy Research and Development Administration under Contract W-7405-ENG-48



DISCLAIMER

MEMORY FACILITATING AND ANTI-AMNESIC EFFECTS OF CORTICOSTEROIDS

James F. Flood, Daniel Vidal

Department of Psychiatry
University of California
Los Angeles, California 90024

Edward L. Bennett, Ann E. Orme

Laboratory of Chemical Biodynamics
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

Sergio Vasquez

Department of Pharmacology
University of California
Los Angeles, California 90024

and

Murray E. Jarvik

Department of Psychiatry &
Department of Pharmacology
University of California
Los Angeles, California 90024

Veterans Administration Hospital-Brentwood
Psychopharmacology Unit
Los Angeles, California 90073

Running title: Corticosteroids and Memory

Send Proofs to: Edward L. Bennett
Laboratory of Chemical Biodynamics
Lawrence Berkeley Laboratory
Berkeley, California 94720

KEY WORDS

Anisomycin, Memory, Protein Synthesis Inhibition, Corticosteroids, Dexamethasone, Hydrocortisone, Corticosterone, Plasma Corticosterone, Cycloheximide, passive avoidance, active avoidance.

ACKNOWLEDGEMENTS

We wish to express our appreciation to Katherine Brentnall for her skilled assistance in the behavioral experiments. The behavioral research was supported by NIMH grant NH 26608-02 to M.E. Jarvik, M.D. and the biochemical research by the Division of Biomedical and Environmental Research of the U. S. Energy Research and Development Administration through the Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory. We wish also to thank Dr. R. George of the UCLA Department of Pharmacology for his support of the corticosteroid assays.

ABSTRACT

The effects of corticosterone, hydrocortisone and dexamethasone on retention of active and passive avoidance training were studied in male mice. Post-training administration of any of the hormones facilitated subsequent retention test performance of poorly trained mice when tested one week after training and drug administration. The optimum dose of dexamethasone was 4 mg/kg, while corticosterone and hydrocortisone were effective at 30 and 40mg/kg respectively. Dexamethasone significantly facilitated retention when administered up to 150 min but not at 210 min after training. It was further determined that dexamethasone blocked the amnesic effect of two but not four successive injections of anisomycin in both active and passive avoidance tasks. Corticosterone and dexamethasone when administered to anisomycin-injected mice caused only a small, transient increase in the protein synthesis inhibition. In saline-injected control mice, the hormones also caused a small inhibition of protein synthesis which disappeared quickly. Plasma corticosterone levels were measured in mice trained and given anisomycin, cycloheximide or saline. Plasma corticosterone levels were reduced 43% by anisomycin and 89% by cycloheximide. In both cases the corticosterone levels were normal by 60 min after the inhibitor injection and elevated by about 2-4 times above control levels at 120 and 180 min after the inhibitor injection. The results are discussed in terms of the effect of central stimulant action of corticosteroids on memory formation.

Since the early 1960's a great deal of research has been done on the role of the pituitary-adrenocortical axis in animal learning where footshock has been the main reinforcement (de Wied, 1969). Recently attention has turned to the effect of ACTH and related compounds on retrieval of stored information through studies of the effect of ACTH peptides on extinction of an avoidance habit (Bohus and de Wied, 1966; Bohus, Gispen and de Wied, 1973; de Wied, 1974; Greven and de Wied, 1973 and Wimersma Greidanus and de Wied, 1971). In most cases, it was found that ACTH peptides delay the extinction of an avoidance habit. This is believed to reflect increased arousal and improved recall of the learning task. Evidence of effects of ACTH peptide fragments on memory formation was reported by Flood, Jarvik, Bennett and Orme (1976). They showed that the post-training injection of $ACTH_{4-10}$-L-Phe^{7} facilitated retention when tested one week after training and drug administration.

Corticosteroids are normally released in response to ACTH secretion. It is surprising given the work done on ACTH and its fragments that corticosteroids have received little attention as to their possible function in memory processing. Bohus (1970) reported that daily administration of cortisol accelerated the rate of extinction of a shock avoidance habit. Wimersma Greidanus (1970) reported that corticosterone, dexamethasone, progesterone, pregnenolone but not cholesterol, testosterone or estradiol accelerated the rate of extinction for a shock avoidance habit. The conclusion reached from this research was that ACTH and corticosteroids have opposing effect on retention.

However, two reports of <u>improved</u> retention as the result of corticosterone and hydrocortisone administration question this generalization. Barondes and Cohen (1968) reported that a mixture of corticosterone and hydrocortisone blocked amnesia which would otherwise have occurred in mice treated with a brain protein synthesis inhibitor--cycloheximide. More recently, Nakajima (1975) confirmed the findings that corticosterone and hydrocortisone blocked

cycloheximide induced amnesia when administered after passive avoidance training. It was also found that intra-hippocampal injections of hydrocortisone blocked cycloheximide induced amnesia (Nakajima, 1976). If corticosteroids interfere with memory processing as suggested by the extinction studies, then one would not expect them to have anti-amnesic effects. This seems particularly strange since drugs like d-amphetamine, strychnine and picrotoxin act as anti-amnesics in mice given protein synthesis inhibitors (Barondes and Cohen, 1968; Gibbs, 1976; Flood, Jarvik, Bennett, Orme and Rosenzweig, 1977) and also improve retention of non-inhibited subjects (McGaugh and Petrinovich, 1965; Breen and McGaugh, 1961; Andry and Luttges, 1971; McGaugh and Krivanek, 1970; Krivanek and McGaugh, 1969; Del Rio, 1971; Castellano, 1974).

The purpose of our research was to determine if corticosteroids would improve retention in poorly trained mice as well as block amnesia in mice treated with another protein synthesis inhibitor--anisomycin. Biochemical determinations were also made of the effects of corticosteroid administration on protein synthesis and also of training and protein synthesis inhibition on plasma corticosterone levels.

MATERIALS AND METHODS

Behavioral Experiments

For the behavioral experiments, CD-1 male mice from Charles River Breeding Laboratories, Wilmington, MA were obtained at 6 weeks of age. After 1 week in the laboratory, the mice were individually caged until trained 1 week later on a T-maze active avoidance task or on a one-trial, step-through passive avoidance task. Mice were trained between 0800 and 1400 hrs.

T-Maze Active Avoidance

The T-maze apparatus and training have been previously described (Flood, Bennet Orme and Rosenzweig, 1975). The apparatus consisted of a black Plexiglass alley with a start box at one end and two goal boxes at the other. A brass floor grid ran throughout the entire maze. Each goal box was fitted with a clear Plexiglass liner, the bottom of which went below the shock grid. This was used to remove the animal from the goal box. The start box was separated from the start alley by a black plastic guillotine door which prevented the mouse from moving down the alley until the trial started. Mice were not permitted to explore the maze prior to training. The conditioned stimulus was a door bell-type buzzer. The intertrial interval was about 40 sec; 0.36 ma footshock was used in all active avoidance experiments.

A training trial consisted of placing the mouse in the start box, then raising the guillotine door and simultaneously sounding the buzzer. Mice not moving to the correct goal box within 5 sec were shocked until they did so. The side preference was determined on the first training trial by forcing all mice to go to the side opposite to their first response. On subsequent trials, the correct goal box was the non-preferred side for each mouse. At the end of each trial, the mouse was removed to its home cage by carefully removing the liner and placing it into the mouse cage. As the training proceeded

a mouse could make one of two types of responses: (a) a response latency less than or equal to 5 sec was an avoidance, (b) a response latency longer than 5 sec was an escape. The retention test given 1 week after training consisted of retraining to a criterion of 1 avoidance response. Mice requiring more than 3 trials to make the first avoidance response were scored as amnesic.

Passive Avoidance

The passive avoidance training and apparatus have been described previously (Flood, Bennett, Rosenzweig and Orme, 1972). In brief, the apparatus consists of a 44 cm long alley divided into a small, black start box and a longer white shock compartment. The two compartments are separated by a panel which contains a mouse hole. Entry into the white compartment was prevented until the appropriate time by a translucent guillotine door. The shock was administered by a high voltage, constant current 18 pole shock scrambler through a brass floor grid in the white box. The footshock intensity was 0.33 ma.

The training trial consisted of placing the mouse in the black start box for 20 sec, then illuminating the white shock box and the mouse hole for an additional 20 sec. Next, the guillotine door was removed while the mouse was facing away from it. The latency-to-enter was determined from the time the mouse oriented toward the mouse hole until it entered completely the white compartment. The shock was turned on when the mouse was half-way down the alley (about 5 sec after entry), and was left on until the mouse escaped from the white compartment (latency-to-escape) and was then returned to its own cage.

The retention test followed the same procedure as for training except that no footshock was given. Mice not entering into the white compartment within 180 sec were removed and given a score of 180 sec. Amnesia was defined as entering into the white shock compartment in 20 sec or less. Retention

was defined as not entering the shock compartment within 20 sec. The basis for choosing this criteria for amnesia and retention have been discussed (Flood et al., 1977).

Drugs and Injections

All drugs were administered subcutaneously in the following doses: anisomycin (ANI) at 20 mg/kg; hydrocortisone succinate or hydrocortisone phosphate at 42 mg/kg equivalent to 30 mg/kg of hormone. Hydrocortisone and corticosterone were suspended in 5% Tween-80 and used at a dose of 30mg/kg. Dexamethasone (DEX) was prepared in saline and administered at doses of 0.5 to 8.0 mg/kg. When ANI or saline was administered prior to training, the subject was lightly anesthetized with ether.

To dissolve ANI, an approximately equal molar amount of 3N HCl, was added to a suspension of ANI in saline, and the pH was finally adjusted to 6-7. The final solution was 2.0 mg/ml in 0.9% NaCl. ANI was obtained as a gift from Pfizer Inc. and is now available commercially through Pfizer Diagnostics, Clifton, N.J. 07012. Corticosterone and all the hydrocortisones were obtained commercially from Sigma Chemical Co. Dexamethasone was generously supplied by Merck, Sharp & Dohme through D. Brown.

BEHAVIORAL EXPERIMENTS AND RESULTS

Experiment 1: Effects of Corticosteroids on Retention of T-Maze Avoidance

The purpose of this experiment was to determine if dexamethasone, hydrocortisone and corticosterone had any effect on retention when administered after T-maze active avoidance conditioning. All subjects were given 4 training trials with the buzzer muffled so that control subjects would show poor retention. Immediately after training subjects were administered either saline 5%, Tween 80, dexamethasone (doses of 0.5, 1.0, 2.0, 4.0 8.0 mg/kg), hydrocortisone phosphate (42 mg/kg), hydrocortisone-succinate (42 mg/kg), hydrocortisone (30 mg/kg) in 5% Tween-80 or corticosterone (30 mg/kg) in 5% Tween-80. The retention test was given one week after training and drug treatment. The N per group was 20.

Results

Post-training injections of DEX at 4.0 or 8.0 mg/kg significantly decreased the percentage of subjects classed as forgetting (DEX 4 mg/kg vs. saline, $P < .005$, $\chi^2 = 10$. DEX 8 mg/kg vs. saline, $P < .001$, $\chi^2 = 12.3$). Lower doses of dexamethasone were found not to significantly effect retention (Table 1). All of the hydrocortisone groups and the corticosterone group had significantly fewer subjects classed as forgetting than either the saline or Tween-80 control groups (Table 1).

Table 1 about here

Experiment 2: Time Dependency of DEX on T-Maze Avoidance

The purpose of this experiment was to determine if dexamethasone (4 mg/kg) facilitated retention in a time dependent manner. Mice were administered dexamethasone either 0, 30, 90, 150, or 210 min after training and saline was administered at either 0 or 210 min after training. The other experimental conditions were the same as in Exp. 1. The N per group was 20. Subjects were tested one week after training.

Results

Dexamethasone significantly improved retention test performance when administered 0 to 150 min after training, but did not improve retention when administered 210 min after training (Table 2). The DEX-150 min group differed from the combined saline control at $P < .05$, $\chi^2 = 5.98$. Thus the results of Exp. 1 and 2 show that improved retention from dexamethasone was not due to a proactive influence of the drug at the time of the retention test.

Table 2 about here

Experiment 3: Anti-amnesic Effect of Dexamethasone for T-Maze Avoidance

The purpose of this experiment was to determine if dexamethasone would block amnesia induced by a series of anisomycin (ANI) injections. So that control subjects would now remember and we could demonstrate ANI-induced amnesia, the training conditions were changed as follows: the number of training trials was 5 instead of 4, and the buzzer was loud instead of muffled. In addition, sensitivity to footshock was increased by the pretraining injection of ANI or saline in this experiment. Other conditions of training and testing were described above. ANI was administered either 2, 3, or 4 times as follows: 15 min prior to training, 1-3/4 hrs, and if given, 3-3/4 and/or 5-3/4 hrs after training. Saline was administered to control groups at the same time. Dexamethasone was administered immediately after training at 4 mg/kg. Saline was administered to control for stress of the dexamethasone injection. The N was 20 per group.

Results

Two, three, or four successive injections of ANI caused significantly poorer retention than four successive injections of saline (Table 3); P values are all greater than .001, χ^2 test. When dexamethasone was administered to mice receiving two successive injections of ANI, the percentage of mice classed as forgetting was significantly reduced [Ani(saline)Ani = 70% forgetting vs ANI(DEX)ANI = 10% forgetting; χ^2 Test, $P < .001$]. The administration of dexamethasone was less effective in reducing the percent forgetting in mice receiving three ANI injections (Table 3, $P < .05$, $\chi^2 = 6.6$). Dexamethasone did not reduce the forgetting of mice given four successive injections of ANI. Thus, additional injections of ANI which increased the duration of inhibition of protein synthesis consistently reduced the anti-amnesic effect of dexamethasone (Table 3).

Table 3 about here

Experiment 4: Anti-Amnesic effect of Dexamethasone for Passive Avoidance

The purpose of this experiment was to determine if dexamethasone would have the same effect on ANI-induced forgetting in a passive avoidance situation as it had in an active avoidance situation (Exp. 1). The training was as described above. The subjects received ANI (2 to 4 times) according to the injection times given in Exp. 3. DEX (4 mg/kg) or a control saline injection was given 30 min after passive avoidance training. The N per group was 20. The subjects were tested one week after training.

Results

Two, three or four successive injections of ANI caused significantly more forgetting than four successive injections of saline (Table 4; χ^2 Test, $P < .001$). DEX significantly reduced forgetting when mice were given two injections ($P < .001$; $\chi^2 = 14.5$), but not when given three or four successive injections of ANI. This decreased ability of DEX to reduce forgetting in this experiment is probably due to the administration of DEX 30 min rather than immediately after training so that the injection procedure itself did not affect retention test scores. As has been published previously a single injection of ANI at this level of training and this dose does not cause significant forgetting (Flood et al., 1974, 1975a,b).

Table 4 about here

BIOCHEMICAL EXPERIMENTS

In order to interpret the behavioral results, it is essential to determine what effect the corticosteroids had on protein synthesis and on protein synthesis inhibition induced by ANI. If the steroids blocked inhibition of protein synthesis, then one could readily account for the results under the hypothesis that protein synthesis is required for long-term memory trace formation. If this is not the case, then we must seek an explanation of the phenomenon in terms of other possible mechanisms of action of the steroids.

A second question studied was the degree to which ANI inhibits the expected increases in plasma corticosterone levels following training. The consistency, degree and time course of corticosterone levels was determined.

PROCEDURES

Animals

The mice used for the biochemical experiments on protein synthesis were male Swiss Webster mice; they were from the first or second generation of a stock obtained from the Charles River Breeding Laboratories and raised at Lawrence Berkeley Laboratory. Recent behavioral and biochemical comparisons of Swiss Webster mice bred in our laboratory and those obtained directly from Charles River showed no significant differences. The mice used in determining the plasma corticosterone levels were obtained from Charles River and housed and trained in the behavioral laboratory. At the time of the experiments mice were 60-80 days of age and weighed about 35g.

Determination of Protein Synthesis

Radioactive [U-^{14}C]-L-valine was injected subcutaneously 20 min prior to sacrifice. Protein synthesis was determined by the ratio of (a) radioactivity resulting from incorporation of the label into the trichloracetic acid insoluble fractions to (b) the total radioactivity in the brain sample. The percent

inhibition or stimulation was determined by comparison of this ratio in the control and experimental mice. The experimental procedures have been described in detail (Flood et al., 1972). Three or 4 mice were used for each data point, 5 or 6 mice were used for the saline or ANI reference controls. Duplicate fractions and determinations of radioactivity were made for each mouse brain. DEX, corticosterone and ANI were obtained from the same sources and administered in the same manner as described in the behavioral Experiments. The [U-^{14}C]-L-valine was obtained from New England Nuclear Corp.

Results

In the first experiment the effects of corticosterone (30 mg/kg) and dexamethasone (4.0 mg/kg) on protein synthesis were determined at 20 or 60 min after the drug or Tween-80 was administered. The results showed that an injection of Tween-80 inhibited protein synthesis 9.6% twenty minutes after administration but by 60 min there was no effect (0.6% inhibition). Corticosterone inhibited protein synthesis 18% at 20 min after administration but it declined to 2.3% by 60 min after training. DEX inhibited protein synthesis by 6.6% and this declined to 1.6% by 60 min after administration. Thus the vehicle, corticosterone, and DEX caused only small, short-term reductions in protein synthesis which did not last even 60 min after drug administration.

In the second experiment the effect of corticosterone and DEX on ANI-induced inhibition of protein synthesis was determined. ANI was administered at the time 0 min with corticosterone or DEX being administered at either 15 or 45 min later (times correspond to those in Exp. 3 and 4 of the behavioral studies). Subjects were sacrificed at 180 min after the ANI injection. ANI alone resulted in 40% inhibition of protein synthesis 3 hrs after its administration Corticosterone plus ANI resulted in a slightly higher level of inhibition (57% for both the 15 min and the 45 min group). DEX plus ANI also caused a slightly higher level of inhibition (51% for the 15 min group and 50% for the 45 min group).

The biochemical study showed that the anti-amnesic and retention facilitating effects of the corticosteroids was not mediated by directly affecting protein synthesis. We have observed similar biochemical and behavioral effects with stimulants and ACTH peptide fragments (Flood, et al., 1976, 1977a,b).

Determination of Plasma Corticosterone

Plasma corticosterone levels were determined in trained and drug injected mice. Mice received either an injection of ANI (20 mg/kg) or cycloheximide (CYCLO) (100 mg/kg) 15 min prior to receiving 5 training trials in the T-maze. Subjects were sacrificed immediately, 60, 120, 140, 180 min after training. After decapitation, blood was collected from the head and the trunk and treated with heparin in saline. In order to obtain sufficient plasma, the blood from two mice given the same treatment was pooled, stored, centrifuged and the plasma frozen and stored until assayed. The assay used was a microadaption of a fluorescence procedure described by Silber et al. (1958) modified by Guillemin et al. (1959). In brief, frozen mouse plasma samples were thawed and 0.1 ml of plasma was added to 2.4 ml of distilled water. The samples and corticosteroid standards were washed briefly with petroleum ether and extracted into methylene chloride. Corticosterone was then extracted from the methylene chloride into a solution of sulfuric acid with ethanol. Fluorescence was allowed to develop for 1 hr and determined in an Aminco Spectrophotofluorometer with excitation and emission wavelengths of 463 and 525 mu. The apparatus was set with the sensitivity at 70, the high voltage at 0.7, and the slit at 5 mm. The standard corticosterone curve was linear in the concentration range of 5-60 µg/100ml plasma.

Results

The plasma corticosterone levels were studied most closely immediately after training; 5 determinations for 5 pairs of trained mice were made. The data showed that ANI and CYCLO prevented increases in corticosterone level relative to the saline controls. ANI reduced corticosterone levels by 43% and CYCLO by 89%. The mean percent μg (μg/100ml plasma) was 13.0 for saline, 7.4 for ANI and 1.5 for CYCLO.

A few determinations were made at 60, 120, 140 and 180 min after training. The saline controls showed a rapid decrease in plasma corticosterone level up to 120 min; thereafter the level was stable. The ANI and CYCLO groups did not differ from saline at 60 min but by 120 min ANI showed a corticosterone level 2.5 times greater than saline while the CYCLO group was 6.3 times greater. An additional injection of ANI given 2 hrs after the first (ANI+ANI) did not depress corticosterone levels measured at 140 or 180 min. In fact, corticosterone levels remained elevated at 2.4 times greater than saline.

The results showed that the inhibitors of protein synthesis, ANI and CYCLO, caused a transient depression of plasma corticosterone level which was followed by an increase in corticosterone level for about 180 min after the first ANI or CYCLO injection. The recovery of protein synthesis did not appear to trigger this large increase in corticosterone level since this increase occurred when protein synthesis inhibition was maintained by an additional injection of ANI (ANI+ANI yielding 240 min of inhibition of protein synthesis of 80% or greater).

DISCUSSION

Summary of Findings

Post training administration of dexamethasome facilitated retention test performance in a dose response and time dependent manner. Hydrocortisone and corticosterone facilitated retention at appropriately higher doses. A dose of dexamethasone that facilitated retention in poorly trained mice also blocked amnesia in ANI injected mice. The anti-amnesic effect was not task dependent since it was demonstrated to occur in both active and passive avoidance situations. The anti-amnesic effect was blocked by giving additional injections of ANI which resulted in increased durations of protein synthesis inhibition.

The administration of corticosterone or dexamethasone caused a slight but transient inhibition of protein synthesis in saline-injected mice and slightly higher levels of protein synthesis inhibition in ANI injected mice. In neither case was the magnitude of the effect of the hormones on protein synthesis regarded as sufficient to account for the observed effect on retention test performance. ANI and CYCLO caused only a transient depression on plasma corticosterone levels of less than 40 min followed by an increase in its level to above the saline control level.

Related Findings

Extinction Studies: Bohus (1970) and Wimersma Greidanus (1970) reported the effect of chronic and acute administration of corticosteroids on extinction of a shock avoidance habit. The chronic studies involved training rats to make a pole jump response over several daily sessions. Cortisol was implanted. This was followed by retraining and then 7 daily extinction sessions; the rats were under the influence of cortisol all the time. Cortisol administration resulted in more rapid extinction.

In the acute study, rats were trained and then given 2 extinction sessions about 4 hrs apart. Corticosterone, dexamethasone, progesterone, or pregnenolone were administered after the first extinction session and it was found that these hormones resulted in a more rapid extinction. Cholesterol, testosterone or estradiol did not affect extinction. Both the chronic and acute studies were interpreted as showing that corticosteroids impaired memory functioning.

It should be pointed out that extinction of a previously learned response habit involves the acquisition of new information (i.e., learning). In this case, the animal must learn that the conditioned stimulus is no longer followed by footshock for failing to make an avoidance response. Several interpretations of the results of the extinction studies are possible. First, corticosteroids might have impaired retrieval of memory for the active avoidance conditioning. Thus the subjects are less motivated to make avoidance responses and extinguish more quickly. This seems unlikely since if memory retrieval were impaired it would be more likely to affect the more recent memory for extinction and thus result in a slower, rather than accelerated extinction. Second, corticosteroids might have enhanced acquisition of extinction, thus a decreased rate of responding would result. We trained 20 naive mice on the T-maze active avoidance task and found that 4 mg/kg of DEX given 1 hr prior to training did not affect the rate of acquisition (DEX = 5.1 trials to 1st CR and Saline = 5.3). Thus there was no indication of a general improvement of acquisition; in fact, there is some indication that corticosteroids can impair acquisition (McEwen and Weiss, 1970). Third, corticosteroid administration could have altered performance variables such as arousal level, mobility or perception. While no impairment was observed when we were training DEX injected mice, it is

possible that in the non-shock extinction situation subtler changes in performance variables could affect the rate of extinction. Fourth, corticosteroids might have enhanced memory formation and thereby have led to a more rapid extinction. In this connection, Bohus (1970) found that cortisol implants in the reticular formation at the thalamic and mesencephalic levels were particularly effective in enhancing extinction. Increased excitability of the CNS with stimulants has been reported frequently to enhance memory processing (McGaugh, 1973; Dawson and McGaugh, 1973 for recent reviews).

While it is tempting to consider pituitary hormones (ACTH) and adreno-corticosteroid hormones as having opposing effects on retention because they have regulating influences on each other, it presents a logical problem in that it is difficult to understand why corticosteroids would be secreted shortly after training at a time when memory processing is thought to be occurring. We suggest that the regulatory mechanisms of the pituitary-adrenal axis are independent of their effect on memory formation and that both ACTH and corticosterone, to the extent that they can affect retention under normal circulating levels, act in a general way to improve memory formation.

Anti-Amnesic Effects: Barondes and Cohen (1968), Nakajima (1975) and our results showed that corticosteroids can block the amnesic effect of CYCLO or ANI. We further showed that extending the duration of inhibition prevented the anti-amnesic effect of DEX. Nakajima suggested that the anti-amnesic effect of corticosteroids showed that inhibitors of protein synthesis might cause amnesia by preventing steroid synthesis or release. This hypothesis seems unlikely since it places the mechanism of action of the inhibitors peripheral to the CNS. Studies have shown that CYCLO and ANI cause amnesia when administered directly into the brain. The doses used were too low to cause significant amnesia when given peripherally (Eichenbaum, 1975; Flood et al., unpublished

observations). In addition, it was reported that aminoglutethimide depleted corticosterone as much as CYCLO but did not inhibit protein synthesis and did not cause amnesia (Squire, St. John and Davis, 1976). Squire et al. (1976) also showed that when a sufficient amount of corticosterone (1.2 mg/kg) was administered to compensate for the loss caused by CYCLO, amnesia still occurred. It should be stressed that the 30 mg/kg dose of corticosterone used to block amnesia elevated plasma corticosterone levels in CYCLO-treated mice about 7 times higher than normal (Squire et al. (1976).

Nakajima (1976) found that corticosterone levels were suppressed in CYCLO-injected mice 10 and 30 but not 60 min after training. Over the 30 to 60 min period corticosterone levels more than doubled in CYCLO-injected mice suggesting delay, rather than a complete blocking of training-induced increases in plasma corticosterone levels.

The present study provides additional evidence that does not support the hypothesis that protein synthesis inhibitors cause amnesia by blocking adrenocorticosteroid function. The retention of poorly trained mice, not given a protein synthesis inhibitor, was improved by a post-training injection of dexamethasone, corticosterone or hydrocortisone. Therefore, improvement of retention with these hormones need not involve inhibitor-depressed plasma corticosterone levels. With respect to the inhibitor studies, corticosterone levels were found to be depressed by CYCLO or ANI but the effect was only temporary (lasted less than 40 min). If the critical period of corticosterone depression was that immediately following training, then a single injection of ANI should have been sufficient to cause amnesia. It has been reported previously that a single injection of ANI at 20 mg/kg given prior to active avoidance does not induce amnesia (Flood et al., 1975; 1976, Exp. 8 and 1977) nor does a single injection of ANI necessarily cause amnesia for passive avoidance training (Flood et al., 1973). Neither can the anti-amnesic effect

of the steroids be explained on the basis of altered protein synthesis since it was found that hormone administration only altered inhibition of protein synthesis slightly and then in the wrong direction (i. e., increasing inhibition).

More recently Nakajima has carried the steroid hypothesis into the central nervous system. He has reported that intra-hippocampal injections of hydrocotisone block CYCLO-induced amnesia from occurring, while septal and hypothalamic injections were ineffective. Nakajima suggests that protein synthesis suppression of corticosteroid release prevents the steroids from reaching steroid-receptors in the hippocampus. The hypothesis still fails to account for the following findings: (a) peripheral protein synthesis inhibitors do not cause amnesia (Dunn, Gray and Iuvone, 1977), (b) suppression of steroidogenesis without protein synthesis inhibition does not cause amnesia (c) the anti-amnesic effect of steroids can be blocked by additional injections of ANI but steroid levels are not depressed, (d) one injection of ANI supresses steroid increases but is not usually found to cause amnesia and (e) steroid administration improved memory of non-inhibited but poorly trained subjects.

Interpretation

We propose as an alternative explanation that the retention improving effects of corticosteroids can be explained on the basis of their stimulant action on the CNS (Feldman and Dafny, 1970). Stimulants may affect long term memory storage by maintaining the life of the short term memory trace upon which long term memory is dependent. Our findings with dexamethasone show a strong similarity with experiments in which CNS excitability was increased by post training administration of stimulants (e.g., d-amphetamine, strychnine, picrotoxin, caffeine, nicotine). All of these stimulants had anti-amnesic

effects against ANI-induced amnesia (Food et al, 1977a,b). An additional similarity between stimulant, ACTH and corticosteroids is that the anti-amnesic effect could be blocked by giving additional injections of ANI which lengthens the duration of inhibition. We believe that these stimulants increase the life of the short term memory trace by effecting a greater interaction between the neurotransmitter and its receptor. Assuming that long term memory trace formation depends upon the presence of the short-term memory trace at a time when protein synthesis is possible, then with a given duration of inhibition, amnesia does not occur because stimulants have increased the life of the short-term memory beyond the inhibition period (ANI-DEX-ANI in Exp. 3 and 4). Increasing the duration of inhibition re-establishes conditions which result in amnesia because the duration of inhibition is again longer than the stimulant-extended life of the short term memory trace (ANI-DEX-ANI+ANI+ANI in Exp. 3 and 4). Corticosteroid release, well known for its association with stress, may modulate arousal and thus provide an optimal level of CNS excitability for long term memory trace formation.

REFERENCES

Andry, D.K. and Luttges, M.W. (1971). Facilitated habituation: Strychnine dose response effects on neural and behavioral habituation. Agents and Actions 2:103-117.

Barondes, S.H. and Cohen, H.D. (1968). Arousal and the conversion of "short-term" to "long-term" memory. Proc. Natl. Acad. Sci. USA 61:923-929.

Bohus, B. (1970). Central nervous structures and the effect of ACTH and corticosteroids on avoidance behaviour: a study with intracerebral implantation of corticosteroids in the rat. In Pituitary, Adrenal and the Brain, edited by D. de Wied and J.A.W.M. Weijnen, Progress in Brain Research 32:171-184. Amsterdam: Elsevier Publishing Co.

Bohus, B., Gispen, W.H. and de Wied, D. (1973). Effect of lysine vasopressin and $ACTH_{4-10}$ on conditioned avoidance behavior of hypophysectomized rats. Neuroendocrinology 11:137-143.

Bohus, B. and de Wied, D. (1966). Inhibitory and facilitatory effect of two related peptides on extinction of avoidance behavior. Science 153:318-320.

Breen, R.A. and McGaugh, J.L. (1961). Facilitation of maze learning with posttrial injections of picrotoxin. J. Physiol. Psychol. 54:498-501.

Castellano, C. (1974). Cocaine, pemoline, and amphetamine on learning and retention of a discrimination test in mice. Psychopharmacologia 36:67-76.

Dawson, R.G. and McGaugh, J.L. (1973). Drug facilitation of learning and memory. In J.A. Deutsch (Ed), The Physiological Basis of Memory, p. 77-111. New York: Academic Press.

Del Rio J. (1971). Facilitation of some chlorpromazine-D-amphetamine mixtures on avoidance learning. Psychopharmcologia 21:39-48.

Dunn, A.J., Gray, H.E., and Iuvone, P.M. (1977), Protein Synthesis and Amnesia: Studies with Emetine and Pactamycin. Pharmacol. Biochem. Behav. 6, 1-4.

Eichenbaum, H. (1975). Localization of memory by regional brain protein inhibition. Doctoral thesis, University of Michigan.

Feldman, S. and Dafny, N. (1970). Effects of adrenocortical hormones on the electrical activity of the brain. In Pituitary, Adrenal and the Brain, edited by D. de Wied and J.A.W.M. Weijnen, Progress in Brain Research 32:90-101. Amsterdam: Elsevier Publishing Co.

Flood, J.F., Bennett, E.L., Rosenzweig, M.R., and Orme, A.E. (1972). Influence of training strength on amnesia induced by pretraining injections of cycloheximide. Physiology and Behavior 9:589-600.

Flood, J.F., Bennett, E.L., Orme, A.E., and Rosenzweig, M.R. (1975). Effects of protein synthesis inhibition on memory for active avoidance training. Physiology and Behavior 14, 177-184.

Flood, J.F., Jarvik, M.E., Bennett, E.L., and Orme, A.E. (1976). Effects of ACTH peptide fragments on memory formation. Pharmacology, Biochemistry and Behavior, 5, Suppl. 1:41-51.

Flood, J.F., Bennett, E.L., Orme, A.E., Rosenzweig, M.R., and Jarvik, M.E. (1977). Effect of the duration of protein synthesis inhibition on pole jump active avoidance and its extinction. Submitted to Pharmacology, Biochemistry and Behavior.

Gibbs, M.E. (1976). Modulation of cycloheximide-resistant memory by sympathomimetic agents. Pharmacology, Biochemistry and Behavior 4:703-707.

Greven, H.M. and de Wied, D. (1973). The influence of peptides derived from corticotrophin (ACTH) on performance: Structural activity studies. In Drug Effects on Neuroendocrine Regulation, edited by F. Zimmermann, W.H. Gispen, B.H. Marks, and D. de Wied., Progress in Brain Research, 39:429-442. Amsterdam: Elsevier Publishing Company.

Guillemin, R., Clayton, G.W., Lipscomb, H.S. and Smith J.D. (1959). Fluorometric measurement of rat plasma and adrenal corticosterone concentration, J. Lab. Clin. Med. 53:830.

Krivanek, J.A. and McGaugh, J.L. (1969). Facilitating effects of pre- and posttrial amphetamine administration on discrimination learning in mice. Agents and Actions 1:36-42.

McGaugh, J.L. and Petrinovich, L.F., (1965). Effects of drugs on learning and memory. Int. Rev.Neurobiol. 8:139-196.

McGaugh, J.L. and Krivanek, J.A. (1970). Strychnine effects on discrimination learning in mice: Effects of dose and time of administration. Physiology and Behavior 5:1437-1442.

McGaugh, J.L. (1973). Drug facilitation of learning and memory. Annual Review of Pharmacology 13:229-241.

Nakajima, S. (1975). Amnesic effect of cycloheximide in the mouse mediated by adrenocortical hormones. J. Comp. Physiol. Psychol. 88:378-385.

Silber, R.H., Busch, R.D. and Oslapas, R. (1958). Practical procedures for estimation of corticosterone or hydrocortisone. Clinical Chemistry 4:278-285.

Squire, L.R., St. John, S. and Davis, H.P. (1976). Inhibitors of protein synthesis and memory: dissociation of amnesic effects and effects on adrenal steroidogenesis. Brain Research 112:200-206.

Wied, D. de (1969). Effects of peptide hormones on behavior. In Frontiers in Neuroendocrinology, edited by W.F. Ganong and L. Martini. New York Oxford University Press, pp. 97-140.

Wied, D. de (1974). Pituitary-adrenal system hormones and behavior. In The Neurosciences Third Study Program, edited by F.O. Schmidt and F.G. Worden. Cambridge, Mass.: MIT Press, pp. 653-666.

Wimersma Greidanus, Tj.B. van, and Wied, D. de (1971). Effects of systemic intracerebral administration of two opposite acting ACTH-related peptides on extinction of conditioned avoidance behavior. Neuroendocrinology 7:291-301.

Table 1

Effect of Corticosteroids Retention of T-Maze Avoidance

Drug	Forgetting	Drug	Forgetting
Saline	70%	Tween-80	75%
Dexamethasone (mg/kg)		Hydrocortisone-phosphate	20%
0.5	75%		
1.0	60%	Hydrocortisone-succinate	20%
2.0	50%	Hydrocortisone-in Tween-80	25%
4.0	20%		
8.0	15%	Corticosterone in Tween-80	25%

Table 2

Time Dependent Effect of Dexamethasone

(N = 20/group)

Treatment Time after training (min)	Forgetting (%)
Saline	
0	75
210	70
Dexamethasone	
0	20
30	20
90	35
150	45 ($P < .05$, $\chi^2 = 4.43$)
210	70

Table 3

Anti-Amnesic Effect of Dexamethasone: T-Maze Avoidance

(N = 20/group)

Treatment Group	Forgetting (%)
Ani(Sal)Ani	70
Ani(DEX)Ani	10
Ani(Sal)Ani+Ani	80
Ani(DEX)Ani+Ani	40
Ani(Sal)Ani+Ani+Ani	80
Ani(DEX)Ani+Ani+Ani	70
Sal(Sal)Sal+Sal+Sal	5

Table 4

Anti-Amnesic Effect of Dexamethasone: Passive Avoidance

(N = 20/group)

Treatment Group	Forgetting (%)
Ani(Sal)Ani	85
Ani(DEX)Ani	25
Ani(Sal)Ani+Ani	85
Ani(DEX)Ani+Ani	60
Ani(Sal)Ani+Ani+Ani	85
Ani(DEX)Ani+Ani+Ani	80
Sa.(Sal)Sal+Sal+Sal	10

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720