Acquisition and retention effects of ethylestrenol in a food-search task

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Ethylestrenol, a synthetic anabolic steroid, was administered to adult male Long-Evans rats to investigate its role in acquisition and retention of a spatial food-search task. The animals were injected i.p. every other day either with 100 μ g of ethylestrenol or with the vehicle alone. Injections began 1 week prior to testing and continued throughout the study. A modified hole-board task was used. After modest food deprivation, the animals were required to find a food reward placed in a given hole. Acquisition of the task was measured by the time required to reach the food hole. Eight trials were given per day. Following a 10-day period without deprivation (but with 2 injections on separate days), eight retention trials were given. Following this, the rats were trained to find the reward at a new location for 2 subsequent days. There were small differences between the groups in performance during acquisition in the original 5-day period but not in the final 2-day "new-hole" training period. During the retention test, however, the ethylestrenol-treated rats reached the food reward significantly faster than did the vehicle-treated rats. These results indicate that retention was substantially enhanced by the synthetic hormone-like agent under conditions in which there was only limited enhancement of acquisition.

For the past 3 decades, investigations have continued on the effects of hormones and peptides on learning and memory processes (Bohus & Lissak, 1968; de Wied, 1964). The focus in the majority of these studies has been on the roles played by adrenocorticosteroids, adrenomedullary steroids, and the hypothalamic-pituitary axis in tasks related to learning and memory (McGaugh & Gold, 1989; van Wimersma Griedanus & Rigter, 1989). While some studies have examined the role of sex steroids in learning and memory (Flood, Morley, & Roberts, 1992; van Wimersma Griedanus, Wijnen, Deurloo, & de Wied, 1973), overall, less attention has been given to the contribution of these steroids in learning and memory tasks.

The behavioral tasks used in these studies have followed similar paradigms. Typically, active- or passive-avoidance tasks in which the animal either makes or withholds a response, respectively, to avoid footshock (van Wimersma Griedanus & Rigter, 1989) have been used. In addition, the greatest emphasis is usually placed on "retention" paradigms in which the treatment is administered *after* training (McGaugh, 1983; McGaugh, Liang, Bennett, & Sternberg, 1984) and assessment consists of measuring the animal's retention of the task at some later time (McGaugh & Gold, 1989).

While these approaches have contributed considerable information to the understanding of the effects of pituitary-adrenal hormones and peptides on learning and memory, we recently utilized a different approach in our

laboratory to investigate the behavioral effects of the neurosteroid pregnenolone, which serves as a precursor for androgens, estrogens, and progesterone (Isaacson, Yoder, & Varner, 1994). In that study, we systemically administered the hormone treatment *prior* to training in a spatial task and examined acquisition as well as retention. The hormone treatment produced a moderate, but significant, enhancement of retention without altering acquisition. We believed it would also be useful to implement this same procedure to investigate the role of a synthetic anabolic steroid, ethylestrenol (19-nor-17 α -pregn-4-en-17-ol). This is a complex molecule with portions that resemble testosterone, estradiol, and pregnenolone. We felt that if effects on acquisition or retention were found, it might be possible, in subsequent studies, to isolate the characteristic with greatest behavioral potency.

Synthetic anabolic steroids were originally designed in attempts to augment the "building" or constructive metabolic effects of testosterone while producing diminished androgenic properties (Taylor, 1991). In addition, recognizing the likelihood that peptides and hormones are most likely to exert subtle, "fine-tuning" effects on memory (Bohus, 1982), ones that are best observed under conditions in which motivational intensity to perform the task is low, we used minimal food deprivation in the task. As with pregnenolone, the ethylestrenol was administered prior to training in a spatial task in which acquisition and retention were measured.

METHOD

Subjects

Sixteen male, Long-Evans rats, 120 days of age, were used in the experiment. The animals were divided into two groups (8 rats/group). The subjects were injected and tested every other day

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with either ethylestrenol or vehicle treatment. All animals were individually housed and maintained on a 12:12-h light:dark schedule during the study. All behavioral testing was conducted during the light phase of the cycle. Food and water were available ad lib except during testing, when animals were food deprived for 18 h prior to testing. Body weights were monitored weekly. The animals were handled daily for 2 weeks prior to the start of the experiment.

Procedure

Steroid injections. The ethylestrenol (a gift from Organon, Inc., The Netherlands) was mixed in 10% absolute ethyl alcohol and double-distilled water. The rats were injected i.p. with either 100 μ g ethylestrenol or the vehicle (10% absolute ethyl alcohol in double-distilled water) at a volume of .1 ml every other day. Injections were given 3 to 4 h after light onset, and the rats then returned to their home cages in the colony room. Injections began 8 days prior to testing.

Hole-board task. A modified version of a testing procedure described by Kesner, Farnsworth, and DiMattia (1989) was used. Testing was conducted in a white Plexiglas apparatus (71.1 $\times$ 71.1 $\times$ 50.8 cm) located in a quiet room illuminated by dim red fluorescent light. The floor of the apparatus contained 16 holes, 3 cm in diameter, equally spaced across the board. Three of the walls of the apparatus were marked with different black and white geometric patterns; the fourth wall was solid white.

Testing was conducted every other day 4 h after injection. The rats were placed into a circular holding chamber (bottom diameter, 26 cm; top diameter, 36 cm; height, 36 cm) for 20 sec prior to testing and also between trials. They were required to find a food reward (Fruit Loops, Kellogg's Company, Battle Creek) that had been placed in a preselected hole of the board. During the acquisition period, eight trials per day were given for 5 days. The location of the food reward remained constant during this time and was the same for each rat. To begin the trial, the rat was placed in the apparatus at one of the four corners. Order of the corners of entry was determined with a Latin square design. The animals were started twice from each corner each day. The rats were given a maximum of 60 sec to discover the food reward. After finding the Fruit Loop or after 60 sec had elapsed, the rats were placed in the holding chamber for a 20-sec intertrial interval (ITI). During this ITI, the floor of the apparatus was cleaned with a dilute Lysol solution. The time to find the reward was recorded for each trial. Following the last trial, the animals were returned to their home cages, where food and water were available. The order of animal testing was quasi-randomized between groups.

At the end of the 5-day acquisition period, a 10-day interval was given in which the animals received only two injections (4 days apart) but no testing or deprivation. At the end of this period, a retention test was administered, consisting of eight regular training trials. The rat was required to find the food reward in the same hole in which it had been located previously. Again, the time required to find the reward was recorded. Following this retention test, the rats were trained, on 2 consecutive days, with the reward placed in a new, different location. The protocol was the same as that used in the original acquisition period.

Statistical analyses. A repeated measures analysis of variance (ANOVA) was used to analyze all eight trials each day and also to analyze designated segments of trials: Trials 1–3 (first), Trials 4–6 (middle), and Trials 6–8 (last). A sign test evaluated by a binomial expansion ($Q = P = .5$) was used to compare mean scores of the groups over the eight daily trials. A repeated measures ANOVA was also used to analyze any day-to-day differences between the groups. Due to procedural difficulties, one ethylestrenol-treated animal was dropped from the retention test and one control animal was dropped from the subsequent 2-day new-food-location test.

RESULTS

The ethylestrenol-treated animals required less time to find the food reward on the initial trials (1–3) on Day 1 of acquisition [$F(1,14) = 6.75, p < .02$], but, as indicated by the repeated measures ANOVA, there was no difference over all eight trials on Day 1 or on any day of acquisition thereafter for either the entire eight trials or when the trials were subdivided into early, middle, or late trials within a session. Significant trial effects were found consistently each day, with both groups showing reduced time to find the reward on later trials (see Figure 1). There was no significant day-to-day difference in either the control or the treated groups or between the two groups, as indicated by a repeated measures ANOVA. However, on Days 2, 3, and 5, the means of the hormone-treated animals were always below those of the control group ($p < .01$). This suggests a small, but reliable, group tendency for the ethylestrenol-treated group to show enhanced acquisition of the task. On the retention test, the ethylestrenol-treated animals found the location of the food reward faster than did the vehicle-treated controls [$F(1,13) = 12.99, p < .003$] (see Figure 2). No differences were found during the 2 days of training with the food in a new, different location. Body weights did not differ between groups at any time.

DISCUSSION

The administration of ethylestrenol, a synthetic steroid, had only a minimal effect on the acquisition of a spatial food-search task, one that was not shown, by a repeated measures ANOVA, to be statistically reliable. However, the enhanced retention, measured by the amount of time it took to find a food reward in its original location after a 10-day interval, was substantially enhanced in the ethylestrenol-treated animals, to a degree unlikely to be the result of the "marginal" enhancement of original learning.

The retention effects could have been due to a general and increasing facilitation of performance due to a gradual accumulation of the steroid, to its metabolites, or to secondary cellular reactions over the series of injections. This does not seem likely, however, since no differences were found between the two groups on the learning of the new food location 2 days after the retention tests.

While both ethylestrenol- and vehicle-treated rats learned the task equally well, the ethylestrenol group exhibited superior retention, supporting the independence of the physiological bases of acquisition and retention processes. It is possible that both membrane and genomic alterations are responsible for the behavioral differences. Considering the latter hypothesis, ethylestrenol's retention-specific effect may occur because of an augmentation of de novo protein synthesis in the cell body, which may be involved in long-term memory formation rather than influencing acquisition, or short-term memory formation, which occurs more rapidly and

Figure 1. Mean ($\pm$ SEM) seconds to reach the food compartment in the food-search task for the hormone- and vehicle-treated groups for the 5 days of acquisition. Ethylestrenol-treated rats are indicated by the open triangles; control rats are indicated by the solid triangles.

Figure 2. Mean ($\pm$ SEM) seconds required to reach the goal area during the retention test in the food-search task. Ethylestrenol-treated rats are indicated by the open triangles; control rats are indicated by the solid triangles.

is therefore probably regulated by posttranslational changes in proteins in the synapse (Agranoff, 1984). Still another possible explanation for the enhanced retention effect of ethylestrenol is that it may be mediating a type of endogenous state dependency involved in memory modulation as discussed by Izquierdo (1984). Perhaps a humoral state was established during the final training day that may have served as a contextual cue during the retention test that occurred 10 days later. However, the main point of this report concerns the potency of ethylestrenol in enhancing retention. This potency could be attributed to a particular portion of the molecule, and, with the generation of further studies, the issue of the potency of various portions of the molecule should be examined.

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