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$ACTH_{4-9}$ Analog (ORG 2766) Facilitates Acquisition of an Inhibitory Avoidance Response in Rats[1]

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MARTINEZ, J. L., JR., B. J. VASQUEZ, R. A. JENSEN, B. SOUMIREU-MOURAT AND J. L. McGAUGH. *$ACTH_{4-9}$ analog (ORG 2766) facilitates acquisition of an inhibitory avoidance response in rats.* PHARMAC. BIOCHEM. BEHAV. **10**(1) 145–147, 1979.—These experiments examined the effects of an $ACTH_{4-9}$ analog (ORG 2766) on an inhibitory avoidance response in rats. Graded doses of ORG 2766 were administered either 1 hr prior to training, immediately after training, or 1 hr prior to the retention test. The animals were tested 24 hr after training. A 5.0 mg/kg dose was administered prior to training significantly facilitated acquisition of the response. ORG 2766 did not significantly affect retention when administered after training or prior to the retention test. Since ORG 2766 only affected acquisition of the response, it is suggested that the drug acts by influencing sensory, motivational or attentional variables rather than directly affecting memory consolidation or retrieval processes.

Inhibitory avoidance task Rats ORG 2766 $ACTH_{4-9}$ analog

THE FINDINGS of numerous studies conducted during the past several years indicate that learning, extinction and retention are affected by pituitary hormones and hormone analogs [10,11]. Particular attention has been focused on the behavioral effects of adrenocorticotrophic hormone (ACTH) and peptide fragments of ACTH such as $ACTH_{4-10}$. For example, it was reported that $ACTH_{4-10}$ facilitates attentional processes in humans [1,8], and attenuates CO_2-induced amnesia in rats [7]. Other studies have reported that posttraining administration of $ACTH_{4-10}$-L-Phe-7 (in which the phenylalanine at position 7 was in its normally occurring levo-form) improved retention of both an inhibitory and active avoidance task, whereas $ACTH_{4-10}$-D-Phe-7 impaired retention in both tasks [2]. De Wied [9] found that other structural alterations of the $ACTH_{4-10}$ molecule enhance its behavioral potency. He reported that in an $ACTH_{4-9}$ analog, replacement of the lysine at position 8 by a D-isomer and replacement of the tryptophan by a phenylalanine in position 9 potentiated extinction of a pole-jump avoidance response more than a hundred-fold. ORG 2766 (H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH), a hormone analog [6] similar to the $ACTH_{4-9}$ sequence described by De Wied [9] attenuates CO_2-induced amnesia if it is given in close temporal proximity to the retention test [6].

The present study was undertaken to extend the findings of Rigter *et al.* [6] and to determine the effect of ORG 2766 on acquisition, consolidation, or retrieval of an inhibitory avoidance response. It was thought that ORG 2766 might influence memory consolidation processes if it was administered immediately following training in view of the findings that $ACTH_{4-10}$, a similar molecule to ORG 2766, enhances memory [2] as well as evidence that ACTH facilitates retention of an inhibitory avoidance response when administered shortly after training [4].

METHOD

Animals

Fischer 344 (Charles River) rats (90 days old) were housed individually, and maintained on a standard light-dark cycle (LD 12:12; lights on at 7:00 a.m.). Food and water were available ad lib. Following adaptation to laboratory conditions for at least 5 days, the animals were trained in a one-trial inhibitory avoidance step-through task.

Procedure

Training consisted of placing the rats in a white illuminated start compartment facing away from the door. When the rat turned around, the door to the larger dark shock

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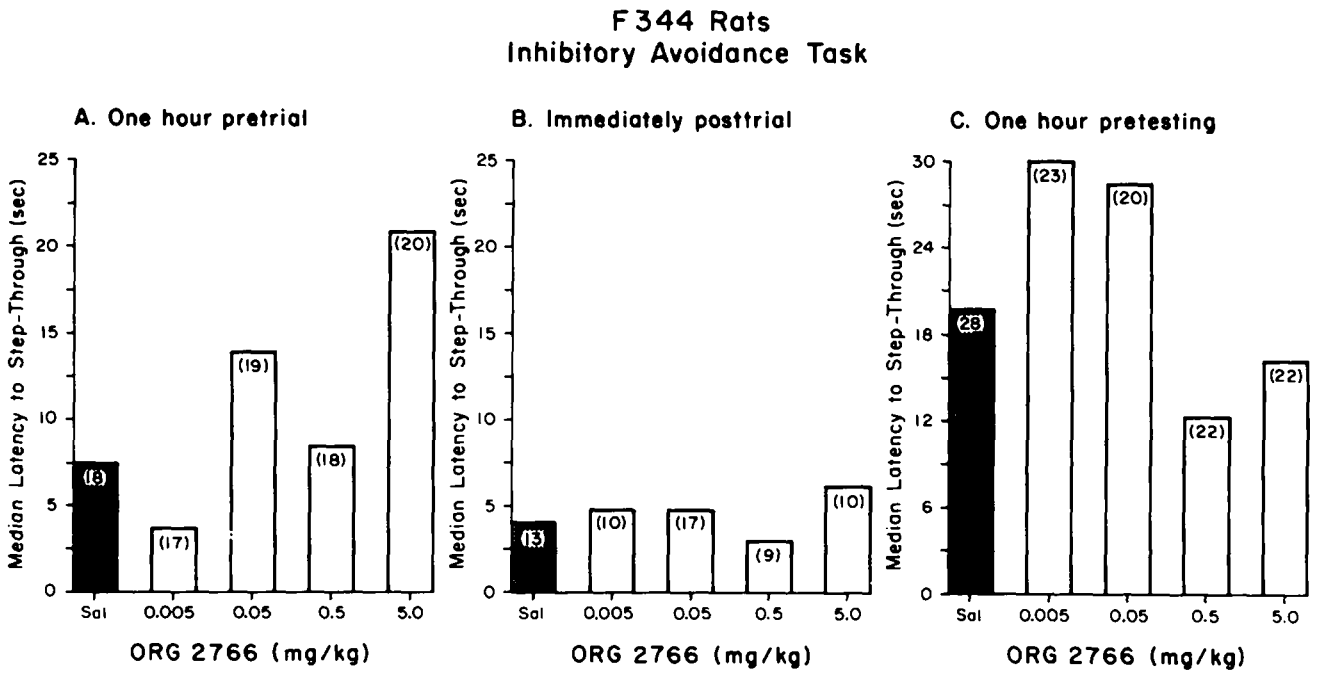

FIG. 1. This figure demonstrates that 5.0 mg/kg ORG 2766 given 1 hr before training significantly ($p<0.05$) facilitates acquisition of an inhibitory avoidance response in rats (Panel A). A four log unit dose range, 0.005–5.0 mg/kg ORG 2766, does not affect retention performance if it is administered either immediately after training or 1 hr before the retention test (Panels B and C). However, an injection given before the retention test seems to be arousing as all of the retention scores in this condition (Panel C) are generally elevated and the saline control group in this condition differs significantly from the combined pre- and immediate post-trial saline control groups ($p<0.01$). Note that the scale on the ordinate of Panel C differs from Panels A and B. The numbers in parentheses, in each bar of the figure, are the number of animals in each experimental condition.

compartment was opened and the rat was allowed to step through. After all four paws contacted the two metal floor plates the door was closed and inescapable 500 μA/5 sec footshock was delivered to the animal [5]. Previous work in this laboratory has shown that using a weak footshock is a particularly effective procedure for demonstrating retrograde enhancement of learning, primarily because of low variability in the distribution of retention scores [3,4]. The level of the constant current sinusoidal footshock (Lafayette Instrument Co.) was determined by the root mean square of the sine wave. A retention test was given 24 hr following training. If the rat failed to step through to the dark side within 600 sec, it was removed from the apparatus, and assigned a score of 600. Training and testing were performed between the hours of 1:00 and 5:00 p.m. Analysis of the retention latencies was performed with Mann-Whitney U-test comparisons, since some ceiling scores were observed producing a truncated distribution.

ORG 2766 was dissolved in distilled water in polyethylene containers immediately prior to injection, and kept cold. It was injected SC at the following doses (mg/kg) and times: (a) 1 hr before training, saline control (n=18), 0.005 (n=17), 0.05 (n=19), 0.5 (n=18), 5.0 (n=20); (b) immediately following training, saline control (n=15), 0.005 (n=10), 0.05 (n=17), 0.5 (n=9), 5.0 (n=10); (c) 1 hr before the retention test, saline control (n=28), 0.005 (n=23), 0.05 (n=20), 0.5 (n=22), 5.0 (n=22).

RESULTS

The median retention latencies for all of the experimental groups are shown in Fig. 1. A comparison of the response latencies of the rats that received saline 1 hr before training or immediately following training indicated that these groups did not differ significantly, $U(15,18)=87.5$, $p>0.05$. Therefore, these two saline groups were combined for all further U-test comparisons. The retention latencies of the rats given 5.0 mg/kg of ORG 2766 1 hr before training were significantly greater than those of the combined saline control group ($U=206.0$, $Z=2.27$, $p<0.05$, two-tailed test). A one-way analysis of variance was performed on the initial step-though entrance latencies of the rats because there were no ceiling scores as in the case of the retention test and a parametric analysis is appropriate in this case. ORG 2766 given 1 hr before training did not produce entrance latencies that differed significantly from each other, $F(4,58)=1.30$, $p>0.05$. The mean initial step-through entrance latencies in seconds for the five groups were: saline=8.52, ORG 2766; 0.005 mg/kg=8.19; 0.05 mg/kg=19.1; 0.5mg/kg=15.87;5.0 mg/kg=12.91. Finally, comparisons with the animals that received ORG 2766 immediately following training to the combined saline control group, and comparisons between the animals that received ORG 2766 1 hr prior to the retention test to their saline control group, indicated that ORG 2766 produced no significant change in these animals.

As shown in Fig. 1, the animals that received an injection of saline 1 hr before the retention test had elevated latency scores in comparison with animals that received injections before or shortly after the training experience. In fact, a saline injection given 1 hr prior to the retention test significantly increased the step-through latencies of these rats

compared to those of the combined saline (1 hr pre- and immediately posttrial) group ($U=276$, $Z=2.69$, $p<0.01$).

DISCUSSION

These findings indicate that a 5.0 mg/kg dose of ORG 2766 administered to rats 1 hr before training significantly facilitated acquisition of an inhibitory avoidance task, and that an injection given 1 hr before the retention test was generally arousing since the saline control group in this condition differed significantly from the combined pre- and immediate posttrial saline control groups (see Fig. 1). Taken together, the results showed that ORG 2766 facilitated acquisition of an inhibitory avoidance response, but it did not influence consolidation or retrieval, at least under the conditions used in this study. The memory modulatory effects of $ACTH_{4-10}$ and ORG 2766 may be different since it has been shown that $ACTH_{4-10}$ administered after training facilitates consolidation of both inhibitory and active avoidance tasks [2]. However, since the memory facilitating properties of $ACTH_{4-10}$ were not directly tested in this study, this conclusion must be viewed with caution.

The increased behavioral potency of ORG 2766 over $ACTH_{4-10}$, reported to be a thousand-fold [11], may be task and situation specific. This conclusion is supported by the fact that only a dose of 0.001 μg/rat ORG 2766 had to be used to significantly attenuate CO_2-induced amnesia in a pre-retention test situation [6], whereas our effective dose of 5.0 mg/kg, in a preacquisition condition, required to enhance acquisition, was considerably greater. However, it should be noted that Rigter *et al.* [6] did not investigate doses as great as 5.0 mg/kg, nor did we investigate doses as low as 0.001 μg/rat.

Since ORG 2766 is devoid of endocrine action [11], these data indicate that the drug acted centrally to produce its effect. The finding that ORG 2766 affected only the acquisition of the avoidance response when given 1 hr before training suggests that it acted by influencing sensory, motivational or attentional variables rather than memory storage processes, and agrees with Rigter *et al.* [6] who concluded that in general, ACTH-like peptides improve selective attention.

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