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Dose-Related Facilitation and Inhibition of Passive Avoidance Behavior by the ACTH 4–9 Analog (ORG 2766)

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FEKETE, M. AND D. DE WIED. *Dose related facilitation and inhibition of passive avoidance behavior by the ACTH 4–9 analog (ORG 2766).* PHARMAC. BIOCHEM. BEHAV. 17(2) 177–182, 1982.—The effects of "low" and "high" doses of the ACTH 4–9 analog (ORG 2766) were studied on passive avoidance behavior of rats compared to ACTH 4–10 and [D-Phe^7] ACTH 4–10. All peptides increased avoidance latency in a dose-dependent manner. However, "high" doses of ORG 2766 (500 and 1000 ng/rat) inhibited passive avoidance retention. "High" amounts of ACTH 4–10 and [D-Phe^7] ACTH 4–10 still facilitated passive avoidance behavior. "High" doses of ORG 2766 like "low" amounts of this peptide delayed extinction of active avoidance behavior. "High" doses of [D-Phe^7] ACTH 4–10 like "low" amounts of this peptide facilitated extinction of active avoidance behavior. The substituted analog apparently carries a dual effect on passive avoidance behavior.

ACTH 4–9 analog Passive avoidance behavior Active avoidance behavior Dose-dependent effects

N-TERMINAL ACTH fragments, like ACTH 1–10, ACTH 4–10, ACTH 4–7 affect acquisition and maintenance of active and passive avoidance behavior in the rat [2, 3, 4, 5, 6, 14, 15]. The introduction of three structural modifications in ACTH 4–9 (H-Met/O_2/-Glu-His-Phe-D-Lys-Phe-OH; ORG 2766), resulted in an increase in behavioral activity by at least a factor of one thousand as determined on extinction of pole-jumping avoidance behavior [7, 14, 15]. A marked dissociation between behavioral and intrinsic endocrine effects was achieved since the steroidogenic, melanocyte-stimulating, opiate-like and lipolytic effects of this neuropeptide are markedly reduced by these substitutions [14, 20, 23, 26].

In previous studies the only difference between ORG 2766 and ACTH 4–10 appeared to be a longer lasting behavioral effect of the substituted compound [9]. The present study shows that while ORG 2766 facilitates passive avoidance behavior in "low" amounts, it acted in an opposite way when "high" doses were administered. This differential effect of ORG 2766 was not observed in active avoidance behavior and absent with ACTH 4–10 and [D-Phe^7] ACTH 4–10 when tested in "low" and "high" doses on passive avoidance behavior.

METHOD

Animals

Male Wistar rats of an inbred strain (CPB-TNO, Zeist, The Netherlands) weighing 140–160 g, were used. The animals were housed 6 per cage and housed at room temperature (20–21°C). All animals had access to commercial food and tap water ad lib and were kept on a controlled illumination schedule (lights on between 6 a.m. and 8 p.m.).

Behavioral Procedures

Passive avoidance behavior. Animals were trained in a step-through type one-trial learning passive avoidance test [1]. The training was started between 3 p.m. and 7 p.m. The experimental apparatus consisted of an illuminated platform attached to a large, dark compartment equipped with a grid floor. After habituation to the dark compartment (2 min), rats were placed on the platform and allowed to enter the dark compartment; since rats prefer dark to light, they normally entered within 15 sec. On the next day after three more trials, unavoidable scrambled footshock (0.25 mA, 2 sec) was delivered through the grid floor of the dark compartment (learning trial). Rats were removed from the shock box 10 sec after the termination of the shock. Passive avoidance latencies were tested 24 hours (first retention test) and 48 hours (second retention test) after the learning trial; the rat was placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 sec. Treatments with peptide or vehicle were given 30 sec after the learning trial (postlearning treatment) or 1 hr before the first retention test (preretention treatment).

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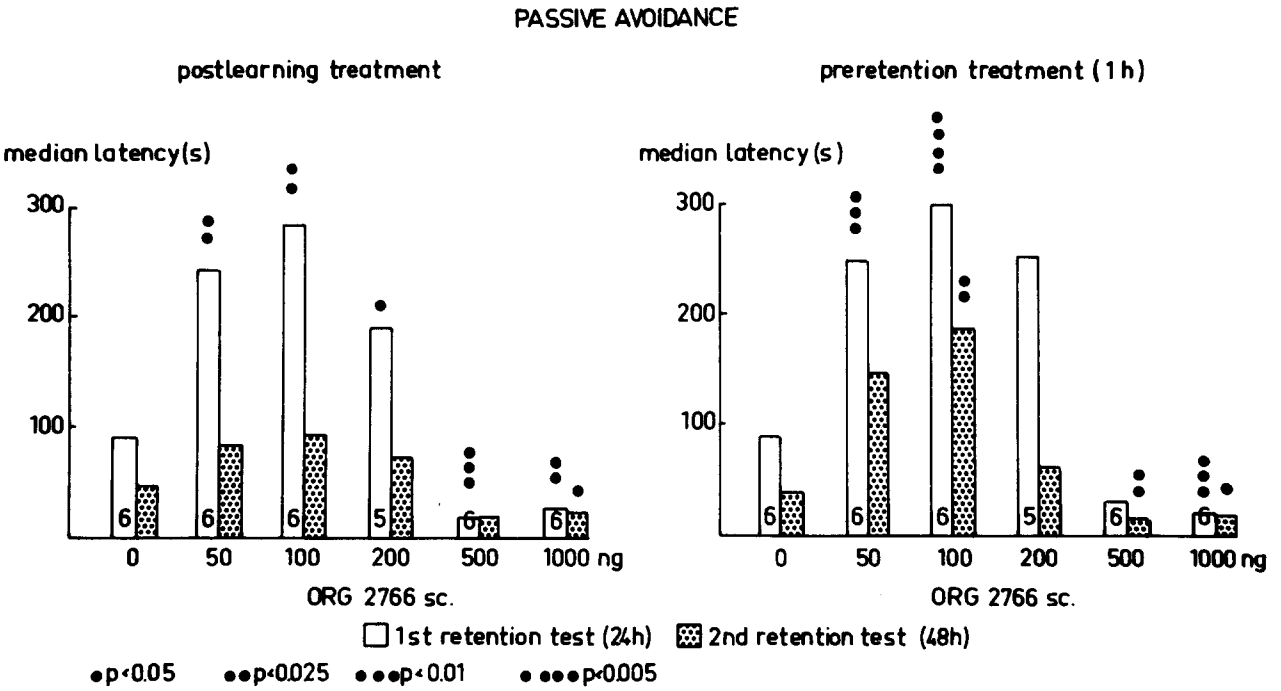

FIG. 1. Effects of graded doses of ORG 2766 on retention of one-trial learning passive avoidance response in rats.

Active avoidance behavior. Active avoidance behavior was studied in a pole-jumping situation as described previously [3,25]. Rats were conditioned to avoid the unconditioned stimulus (US) of an electric footshock (0.20 mA, AC) by jumping onto a pole (diameter 1.5 cm) located in the center of the box (30×30×40 cm). The conditioned stimulus (CS) was a light signal. The US was applied if an avoidance response had not occurred within 5 sec after the onset of the CS. The CS remained on during presentation of the US. Ten acquisition trials were given daily. Acquisition training for 3 days was followed by extinction sessions on day 4 and 5. In [D-Phe^7] ACTH 4–10 experiments 4 days of acquisition were used to make the rats more resistant to extinction. Extinction sessions were run on day 5 and 6. Ten reinforced trials were presented per session in which the CS was terminated immediately after the rat had jumped onto the pole within 5 seconds (positive response, conditioned avoidance response) or after 5 seconds in the absence of avoidance. Those animals which made 8 or more avoidances at the first extinction session on day 4 (or 5), were used for further experimentation. The rats received peptide or saline in a volume of 0.5 ml per rat SC. Thirty sec after completion of the first extinction session and two more extinction sessions were run at 2 and 4 hours after the first one. The training and extinction sessions were started between 7 a.m. and 11 a.m.

Peptides

ACTH 4–10 (H-Met-Glu-His-Phe-Arg-Trp-Gly-OH), [D-Phe^7] ACTH 4–10 (H-Met-Glu-His-D-Phe-Arg-Trp-Gly-OH) and ACTH 4–9 analog (H-Met/O_2/-Glu-His-Phe-D-Lys-Phe-OH; ORG 2766) were dissolved in one drop of 10^{-5} N HCl then diluted with 0.9% saline (pH: 6.5–6.7). All injections were given subcutaneously in a volume of 0.5 ml. Control animals received the same volume of the vehicle.

Statistical Analysis

Mann-Whitney's non-parametric ranking test was used for statistical analysis.

RESULTS

Postlearning treatment with ORG 2766 in 50, 100 and 200 ng doses significantly facilitated passive avoidance while 500 and 1000 ng inhibited avoidance latency at the first retention test. The 1000 ng dose of ORG 2766 was also effective at the second retention test (Fig. 1). Treatment with 50 and 100 ng of ORG 2766 1 hour before the first retention test, i.e., 23 hours after the learning trial significantly facilitated passive avoidance behavior at the first retention test (Fig. 1). A dose of 100 ng ORG 2766 also increased passive avoidance latency at the second retention test. Administration of 200 ng increased avoidance latency but due to large variations in avoidance latencies, the difference between ORG 2766 and saline treated groups was not significant. However, 500 ng (at the second retention test) and 1000 ng (at both the first and second retention test) inhibited passive avoidance behavior.

Postlearning treatment with 50, 100 and 200 μg doses of ACTH 4–10 failed to affect passive avoidance latency, while massive doses of this peptide (500 and 1000 μg/rat) significantly increased avoidance latency at both retention tests (Fig. 2). Treatment with the lower amounts of ACTH 4–10 1 hour before the first retention test significantly facilitated passive avoidance behavior. The higher doses also facilitated passive avoidance behavior at the second retention test (Fig. 2).

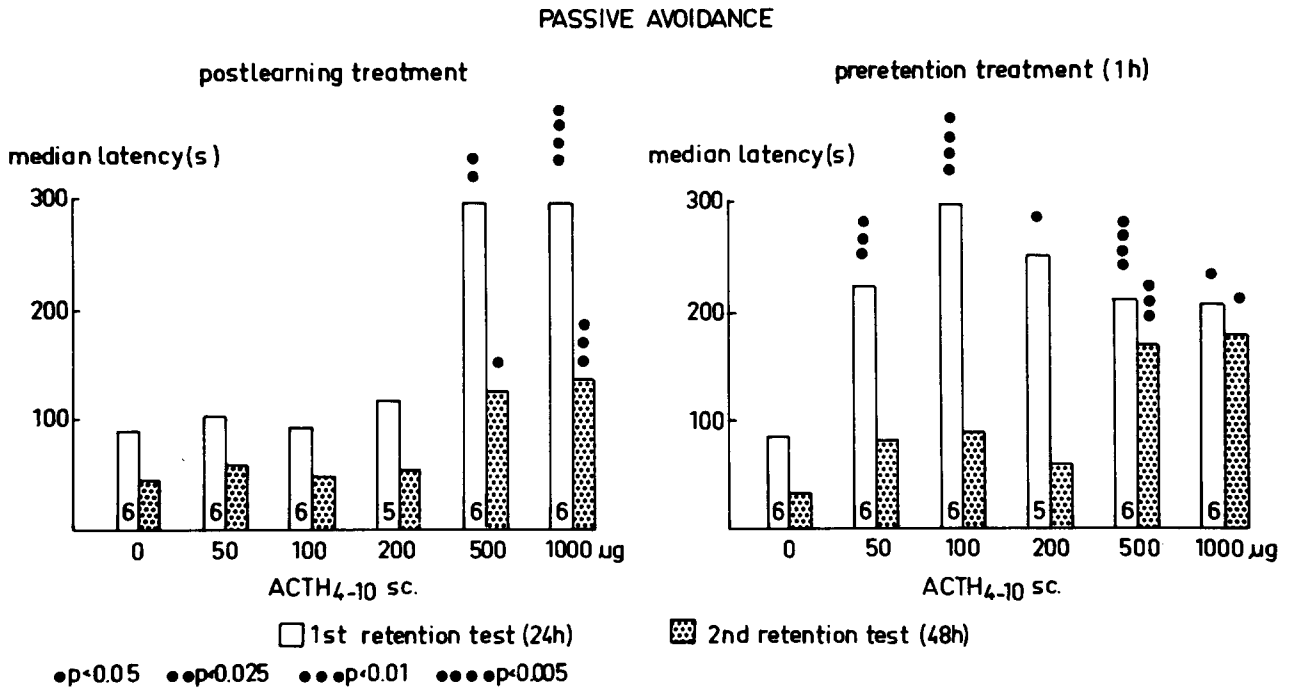

FIG. 2. Effects of graded doses of ACTH 4–10 on retention of one-trial learning passive avoidance response in rats.

Postlearning administration of 50, 100, 200 and 1000 µg doses of [D-Phe^7] ACTH 4–10 significantly increased passive avoidance latency at the first retention test. No effect was found at the second retention (Fig. 3). Treatment 1 hour before the first retention test with the same amounts of [D-Phe^7] ACTH 4–10 significantly facilitated passive avoidance behavior at both retention tests (Fig. 3).

As can be seen from Table 1, subcutaneous treatment immediately after the first extinction session with ORG 2766, in 100, 300 and 1000 ng doses, delayed extinction of the pole-jumping avoidance response 2, 4 and 24 hours later. Subcutaneous injection immediately after the first extinction session with [D-Phe^7] ACTH 4–10, in 10 and 30 µg doses facilitated extinction of the pole-jumping avoidance response 2 and 4 hours later (Table 1).

DISCUSSION

The present experiments show that ORG 2766, ACTH 4–10 and [D-Phe^7] ACTH 4–10 dose dependently facilitate passive avoidance behavior. As found in previous studies, ORG 2766 facilitated passive avoidance behavior at one thousandth the dose and possessed a longer lasting effect than ACTH 4–10 [9, 14, 15, 21]. "High" amounts of ORG 2766 had an opposite effect in that it inhibited passive avoidance behavior. This dual effect of ORG 2766 may have to do with the level of arousal of the animal, since an inverted U-shaped relationship exists between behavioral efficiency and arousal. An increase in arousal on the ascending part of the curve may stimulate behavior and an increase at the peak or thereafter may have an inhibitory effect [16]. Such an U-shaped effect has been found for ACTH 4–10 on self-stimulation behavior [19] for ACTH on active and passive avoidance behavior [13,17] and for ACTH-related peptides on lordosis behavior in rats [24]. However, facilitation and inhibition of passive avoidance behavior seems to depend on the dose of ORG 2766 rather than on the level of arousal. This dual effect seems to be specific for passive avoidance behavior, since "low" and "high" doses of ORG 2766 affect extinction of pole-jumping active avoidance behavior in the same direction. In addition, it is a specific action of ORG 2766, since ACTH 4–10 or [D-Phe^7] ACTH 4–10 affected passive avoidance behavior in the same direction irrespective whether "low" or "high" dose were used. [D-Phe^7] ACTH 4–10 facilitates extinction of pole-jumping active avoidance behavior in relatively low doses (1 and 3 µg/rat [9]. The effect could not be reversed by amounts thirty times those needed to facilitate extinction.

[D-Phe^7] ACTH 4–10 as well as ORG 2766 when injected immediately after the learning trial facilitated passive avoidance behavior. This might suggest that these peptides affect memory processes. However in previous studies it has been shown that the influence of [D-Phe^7] ACTH 4–10 is on retrieval rather than on consolidation processes, as was also found with ACTH 4–10 [9]. The "illusory" posttrial effect of ORG 2766 as well as of [D-Phe^7] ACTH 4–10 is a consequence of the 24 hour lasting effect of these peptides on passive avoidance behavior as shown earlier in a previous paper [9].

Our data are, however, at variance with those of others. Martinez *et al.* [18] have shown that a rather high dose of ORG 2766 (5.0 mg/kg) administered prior to the learning trial facilitated acquisition of passive avoidance behavior. It had no effect when administered immediately after the learning trial or 1 hour prior to the retention test. Their control rats, however, showed much lower avoidance latencies (median latency to step through was 7.5 and 4 sec, respectively) than those found in the present experiments. Flood *et al.* [12]

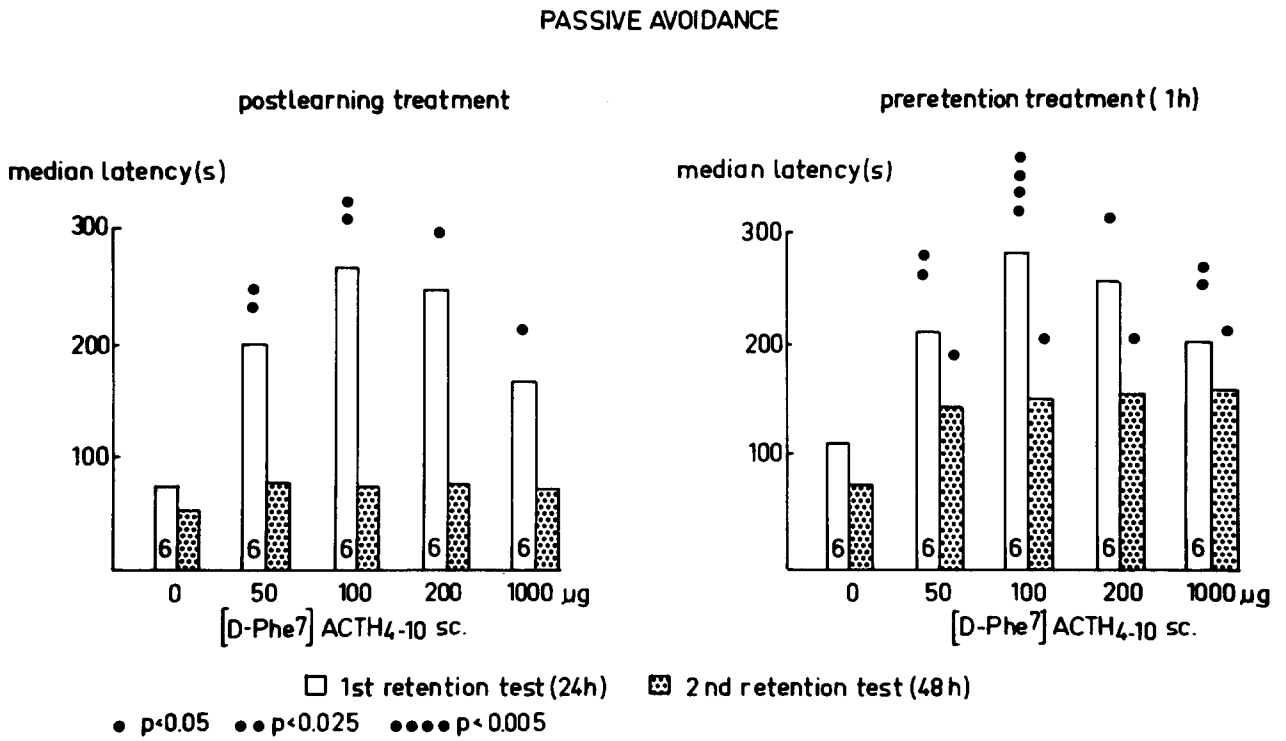

FIG. 3. Effects of graded doses of [D-Phe^7] ACTH 4–10 on retention of one-trial learning passive avoidance response in rats.

TABLE 1

EFFECT OF ORG 2766 AND [D-Phe^7] ACTH 4–10 ON THE RATE OF EXTINCTION OF POLE-JUMPING AVOIDANCE BEHAVIOR IN RATS

		Number of Avoidances				
Treatment	n	0	2	4	24	48*
ORG 2766						
100 ng†	6	8.5 ± 0.2‡	8.2 ± 0.3§	7.8 ± 0.3§	5.2 ± 0.5§	1.3 ± 0.3
300 ng	6	8.5 ± 0.2	8.8 ± 0.5§	9.0 ± 0.3§	5.2 ± 0.5§	1.3 ± 0.3
Saline, 0.5 ml	6	8.5 ± 0.3	4.7 ± 0.3	2.5 ± 0.6	1.5 ± 0.2	1.5 ± 0.6
ORG 2766						
1000 ng	6	8.8 ± 0.3	8.7 ± 0.4§	8.2 ± 0.5§	5.5 ± 0.4§	1.8 ± 0.3
Saline, 0.5 ml	6	8.6 ± 0.3	4.5 ± 0.6	1.7 ± 0.3	1.7 ± 0.5	1.2 ± 0.3
[D-Phe^7] ACTH 4–10						
10 µg	8	8.8 ± 0.3	4.0 ± 0.7¶	2.0 ± 0.6¶	1.3 ± 0.4	N.D.#
30 µg	8	9.1 ± 0.2	3.9 ± 0.7¶	1.6 ± 0.4¶	1.4 ± 0.3	N.D.
Saline, 0.5 ml	7	9.3 ± 0.3	8.9 ± 0.5	7.7 ± 0.5	3.0 ± 1.0	N.D.

*Hours after injection.
†Dose per rat SC.
‡Mean ± S.E.
§$p<0.005$ vs saline-treated rats.
¶$p<0.001$ vs saline-treated rats.
#Not determined.

have shown that high dose of [D-Phe^7] ACTH 4–10 (0.3, 1.0 and 3.0 mg/kg) administered after the learning trial impaired passive avoidance behavior of mice. Our experiments failed to show attenuation of passive avoidance behavior in rats even when similar amounts of [D-Phe^7] ACTH 4–10 were used as employed by Flood *et al.* [12]. Recently, Soumireu-Mourat *et al.* [22] showed that a dose of 10 ng of ORG 2766 injected either subcutaneously or intracerebroventricularly facilitated extinction of a one way active avoidance behavior of mice. The same authors reported that posttraining injection of the same dose of ORG 2766 impaired the retention of a continuously reinforced appetitive operant conditioning task after peripheral administration but had the opposite effect after intracerebroventricular administration. On the other hand, we found that ORG 2766 in "low" doses (15–50 ng/rat SC) enhanced lever pressing for low intensity stimulation, but attenuated self-stimulation at higher current intensities in a continuous reinforced electrical self-stimulation behavior elicited from the medial septal area using an ascending sequence of stimulus intensities within a session [8]. The differences between these findings may be ascribed to differences in the dose, the species and the paradigms used.

The dose dependent facilitation and inhibition of passive avoidance behavior by ORG 2766 suggest the presence of two different intrinsic activities in the same molecule. It is noteworthy that the influence of ACTH-related peptides on social interaction in rats is more or less similar to that found on passive avoidance behavior. Both ACTH 4–10 and [D-Phe^7] ACTH 4–10 decreases social interaction, although [D-Phe^7] ACTH 4–10 affects social interaction in an unfamiliar situation only [10]. Recently File [11] found that ORG 2766 facilitates social interaction. To facilitate social interaction a thousand times lower amount was needed than that of ACTH 4–10 to exert an opposite effect. However, the present experiments show that the opposite effect of ORG 2766 on passive avoidance behavior was found only following "high" doses of the compound.

The attenuating effect of "high" (500 and 1000 ng) doses of ORG 2766 on retention of passive avoidance behavior may be explained by a competitive action on ACTH sensitive brain structures, or by a functional antagonistic action. It is also possible that the attenuating effect is caused by interference with endogenous (enzymic?) processes to prevent the formation of endogenous neuropeptides related to ACTH or by release of neuropeptide(s) with amnestic properties. However, no explanation can be offered at present for the observation that ORG 2766 has a dual action on passive avoidance behavior only. "Low" amounts of ORG 2766 mimic the effect of ACTH 4–10 and related peptides on active and passive avoidance behavior. ACTH 4–7 is as active as ACTH 4–10 on extinction of pole-jumping active avoidance behavior [7], thus the inhibitory effect of ORG 2766 on passive avoidance behavior probably is located in the C-terminal part of the peptide.

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