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Potency and Duration of Action of the ACTH 4–9 Analog (ORG 2766) as Compared to ACTH 4–10 and [D-Phe^7] ACTH 4–10 on Active and Passive Avoidance Behavior of Rats

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FEKETE, M AND D DEWIED *Potency and duration of action of the ACTH 4–9 analog (ORG 2766) as compared to ACTH 4–10 and [D-Phe^7] ACTH 4–10 on active and passive avoidance behavior of rats* PHARMAC BIOCHEM BEHAV **16**(3) 387–392, 1982 —Experiments were performed to examine the potency and duration of action of various ACTH analogs on active and passive avoidance behavior of rats ACTH 4–10 and the ACTH 4–9 analog (ORG 2766) delayed extinction of pole-jumping avoidance behavior and facilitated passive avoidance responding [D-Phe^7] ACTH 4–10 facilitated extinction of pole-jumping avoidance behavior and facilitated passive avoidance responding ORG 2766 was a thousand times more active than ACTH 4–10 The effect of ORG 2766 on extinction of pole-jumping avoidance behavior and on passive avoidance behavior was of longer duration than that of ACTH 4–10 As determined more precisely in the passive avoidance test it appeared that the action of ACTH 4–10 lasted 3 to 6 hours, while that of ORG 2766 amounted to at least 24 hours Although [D-Phe^7] ACTH 4–10 was a thousand times less active than ORG 2766 in the passive avoidance paradigm, its duration of action was of the same magnitude In view of this, the marked increase in potency of the ACTH 4–9 analog cannot be explained only on the basis of its metabolic stability but also by an increased intrinsic activity

ACTH 4–9 analog Active avoidance behavior Passive avoidance behavior Potency Duration of action

THE ACTH 4–9 analog ORG 2766, which has reduced steroidogenic, melanocyte-stimulating and lipolytic and no opiate-like effects, has a markedly increased behavior potency, as compared to ACTH 4–10, as determined on extinction of pole-jumping avoidance behavior [7, 11, 12] The half life of ORG 2766 in comparison to ACTH 4–10 is markedly prolonged [19] However, comparative studies on the *in vivo* duration of action for these two peptides are missing The present study was undertaken to compare potency and duration of action of ACTH 4–10 and ORG 2766 on active and passive avoidance behavior In addition the effect of [D-Phe^7] ACTH 4–10 was measured in the same two paradigms because it contains a D-enantiomer amino acid like ORG 2766 and was found in previous studies to exert a prolonged effect on passive avoidance responding [11]

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 5 a m and 7 p m)

Behavioral Procedures

Active avoidance behavior Active avoidance behavior was studied in a pole-jumping situation as described previously [4,18] Rats were conditioned to avoid the uncon-

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TABLE 1

EFFECT OF ACTH 4–10 AND ORG 2766 ON THE RATE OF EXTINCTION OF POLE-JUMPING AVOIDANCE BEHAVIOR IN RATS

Treatment	n	Number of avoidances 0	2	4	24	48*
ACTH 4–10						
1 μg†	12	8 8 ± 0 3‡	6 9 ± 0 4¶	4 8 ± 0 3#	1 4 ± 0 2	N S **
3 μg	12	8 8 ± 0 2	8 3 ± 0 3#	7 4 ± 0 3#	2 3 ± 0 6	N S
Saline, 0 5 ml	12	8 7 ± 0 2	4 2 ± 0 4	2 0 ± 0 4	1 1 ± 0 3	N S
ACTH 4–10						
1 μg	8	8 8 ± 0 3	N S	N S	3 1 ± 0 4	1 9 ± 0 5
3 μg	9	8 8 ± 0 2	N S	N S	3 3 ± 0 7	1 7 ± 0 4
Saline, 0 5 ml	8	8 5 ± 0 3	N S	N S	3 0 ± 0 6	1 8 ± 0 5
ORG 2766						
1 ng	10	8 6 ± 0 4	7 0 ± 0 7§	4 3 ± 0 7#	3 4 ± 0 6¶	1 0 ± 0 4
3 ng	10	8 7 ± 0 4	8 3 ± 0 4#	6 6 ± 0 6#	5 3 ± 0 5#	1 4 ± 0 2
Saline, 0 5 ml	10	8 3 ± 0 2	4 4 ± 0 6	1 8 ± 0 5	1 1 ± 0 3	0 9 ± 0 2
ORG 2766						
1 ng	8	8 9 ± 0 2	N S	N S	4 1 ± 0 5	1 6 ± 0 5
3 ng	9	8 6 ± 0 2	N S	N S	5 1 ± 0 7§	1 9 ± 0 5
Saline, 0 5 ml	8	8 5 ± 0 3	N S	N S	3 0 ± 0 6	1 8 ± 0 5

*Hr after injection
†Dose per rat SC
‡Mean ± S E
§$p<0$ 05 vs saline-treated rats
¶$p<0$ 005 vs saline-treated rats
#$p<0$ 001 vs saline-treated rats
**Not studied

ditioned 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 seconds 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 nonreinforced 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 immediately after completion of the first extinction session and two more extinction sessions were run at 2 and 4 hours after and/or 24 and 48 hours after the first one The training and extinction sessions started between 7 a m and 11 a m

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) (The median entrance latencies at the learning trial for the different groups in the various experiments ranged from 3 to 10 secs, and the group differences were not significant) Rats were removed from the shock box 10 sec after the termination of the shock Passive avoidance latencies were tested 24 hours and/or 48 hours 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 immediately after the learning trial (postlearning treatment) or at different times before the first retention test (given in the Tables)

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

TABLE 2

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

Treatment	n	Number of avoidances 0	2	4	24	48*
[D-Phe^7] ACTH 4–10						
1 μg†	8	8 5 ± 0 3‡	6 5 ± 0 5¶	4 4 ± 0 8#	2 1 ± 0 3	N S ††
3 μg	8	8 4 ± 0 2	5 4 ± 0 7#	2 4 ± 0 4**	2 0 ± 0 3	N S
Saline, 0 5 ml	7	8 6 ± 0 2	8 3 ± 0 3	7 4 ± 0 3	2 7 ± 0 3	N S
[D-Phe^7] ACTH 4–10						
1 μg	8	9 0 ± 0 3	N S	N S	4 4 ± 0 5	2 0 ± 0 3
3 μg	8	8 9 ± 0 4	N S	N S	3 1 ± 0 4§	1 6 ± 0 3
Saline, 0 5 ml	8	8 6 ± 0 2	N S	N S	5 4 ± 0 7	1 8 ± 0 5

*Hr after injection
†Dose per rat SC
‡Mean ± S E
§$p<0$ 025 vs saline-treated rats
¶$p<0$ 01 vs saline-treated rats
#$p<0$ 005 vs saline-treated rats
**$p<0$ 001 vs saline-treated rats
††Not studied

RESULTS

Active Avoidance Behavior

As can be seen from Table 1, subcutaneous treatment immediately after the first extinction session with μg doses of ACTH 4–10 delayed extinction of the pole-jumping avoidance response 2 and 4 hours later, but not 24 hours later Under the same conditions ORG 2766, in ng doses, delayed extinction of the pole-jumping avoidance response at 2, 4 and 24 hours later If the 2 and 4 hour extinction sessions had been omitted, ORG 2766 (but not ACTH 4–10) delayed extinction of the pole-jumping avoidance response 24 hours later The effect of the peptide had disappeared 48 hours after injection On a weight basis ORG 2766 appeared to be a thousand times more potent than ACTH 4–10

As can be seen from Table 2, subcutaneous injection immediately after the first extinction session with [D-Phe^7] ACTH 4–10, in μg doses, facilitated extinction of the pole-jumping avoidance response 2 and 4 hours later The number of avoidances of peptide treated rats did not differ significantly from those of saline treated controls 24 hours after injection If the 2 and 4 hour extinction sessions had been omitted, [D-Phe^7] ACTH 4–10 also facilitated extinction of the pole-jumping avoidance response 24 hours later

Passive Avoidance Behavior

The results of postlearning and preretention injections of ACTH 4–10, ORG 2766 and [D-Phe^7] ACTH 4–10 on the retention of passive avoidance behavior are shown in Table 3 Postlearning treatment with ACTH 4–10 in various doses did not significantly affect the 24 hour avoidance latency However, treatment with the same amounts of ACTH 4–10 1 hour before the first retention test 24 hours after the learning trial significantly facilitated passive avoidance behavior in a dose dependent manner No effect was found at the second retention test Postlearning treatment with various amounts of ORG 2766 significantly increased passive avoidance latency at the first retention test (Table 3) The magnitude of the action was related to the dose of peptide The effect was absent at the second retention test The same amounts of ORG 2766 given 1 hour before the first retention test facilitated passive avoidance behavior at the first retention test The highest dose of the peptide (90 ng) also significantly increased avoidance latency at the second retention session 24 hours later Also in these studies ORG 2766 appeared to be a thousand times more potent than ACTH 4–10

Postlearning treatment with 30 and 90 μg doses of [D-Phe^7] ACTH 4–10 increased passive avoidance latency at the first retention test The effect was absent at the second retention test Treatment with 10, 30 and 90 μg doses of [D-Phe^7] ACTH 4–10 1 hour before the first retention test significantly facilitated passive avoidance behavior The highest dose of the peptide (90 μg) also significantly increased avoidance latency at the second retention session

To determine the duration of the action of ACTH 4–10, the highest dose (90 μg) was administered at 1, 3 6 hours before the 24 hour retention test As can be seen from Table 4, a significant effect was found when the treatment was given at 1 and at 3 hours prior to the retention test, but not when the treatment was given at 6 hours before the retention test This suggests that the duration of action of ACTH 4–10 lies between 3 and 6 hours

Administration of the 90 ng dose of ORG 2766 immediately after the learning trial, 12 or 18 hours later failed to affect avoidance latency at the retention test 48 hours later (Table 5) Only if given 24 hours after the learning trial and latency was measured 24 hours later a significant increase in avoidance latency was found Thus, the duration of action of ORG 2766 amounted to approximately 24 hours

To determine the duration of the action of [D-Phe^7] ACTH 4–10 the highest dose (90 μg) was administered at 48, 42, 36, 30 and 24 hours before the 48 hour retention test

TABLE 3

EFFECT OF SUBCUTANEOUS TREATMENT WITH ACTH 4–10, ORG 2766 AND [D-Phe^7] ACTH 4–10 ON RETENTION OF ONE-TRIAL LEARNING PASSIVE AVOIDANCE RESPONSE IN RATS

	n	First retention test (24 hr)	p*	Second retention test (48 hr)	p*
Postlearning treatment					
ACTH 4–10					
10 μg†	5	110‡	>0 05	46	>0 05
30 μg	5	95	>0 05	48	>0 05
90 μg	5	118	>0 05	60	>0 05
Saline 0 5 ml	5	100		53	
ORG 2766					
10 ng	5	169	<0 025	72	>0 05
30 ng	5	194	<0 01	57	>0 05
90 ng	5	300	<0 005	72	>0 05
Saline, 0 5 ml	5	96		53	
[D-Phe^7] ACTH 4–10					
10 μg	6	118	>0 05	59	>0 05
30 μg	6	208	<0 05	64	>0 05
90 μg	6	226	<0 05	65	>0 05
Saline, 0 5 ml	6	92		48	
Pre-retention treatment					
ACTH 4–10					
10 μg	7	156	<0 001	33	>0 05
30 μg	7	255	<0 001	34	>0 05
90 μg	7	300	<0 001	41	>0 05
Saline, 0 5 ml	7	97		40	
ORG 2766					
10 ng	7	147	<0 05	68	>0 05
30 ng	7	235	<0 001	125	>0 05
Saline, 0 5 ml	6	91		30	
ORG 2766					
90 ng	8	300	<0 001	171	< 0 001
Saline, 0 5 ml	7	97		40	
[D-Phe^7] ACTH 4–10					
10 μg	6	154	<0 05	52	>0 05
30 μg	6	233	<0 05	70	>0 05
90 μg	6	256	<0 01	137	<0 05
Saline, 0 5 ml	6	97		53	

*Differences between peptide- and saline-treated rats are expressed as the level (p) of statistical significance (Mann-Whitney U-test)
†Dose per rat, SC
‡Median latency in sec

Administration of the peptide immediately after the learning trial, 6, 12 or 18 hours later failed to affect avoidance latency at the 48 hour retention test (Table 6) Only if given 24 hours after the learning trial, i e , 24 hours before the 48 hour retention test a significant increase in avoidance latency was found Thus, the duration of action of [D-Phe^7] ACTH 4–10 amounted to approximately 24 hours

DISCUSSION

The present experiments confirm earlier findings that both ACTH 4–10 and ORG 2766 delay extinction of pole-jumping active avoidance behavior and that ORG 2766 is a thousand times more active than ACTH 4–10 [5, 6, 7, 8, 11, 12, 19] In addition it was found that ORG 2766 had a much longer lasting effect than ACTH 4–10 The same was found in passive avoidance behavior In this respect it is noteworthy that ORG 2766 also appeared to be a thousand times more potent than ACTH 4–10 in reversing CO_2-induced amnesia [15] ACTH-like peptides given prior to the retention test of a one-trial learning step-through situation facilitated passive avoidance behavior in rats [3,17] As in active avoidance situations, fragments of ACTH such as ACTH 1–10, ACTH 4–10 or ACTH 4–7 facilitate conditioned behavior in the passive avoidance test [11] This effect is of a short term nature The effect of ACTH 4–10 on passive avoidance

TABLE 4

TIME-RELATED EFFECTS OF ACTH 4–10 ON RETENTION OF ONE-TRIAL LEARNING PASSIVE AVOIDANCE RESPONSE IN RATS

Treatment-test interval	Passive avoidance behavior 24 hours after the learning trial (median in sec) Saline	ACTH 4–10 (90 μg SC)	Significance
6 hr	89 (6)*	147 (6)	$p>0.05$
3 hr	99 (6)	234 (6)	$p<0.05$
1 hr	92 (6)	300 (6)	$p<0.01$

*Number of rats

TABLE 5

TIME-RELATED EFFECTS OF ORG 2766 ON RETENTION OF ONE-TRIAL LEARNING PASSIVE AVOIDANCE RESPONSE IN RATS

Treatment-test interval	Passive avoidance behavior 48 hours after the learning trial (median in sec) Saline	ORG 2766 (90 ng SC)	Significance
48 hr	69 (9)*	55 (10)	$p>0.05$
36 hr	79 (9)	104 (10)	$p>0.05$
30 hr	69 (9)	107 (10)	$p>0.05$
24 hr	56 (9)	173 (10)	$p<0.005$

*Number of rats

TABLE 6

TIME-RELATED EFFECTS OF [D-Phe^7] ACTH 4–10 ON RETENTION OF ONE-TRIAL LEARNING PASSIVE AVOIDANCE RESPONSE IN RATS

Treatment-test interval	Passive avoidance behavior 48 hours after the learning trial (median in sec) Saline	[D-Phe^7] ACTH 4–10 (90 μg SC)	Significance
48 hr	54 (7)*	43 (7)	$p>0.05$
42 hr	75 (7)	88 (7)	$p>0.05$
36 hr	66 (7)	56 (7)	$p>0.05$
30 hr	56 (7)	68 (7)	$p>0.05$
24 hr	64 (7)	109 (9)	$p<0.05$

*Number of rats

behavior was still present when the injection of the peptide was given at 1 or 3 but not at 6 hours before the retention test Thus the influence of ACTH 4–10 seems to last between 3 and 6 hours A similar experiment with ORG 2766 showed that this peptide was active when given 24 hours before the retention test Thus the facilitating effect of ORG 2766 on passive avoidance behavior is of a much longer nature than that of ACTH 4–10

Our data are, however, at variance with those of others Martinez *et al* [14] have shown that a rather high dose of ORG 2766 (5 mg/kg) administered prior to the learning trial significantly facilitated acquisition of a passive avoidance response, but not when administered immediately after the learning trial or 1 hour prior to the retention test Their control rats, however, did not show much avoidance behavior (median latency to step-through was 7 5 and 4 sec, respectively), and the doses they used were much higher than the ones employed in the present experiments On the other hand, Rigter *et al* [15] reported that ORG 2766 attenuates CO_2-induced amnesia for a one-trial passive avoidance response when administered prior to the retrieval, but not when given prior to acquisition The latter studies suggested that the duration of action of ORG 2766 was somewhat longer than that of ACTH 4–10 Indeed, the present experiments show that ORG 2766 has a much longer lasting effect than ACTH 4–10

ORG 2766 increased passive avoidance latencies when administered immediately after the learning trial and tested 24 hours later, as well as when injected 24 hours after the learning trial and tested 24 hours after peptide administration These findings not only show that the effect of ORG 2766 is of a much longer duration than that of ACTH 4–10, but also that the influence of this analog is on retrieval rather than on consolidation processes as was also found with ACTH 4–10 [3,17] Rigter *et al* [15] arrived at the same conclusion since ORG 2766 attenuated CO_2-induced amnesia only if administered prior to the retrieval but not when given prior to the learning trial The posttrial influence of massive doses of ACTH 4–10 on passive avoidance behavior of mice as found by Flood *et al* [9] might be explained by a carry-over effect due to the flooding of the organism with the peptide

A key role for the amino acid residue phenylalanine at position 7 in the mediation of the behavioral effect of ACTH-like peptides was first demonstrated using an analog of ACTH 1–10 in which phenylalanine in position 7 was replaced by its D-enantiomer In contrast to ACTH 1–10 which delays extinction of a shuttle-box conditioned avoidance response, [D-Phe^7] ACTH 1–10 administration led to a facilitation of extinction [2] Subsequent observations showed that the heptapeptide [D-Phe^7] ACTH 4–10 and the tetrapeptide [D-Phe^7] ACTH 4–7 were as active as the decapeptide [D-Phe^7] ACTH 1–10 [7,11] The present experiments confirm the earlier findings for the ACTH 4–10 peptides

Interestingly, administration of [D-Phe^7] ACTH 4–10, which in active avoidance situations has an effect in a direction opposite to "all-L" peptides, facilitates passive avoidance behavior [3,11] The behavioral effect of this peptide, however, is of a considerably longer duration, than that of ACTH 4–10 and as long as that of ORG 2766 This was also found in the active avoidance test, when the 2 and 4 hour extinction sessions had been omitted No difference was found between performance of control and [D-Phe^7] ACTH 4–10 treated animals 24 hours later, when they were also tested 2 and 4 hours after treatment, because pole-jumping avoidance behavior of control rats had already been extinguished

The mechanism through which [D-Phe^7] ACTH 4–10 and the "all-L" peptides exert their influence on passive avoidance behavior is probably different Flood *et al* [9] have shown that rather high doses of [D-Phe^7] ACTH 4–10 (0 3, 1 0 and 3 0 mg/kg) administered after the learning trial significantly impaired passive avoidance behavior In the present experiments much lower amounts of [D-Phe^7] ACTH 4–10 were used Our data show that [D-Phe^7] ACTH 4–10 increased passive avoidance latencies when administered immediately after the learning trial and tested 24 hours later, as well as when injected 24 hours after the learning trial and tested 24 hours after the peptide administration Thus our findings indicate that the influence of this analog is on retrieval rather than on consolidation processes as was also found with ACTH 4–10 [3,17]

The long lasting behavioral activity of ORG 2766 may be explained by stabilization of the peptide against proteolytic breakdown [16,19] The increased potency however cannot be explained only on the basis of proteolytic protection of the peptide, since the potency ratio relative to ACTH 4–10 both in active and passive avoidance behavior if determined within 1 hour (passive avoidance behavior) or 2 to 4 hours (pole-jumping active avoidance behavior) was the same In addition, the dose-response curves of ORG 2766 and ACTH 4–10 on extinction of pole-jumping avoidance behavior, after subcutaneous, oral and intraventricular administration showed fairly constant potency ratios [13], indicating that protection against metabolic degradation is not the sole reason of the behavioral potentiation of the peptide Also, the long lasting effect of [D-Phe^7] ACTH 4–10 which is comparable to that of ORG 2766 is not associated with an increased potency on passive avoidance responding Thus, the increased potency of ORG 2766 may be explained by assuming an increased intrinsic activity, resulting from the various substitutions in the peptide molecule [12]

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