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Behavioral Activity of a Short Chain ACTH Analog

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DRAGO, F., G. CONTINELLA AND U. SCAPAGNINI. *Behavioral activity of a short chain ACTH analog*. PHARMACOL BIOCHEM BEHAV **20**(5) 689–695, 1984.—The behavioral activity of $ACTH_{1-17}$ analog (β-Ala_1, Lys_{17}) $ACTH_{1-17}$-4-amino-n-butilamide (Ala_1-Lys_{17}-$ACTH_{1-17}$) has been studied in the rat. Acquisition of shuttle-box active avoidance behavior was facilitated by Ala_1-Lys_{17}-$ACTH_{1-17}$ administered both subcutaneously (SC) and intracerebroventricularly (ICV), and this effect was suppressed by peripheral administration of haloperidol or naltrexone. Extinction of pole jumping active avoidance behavior was delayed by SC administration of the peptide in a dose-dependent manner. Retention of a step-through passive avoidance behavior was facilitated SC or ICV injection of Ala_1-Lys_{17}-$ACTH_{1-17}$. Adrenalectomy failed to modify the effects of the peptide on the retention of passive avoidance behavior. Furthermore, ICV injection of graded doses of Ala_1-Lys_{17}-$ACTH_{1-17}$ induced excessive grooming, and this effect was totally prevented by intraperitoneal (IP) injection of haloperidol or naltrexone SC administration of Ala_1-Lys_{17}-$ACTH_{1-17}$ induced a slight but significant increase in negative responses in a test for behavioral responsiveness to electrical footshock. This effect was totally prevented by IP injection of naltrexone. It is concluded that Ala-Lys_{17}-$ACTH_{1-17}$ shares some of the behavioral effects of $ACTH_{4-10}$ and some $ACTH_{1-24}$, but it seems to be more potent than the latter peptides. Both dopamine and opioid transmission seem to be involved in the behavioral activity of Ala_1-Lys_{17}-$ACTH_{1-17}$.

$ACTH_{1-17}$ analog Active avoidance behavior Passive avoidance behavor Grooming Responsiveness to electrical footshock

A number of structure-activity studies have been performed to determine the regions in the adrenocorticotrophin (ACTH) molecule that are essential for its behavioral activity, namely for the effects on avoidance and grooming behavior. $ACTH_{4-7}$ seems to be the shortest active sequence with essentially the same behavioral effects of ACTH on avoidance behavior [12]. $ACTH_{7-10}$ also exerts some behavioral effects, but its potency is lower than that of $ACTH_{4-10}$ unless the COOH-terminal sequence is elongated to $ACTH_{7-16}$ [23]. Differences have been described among ACTH fragments with respect to the induction of excessive grooming in the rat [9]. Also, it has been reported that $ACTH_{4-10}$ does not affect behavioral responsiveness to electrical footshock [8]. However, this peptide possesses a certain affinity to opiate receptors as well as other ACTH fragments [15].

Among ACTH analogues, ORG 2766 (H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH) was shown to be more potent than $ACTH_{4-10}$ both in active and passive avoidance behavior [5].

The present study was undertaken to investigate the behavioral profile of the new $ACTH_{1-17}$ analog (β-Ala_1-Lys_{17}) $ACTH_{1-17}$-4-amino-n-butilamide (Ala_1-Lys_{17}-$ACTH_{1-17}$). This peptide bearing substitutions of the N- and C-terminal aminoacid residues, resulted in enhanced and prolonged biological activity (in terms of steroidogenic potency) as compared to natural ACTH [16]. The behavioral potency of Ala_1-Lys_{17}-$ACTH_{1-17}$ has been compared to that of $ACTH_{1-24}$ and of $ACTH_{4-10}$.

METHOD

Animals

Male rats of Wistar strain (purchased from Charles River, Como, Italy), weighing 140–150 g, were used. The animals were housed 5 per cage and kept at room temperature (20°C). All animals had free access to commercial food and water, under a constant light-dark cycle (lights on between 8.00 and 20.00). Seven days prior to the experimental session a number of animals were implanted with permanent plastic cannula into their lateral ventricle (foramen interventriculare, König and Klippel, A6360). Another group of animals were adrenalectomized or sham-adrenalectomized. Adrenalectomized rats were given saline solution instead of tap water. Only those animals which showed good physical conditions were used seven days after operation. All animals were used only once in the behavioral experiments.

Drugs

$ACTH_{4-10}$(H - Met - Glu - His - Phe - Arg - Trp - Gly - OH), $ACTH_{1-24}$ (H - Ser - Tyr - Ser - Met - Glu - His - Phe - Arg - Trp - Gly - Lys - Pro - Val - Gly - Lys - Lys - Arg - Arg - Pro - Val - Lys -

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Val-Tyr-Pro-OH), and Ala_1-Lys_{17}-$ACTH_{1-17}$ (H-βAla-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Lys-NH-$(CH_2)_4$-NH) were dissolved in one drop of 10^{-5} N HCl then diluted with o.9% saline (pH: 6.6). Injections were given subcutaneously (SC) in a volume of 0.5 ml or intracerebroventricularly (ICV) in a volume of 3 μl. Control animals received the same volume of the vehicle. Haloperidol (Merck, USA) and naltrexone (Serva, West Germany) were dissolved in saline and injected intraperitoneally (IP) at the dose of 0.5 mg/kg.

Behavioral Procedures

Active avoidance behavior was studied in a shuttle-box and a pole jumping situation. The acquisition of shuttle-box active avoidance behavior was studied as described elsewhere [3]. Briefly, animals were trained to avoid the unconditioned stimulus (US) of a scrambled electrical footshock (0.20 mA, AC) delivered through the grid floor of a box divided into two sections by a barrier. The conditioned stimulus (CS) was a buzzer presented for 5 sec prior to the US. If no escape occurred within 20 sec of CS/US presentation, the shock was terminated. A maximum of twenty conditioning trials were given in a single session with a variable intertrial interval averaging 60 sec. The learning criterion was 5 consecutive conditioned avoidance responses (CARs). For those animals that reached the criterion in less than 20 trials, the remaining trials until 20 were considered as CARs. Indexes of avoidance behavior were the total number of CARs and the number of learners.

Extinction of pole jumping avoidance response was studied in the apparatus described by de Wied [19]. The rats were conditioned to avoid the US of an electrical floor shock (0.25 mA, AC) by jumping onto a pole located in the center of the conditioning apparatus. The CS was a light (40 W) provided above the pole and presented for 5 sec. The US was applied if an avoidance response had not occurred within 5 sec of CS presentation. Ten acquisition trials were given daily. Acquisition training for 3 days was followed by extinction sessions on day 4 and 5. Ten nonreinforced trials were presented per session in which the CS was terminated immediately after the rat had jumped onto the pole within 5 sec (conditioned avoidance response, CAR) or after 5 sec in the absence of avoidance. Those animals which made 5 or more avoidances at the second acquisition sessions were used for further experimentation.

Passive avoidance behavior was studied in a step-through type of passive avoidance behavior [1]. Briefly, the rats were adapted to the apparatus consisting of a large dark compartment equipped with a grid floor and a mesh-covered elevated runway attached to the front center of the dark chamber. Adaptation training was followed by a single trial in which the rats were placed on the elevated platform and allowed to enter the dark box. Three such trials were given on the next day with an intetrial interval of 5 min. After the third trial the rats received a single 2-sec unavoidable scrambled foot shock (0.25 mA, AC) immediately after entering the dark compartment. Retention of the response was tested 24 and 48 hr after the learning trial. The rats were placed on the elevated runway and the latency to re-enter the shock compartment was recorded up to a maximum of 300 sec.

Grooming activity of the rats was scored as described by Gispen *et al.* [9]. The rats were placed individually into boxes with transparent walls, in a low-noise room. The behavior of the rats was sampled every 15 sec beginning immediately after the animals were placed into the boxes. The occurrence of grooming was recorded for 50 min, with a maximum of 200 possible positive grooming scores.

Responsiveness to electrical footshock was studied by using the technique described by Gispen *et al.* [8], slightly modified. Two sets of 10 shock intensities between 38 and 383 μA were given in a random order. Behavioral responses recorded were: no response, flinch, jerk, jump, run, and vocalization. The occurrence of each response from 20 trials was recorded.

Open field behavior was studied in a circular arena as described by Weijnen and Slangen [18]. Ambulation (number of floor units entered), rearing and defecation scores were recorded during a 3-min observation session.

All behavioral experiments were performed between 10.00 and 17.00 at natural light. The number of animals tested in each group is given in Tables 1–5.

Animals bearing ICV cannulas or made adrenalectomized were killed at the end of the behavioral procedures. Localization of cannulas was checked by injecting Evans blue and macroscopical inspection of the colouring of the walls of the ventricular system in formaline fixed brains. Macroscopical inspection was also performed for checking correct adrenalectommy.

Experimental Design

Experiment 1. The influence of Ala_1-Lys_{17}-$ACTH_{1-17}$ on the acquisition of shuttle-box active avoidance behavior was studied in rats injected SC with Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05, 0.1, and 0.2 μg/rat). Comparison was made with animals injected SC with $ACTH_{1-24}$ (0.1 μg/rat) or saline. Injections were performed 1 hr before the beginning of the test. Ala_1-Lys_{17}-$ACTH_{1-17}$ was also injected ICV at the dose of 0.01 μg/3 μl/rat 30 min before the test. This dose has been selected on the basis of experiments concerning ACTH fragments [9]. The possible involvement of dopaminergic and opioid transmission in the behavioral effects of Ala_1-Lys_{17}-$ACTH_{1-17}$ was studied in rats injected IP with haloperidol or naltrexone (0.5 mg/kg) and SC with Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat), respectively 2 hr and 1 hr before the behavioral test. Control animals received IP injection of saline.

Experiment 2. Extinction of pole jumping active avoidance behavior was studied in rats injected SC with Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05, 0.1, and 0.2 μg/rat) 1 hr before the first extinction session. Saline-treated rats served as controls.

Experiment 3. The effect of Ala_1-Lys_{17}-$ACTH_{1-17}$ on the retention of passive avoidance response was studied in rats injected SC with this peptide (0.05 and 0.1 μg/rat). Comparison was made with rats injected with $ACTH_{4-10}$ (0.5 μg/rat) or saline. Injections were performed 1 hr before the first retention test. Ala_1-Lys_{17}-$ACTH_{1-17}$ was also injected ICV at the dose of 0.01 μg/3 μl/rat 30 min before the first retention test. The possible involvement of adrenocorticotrophic activity was studied in adrenalectomized rats injected SC with Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 μg/rat) 1 hr before the first retention test. Adrenalectomized animals injected SC with saline served as controls.

Experiment 4. The effect of Ala_1-Lys_{17}-$ACTH_{1-17}$ on grooming behavior was studied in rats injected with graded doses of this peptide (0.01, 0.05, 0.1, 0.3, and 1 μg/3 μl/rat). Comparison was made with animals injected ICV with $ACTH_{1-24}$ (0.3 and 1 μg/3 μl/rat) or saline. The possible in-

TABLE 1

EFFECTS OF Ala_1-Lys_{17}-$ACTH_{1-17}$ ON THE ACQUISITION OF SHUTTLE-BOX ACTIVE AVOIDANCE BEHAVIOR AND THE INTERACTION WITH HALOPERIDOL AND NALTREXONE

Treatment	(n)	CARs[1]	Percentage of Learners[2]
(1) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 μg/rat)*	(12)	12.2 ± 0.2[3]	58.3
(2) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat)*	(8)	14.9 ± 0.2[3]	87.5[4]
(3) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.2 μg/rat)*	(6)	17.9 ± 0.3[5]	100.0[4]
(4) $ACTH_{1-24}$ (0.1 μg/rat)*	(12)	15.1 ± 0.3[3]	83.3[4]
(5) Saline*	(24)	9.1 ± 0.2	20.8
(6) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/3 μl/rat)**	(12)	15.7 ± 0.4[6]	83.3[4]
(7) Saline†	(12)	8.9 ± 0.2	25.0
(8) Haloperidol (0.5 mg/kg)‡ + Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat)*	(16)	9.2 ± 0.3	20.0
(9) Haloperidol (0.5 mg/kg)‡ + saline*	(16)	9.3 ± 0.3	20.0
(10) Naltrexone (0.5 mg/kg)‡ + Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat)*	(8)	9.0 ± 0.2	25.0
(11) Naltrexone (0.5 mg/kg)‡ + saline*	(8)	8.6 ± 0.1	25.0
(12) Saline‡ + Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat)*	(24)	16.0 ± 0.3[7]	83.3[8]
(13) Saline‡ + saline*	(8)	8.6 ± 0.2	25.0

[1]Values are mean ± SEM.
[2]Values are percentage.
[3]Significantly different as compared to group 5 ($p<0.05$, Dunnett's test).
[4]Significantly different as compared to group 5 ($p<0.05$, Fischer exact t-test).
[5]Significantly different as compared to group 5 ($p<0.01$, Dunnett's test).
[6]Significantly different as compared to group 7 ($p<0.01$, Student t-test).
[7]Significantly different as compared to group 13 ($p<0.05$, Dunnett's test).
[8]Significantly different as compared to group 13 ($p<0.05$, Fischer exact t-test).
*Administered SC 1 hr before the test.
†Administered ICV 30 min before the test.
‡Administered IP 2 hr before the test.

volvement of dopaminergic and opioid transmission in grooming behavior induced by Ala_1-Lys_{17}-$ACTH_{1-17}$ was studied in rats injected IP with haloperidol or naltrexone (0.5 mg/kg) and ICV with Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.3 μg/3 μl/rat), respectively 90 min and 30 min before the behavioral test. Control animals received IP injection of saline.

Experiment 5. The influence of Ala_1-Lys_{17}-$ACTH_{1-17}$ on responsiveness to electrical footshock was studied in rats injected with this peptide (0.05 and 0.1 μg/rat). Control animals received SC injection of saline. Naltrexone (0.5 mg/kg) was injected IP to a group of animals 1 hr before receiving SC administration of Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat) in order to study the possible involvement of opioid transmission in changes of responsiveness to electrical footshock induced by Ala_1-Lys_{17}-$ACTH_{1-17}$.

Experiment 6. The influence of Ala_1-Lys_{17}-$ACTH_{1-17}$ on exploratory behavior was studied in rats injected SC with this peptide (0.05 and 0.1 μg/rat). Control animals received SC injection of saline.

Statistical Analysis

The statistical differences were analysed using Student t-test and Dunnett's test for multiple comparison of parametric data. Mann-Whitney U-test and Steel's test were used for nonparametric data, and Fischer's exact t-test for frequencies. A probability level of 0.05 or less was accepted as significant difference.

RESULTS

Experiment 1

SC injection of Ala_1-Lys_{17}-$ACTH_{1-17}$ facilitated the acquisition of shuttle-box active avoidance behavior in a dose-dependent manner (Table 1). The total number of CARs was higher in rats treated with this peptide, and a higher percentage of these animals reached the learning criterion as compared to saline-treated controls. The effect on acquisition of active avoidance behavior following SC injection of Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat) was of similar order of magnitude than that caused by the same dose of $ACTH_{1-24}$. ICV administration of Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.01 μg/rat) was also followed by a facilitated acquisition of shuttle-box active avoidance behavior, as indicated by the higher number of CARs and the higher percentage of learners found in rats treated with this peptide than in controls. IP administration of haloperidol or naltrexone totally prevented the facilitating effect of Ala_1-Lys_{17}-$ACTH_{1-17}$ on the acquisition of active avoidance behavior.

TABLE 2

EFFECTS OF Ala_1-Lys_{17}-$ACTH_{1-17}$ ON THE EXTINCTION OF POLE JUMPING ACTIVE AVOIDANCE BEHAVIOR

Treatment	(n)	Days of Extinction[1] First	Second
(1) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 μg/rat)*	(8)	4.8 ± 0.4[2]	2.6 ± 0.2
(2) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat)*	(8)	6.7 ± 0.6[2]	3.0 ± 0.4
(3) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.2 μg/rat)*	(8)	7.9 ± 0.5[3]	3.0 ± 0.3
(4) Saline*	(8)	3.0 ± 0.1	2.3 ± 0.7

[1]Values are mean ± SEM.
[2]Significantly different as compared to group 4 ($p<0.05$, Dunnett's test).
[3]Significantly different as compared to group 4 ($p<0.01$, Dunnett's test).
*Administered SC 1 hr before the test.

TABLE 3

EFFECTS OF Ala_1-Lys_{17}-$ACTH_{1-17}$ ON THE RETENTION OF PASSIVE AVOIDANCE BEHAVIOR AND THE ROLE OF ADRENALECTOMY

Treatment	(n)	Retention Tests[1] First	Second
(1) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 μg/rat)*	(12)	188[2]	50
(2) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat)*	(12)	240[3]	54
(3) $ACTH_{4-10}$ (0.5 μg/rat)*	(6)	190[2]	48
(4) Saline*	(24)	84	24
(5) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.01 μg/3 μl/rat)†	(12)	178[4]	54
(6) Saline†	(12)	75	23
(7) Adrenalectomy + Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 μg/rat)*	(12)	144[5]	25
(8) Adrenalectomy + saline*	(12)	62	23

[1]Values are median in sec.
[2]Significantly different as compared to group 4 ($p<0.05$, Steel's test).
[3]Significantly different as compared to group 4 ($p<0.01$, Steel's test).
[4]Significantly different as compared to group 6 ($p<0.05$, Mann-Whitney U-test).
[5]Significantly different as compared to group 8 ($p<0.05$, Mann-Whitney U-test).
*Administered SC 1 hr before the first retention test.
†Administered ICV 30 min before the first retention test.

Experiment 2

Extinction of pole jumping active avoidance behavior was delayed by SC injection of Ala_1-Lys_{17}-$ACTH_{1-17}$ in a dose dependent manner (Table 2). However, the effect of the peptide was found statistically significant only in the first extinction session, and not in the second.

Experiment 3

Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 and 0.1 μg/rat, injected SC) facilitated the retention of passive avoidance response, as indicated by the median latencies to re-enter the shock box (Table 3). The effect following the injection of the peptide (0.05 μg/rat) was of similar order of magnitude than that caused by ten-fold higher dose of $ACTH_{4-10}$. ICV administration of Ala_1Lys_{17}-$ACTH_{1-17}$ (0.01 μg/3 μl/rat) was also followed by a facilitated retention of passive avoidance behavior. Adrenalectomy did not modify the behavioral response of rats treated with Ala_1-Lys_{17}-$ACTH_{1-17}$.

Experiment 4

ICV administration of Ala_1-Lys_{17}-$ACTH_{1-17}$ induced excessive grooming in a dose-dependent fashion (Table 4). The effect following the injection of the peptide (0.1 and 0.3 μg/3 μl/rat) was of similar order of magnitude than those following the administration of three-fold higher dose of $ACTH_{1-24}$ (0.3 and 1 μg/3 μl/rat). IP administration of haloperidol or naltrexone totally abolished the excessive grooming induced by ICV injection of Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.3 μg/3 μl/rat).

TABLE 4

EFFECTS OF Ala_1-Lys_{17}-$ACTH_{1-17}$ ON THE GROOMING BEHAVIOR AND THE INTERACTION WITH HALOPERIDOL AND NALTREXONE

Treatment	(n)	Grooming Score[1]
(1) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.01 μg/3 μl/rat)*	(8)	76.7 ± 5.8[2]
(2) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 μg/3 μl/rat)*	(8)	92.7 ± 5.7[2]
(3) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/3 μl/rat)*	(8)	101.2 ± 5.3[2]
(4) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.3 μg/3 μl/rat)*	(8)	117.4 ± 6.5[3]
(5) Ala_1-Lys_{17}-$ACTH_{1-17}$ (1 μg/3 μl/rat)*	(8)	122.5 ± 6.8[3]
(6) $ACTH_{1-24}$ (0.3 μg/3 μl/rat)*	(8)	100.2 ± 5.3[2]
(7) $ACTH_{1-24}$ (1 μg/3 μl/rat)*	(8)	115.7 ± 6.2[3]
(8) Saline*	(32)	24.5 ± 1.9
(9) Haloperidol (0.5 mg/kg)‡ + Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.3 μg/3 μl/rat)*	(6)	28.6 ± 2.7
(10) Haloperidol (0.5 mg/kg)† + saline*	(6)	22.8 ± 3.0
(11) Naltrexone (0.5 mg/kg)† + Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.3 μg/3 μl/rat)*	(6)	26.8 ± 2.9
(12) Naltrexone (0.5 mg/kg)† + saline*	(6)	24.9 ± 3.0
(13) Saline† + Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.3 μg/3 μl/rat)*	(6)	115.5 ± 6.2[4]
(14) Saline† + saline*	(6)	24.9 ± 2.7

[1]Values are mean ± SEM.
[2]Significantly different as compared to group 8 ($p<0.05$, Dunnett's test).
[3]Significantly different as compared to group 8 ($p<0.01$, Dunnett's test).
[4]Significantly different as compared to group 14 ($p<0.01$, Dunnett's test).
*Administered ICV 30 min before the test.
†Administered IP 90 min before the test.

TABLE 5

EFFECTS OF Ala_1-Lys_{17}-$ACTH_{1-17}$ ON THE RESPONSIVENESS TO ELECTRICAL FOOTSHOCK AND THE INTERACTION WITH NALTREXONE

		Behavioral Response[1]			
Treatment	(n)	No Response	Flinch	Jerk/Jump/Run	Vocalization
(1) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 μg/rat)*	(12)	3.0[2]	2.5	12.5	2.0
(2) Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat)*	(12)	3.5[2]	2.0	11.5	3.0
(3) Saline*	(14)	1.0	3.5	12.0	3.5
(4) Naltrexone (0.5 mg/kg)† + Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat)*	(8)	1.5	3.0	11.5	4.0
(5) Naltrexone (0.5 mg/kg)† + saline*	(8)	1.5	3.0	11.5	4.0
(6) Saline† + Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.1 μg/rat)*	(8)	3.5[3]	2.0	11.5	3.0
(7) Saline† + saline*	(8)	1.0	3.0	12.5	3.5

[1]Values are median of behavioral responses on 20 trials.
[2]Significantly different as compared to group 3 ($p<0.05$, Steel's test).
[3]Significantly different as compared to group 7 ($p<0.05$, Steel's test).
*Administered SC 1 hr before the test.
†Administered IP 2 hr before the test.

Experiment 5

Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 and 0.1 μg/rat) significantly increased the number of no responses in the test for responsiveness to electrical footshock (Table 5). This effect was totally prevented by IP injection of naltrexone.

Experiment 6

Rats injected with Ala_1-Lys_{17}-$ACTH_{1-17}$ (0.05 and 0.1 μg/rat) showed no change in exploratory behavior observed in an open field as compared to animals treated with saline (data are not shown).

DISCUSSION

Ala_1-Lys_{17}-$ACTH_{1-17}$ is a short chain ACTH analog bearing substitutions of the N- and C-terminal aminoacid residues that resulted in enhanced and prolonged biological activity (in terms of steroidogenic potency) as compared to natural ACTH [16]. The present studies showed that Ala_1Lys_{17}-$ACTH_{1-17}$ is a behaviorally potent peptide, whose profile of action is similar to that of some ACTH fragments. It facilitated acquisition and delayed extinction of active avoidance behavior and facilitated retention of passive avoidance behavior. Although ACTH-analogues are not particularly effective in changing acquisition of active avoidance behavior [21], it seems that the use of a shuttle-box test in a single session can demonstrate an influence of ACTH-analogues on this behavior. This holds for $ACTH_{4-10}$ [3], and in the present experiments for Ala_1-Lys_{17}-$ACTH_{1-17}$. Thus, the selection of proper technique is of primary importance to study peptide action on the acquisition of avoidance behavior.

In the present paper, we showed that Ala_1-Lys_{17}-$ACTH_{1-17}$ and $ACTH_{1-24}$ facilitate acquisition of active avoidance behavior and the effect of the latter peptide is dose-dependent. Furthermore, effects on extinction of active avoidance behavior and on retention of passive avoidance behavior following peripheral or central administration of Ala_1-Lys_{17}-$ACTH_{1-17}$ were similar to those many ACTH fragments [21]. However, Ala_1-Lys_{17}-$ACTH_{1-17}$ appeared to be more potent than $ACTH_{4-10}$ in facilitating retention of passive avoidance behavior. Ala_1-Lys_{17}-$ACTH_{1-17}$ increased grooming activity, while this effect is not present after ICV administration of several ACTH fragments such as $ACTH_{4-10}$, $ACTH_{7-10}$, $ACTH_{1-10}$, $ACTH_{7-16}$, and $ACTH_{11-24}$ [9]. An increase in no responses in the responsiveness to electrical footshock was observed following the administration of Ala_1-Lys_{17}-$ACTH_{1-17}$, while responsiveness is not modified by some ACTH fragments, such as $ACTH_{4-10}$ [8].

Among ACTH fragments, $ACTH_{4-10}$ seems to be the shortest sequence that keeps unaltered the behavioral activity of $ACTH_{1-24}$ [12]. $ACTH_{7-10}$ also exerts some behavioral actions, though less potent than $ACTH_{4-10}$ [21]. The residual behavioral potency observed for the sequence $ACTH_{7-10}$ could be increased to the same level as that of the reference $ACTH_{4-10}$ by extending the COOH-terminal sequence to $ACTH_{7-16}$ [23]. In fact, a series of analogues of $ACTH_{7-16}$ were synthetized showing a steady increase in behavioral potency, culminating in a milion-fold potentiation for the sequence H-Met-(O_2)-Ala-Ala-Phe-D-Lys-Phe-Gly-D-Lys-Pro-Val-Gly-Lys-Lys-NH_2 (Org 5042). Omission of either the glycil residue in position 10 or a lysil residue in position 16 was accompanied by a drastic decrease in potency [11,22]. It is worth mentioning that substitution of arginine by lysine in position 17 increases the resistance of Ala_1-Lys_{17}-$ACTH_{1-17}$ to degradation by carboxypeptidase, hence protecting lysine in position 16 from rapid cleavage [13]. This holds also for enzymatic activity on N-terminal sequence of the peptide, that is diminished by substitution of serine by β-alanine in position 1 [2,13].

Although elongation of COOH-terminal of $ACTH_{7-10}$ to $ACTH_{7-16}$ restores full behavioral activity of the fragment as compared to $ACTH_{4-10}$ [23], it fails to modify the capacity of the peptide to influence grooming behavior. In fact, either $ACTH_{7-10}$ or $ACTH_{7-16}$ were found to be inactive in inducing excessive grooming in the rat [9]. Structure-activity studies suggested that others than the sequence 4–10 is the carrier of information for onset of excessive grooming. $ACTH_{4-10}$ itself failed to influence grooming behavior of rats [9]. Since $ACTH_{1-13}$ and $ACTH_{5-14}$ showed only border-line activity, it was suggested that the minimal sequence required for the capacity to induce grooming is the fragment 5–10 elongated at the COOH-terminal either by the ACTH sequence 11–13 or by a corresponding part of the β-MSH sequence 13–18, possibly to endow the peptide with an improved resistance to metabolic break-down [9]. Thus, Ala_1-Lys_{17}-$ACTH_{1-17}$ induced excessive grooming, unlike $ACTH_{7-10}$ or $ACTH_{7-16}$, as it contains the aminoacid sequence from residue 5 to 16. Furthermore, present results showed that Ala_1-Lys_{17}-$ACTH_{1-17}$ was three-fold more potent than $ACTH_{1-24}$ in inducing excessive grooming. Interestingly, the fragment 1–16 of ACTH molecule also induces excessive grooming but its potency is lower than that of $ACTH_{1-24}$ [9].

The facilitated retention of passive avoidance behavior induced by Ala_1-Lys_{17}-$ACTH_{1-17}$ was not changed in adrenalectomized rats seven days after surgery. This finding is in agreement with the concept that adrenalectomy per se does not change responding in conditioned avoidance situations [21], and suggests that the influence of Ala_1-Lys_{17}-$ACTH_{1-17}$ on avoidance behavior is due to an extra-adrenal effect presumably located in the central nervous system. Experiments with other ACTH-analogues practically devoid of corticotrophic effects [20] substantiate this hypothesis.

Both Ala_1-Lys_{17}-$ACTH_{1-17}$-induced effects on avoidance behavior and grooming seem to involve dopaminergic and opioid neurotransmission. Also $ACTH_{1-24}$-induced behavioral effects possibly involve dopaminergic and opioid neurotransmission [4]. Particularly, $ACTH_{1-24}$-induced excessive grooming can be suppressed by peripheral application of both dopamine receptor antagonist haloperidol [24] and opiate receptor antagonist naloxone [7]. Thus, it can be assumed that Ala_1-Lys_{17}-$ACTH_{1-17}$ and $ACTH_{1-24}$ share a similar neural substrate in inducing behavioral effects. The finding that naltrexone suppresses the effects of Ala_1-Lys_{17}-$ACTH_{1-17}$ on acquisition of active avoidance behavior is consonant with other data suggesting an involvement of opioid mechanisms in active avoidance conditioning [26].

Although none of the peptides $ACTH_{1-24}$, $ACTH_{1-16}$ and $ACTH_{4-10}$ exerts analgesic effect in the hot plate test [25], they show appreciable affinity for brain opiate receptors [15]. Thus, it is possible that $ACTH_{4-10}$ and Ala_1-Lys_{17}-$ACTH_{1-17}$ share also a common substrate, eventually involving opioid mechanisms. However, $ACTH_{4-10}$ fails to alter responsiveness to electrical footshock in rats [8], but the analog of $ACTH_{4-9}$ Org 2766 induces profound analgesia after central administration [17]. Thus, it seems that ACTH analogues can be peptides with mixed agonist-antagonist properties on the opiate receptors [14]. As already suggested [10], either the agonist or the antagonist properties will be dominant depending on the test system used. $ACTH_{1-24}$ exerts agonist property in what it induces excessive grooming and antagonist property in what it counteracts morphine-induced analgesia [25]. Ala_1-Lys_{17}-$ACTH_{1-17}$ induced an increase in the number of no responses in the test for responsiveness to electrical footshock and this effect can be regarded as an analgesic effect. In fact, peripheral administration of naltrexone totally suppressed changes induced by this peptide in responsiveness to electrical footshock. However, since the involvement of opioid mechanisms in the effect on extinction of active avoidance behavior and in retention of passive avoidance behavior have not been studied, the profile of interactions of Ala_1-Lys_{17}-$ACTH_{1-17}$ with central

opioids remains to be defined. The effects of Ala_1-Lys_{17}-$ACTH_{1-17}$ on responsiveness to electrical footshock can be linked, once again, to lysine residue in position 17 as $ACTH_{1-16}$ is totally devoid of analgesic properties [25].

The possible involvement of dopamine transmission in behavioral effects of Ala_1-Lys_{17}-$ACTH_{1-17}$ arises from the finding that dopamine receptor antagonist, haloperidol suppressed facilitated acquisition of active avoidance and induction of excessive grooming behavior. $ACTH_{1-24}$-induced excessive grooming is also suppressed by haloperidol [24]. Furthermore, several data demonstrated an interaction of ACTH-related peptides and brain dopamine [4]. Thus, it is possible that a common dopaminergic mechanism underlies the behavioral effects of ACTH-related peptides.

In the present study Ala_1-Lys_{17}-$ACTH_{1-17}$ failed to change exploratory behavior of rats tested in the open field test. ACTH fragments such as $ACTH_{1-24}$ and $ACTH_{4-10}$ do not modify exploratory behavior of rats [6]. This finding is of interest in interpreting the effects of Ala_1-Lys_{17}-$ACTH_{1-17}$ on avoidance behavior which cannot depend on an influence on locomotor activity of the animals.

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