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BRIEF COMMUNICATION

Differential Behavioral Effects of ACTH 4–10 and [D-Phe^7]ACTH 4–10[1]

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WIEGANT, V. M., D. COLBERN, TJ. B. VAN WIMERSMA GREIDANUS AND W. H. GISPEN. *Differential behavioral effects of ACTH 4–10 and [D-Phe^7] ACTH 4–10.* BRAIN RES. BULL. 3(2) 167–170, 1978. – Intraventricular administration of an access of ACTH 4–10 does not interfere with the excessive grooming behavior of rats, elicited by intraventricular administration of [D-Phe^7] ACTH 4–10. In an avoidance extinction paradigm, the two ACTH analogs have opposite effects. ACTH 4–10 counteracts the facilitation of extinction seen after [D-Phe^7] ACTH 4–10, only under conditions that treatment with ACTH 4–10 alone results in retardation of that extinction. The data are discussed in terms of a multiple interaction of these peptides with brain function.

Grooming Avoidance-extinction ACTH 4–10 [D-Phe^7] ACTH 4–10 Neuropeptides

THE BEHAVIORAL effects of peptides structurally related to ACTH and MSH, i.e., induction of the stretching and yawning syndrome (SYS) and delay of avoidance extinction, were first ascribed to the presence of the sequence ACTH 4–10 [4,8]. However, with regard to delaying avoidance extinction in the rat, ACTH 4–7 was found to be the smallest peptide sequence to have essentially the same potency as ACTH 4–10 [6,12]. Yet, other fragments from the N-terminus such as ACTH 7–10, ACTH 11–13-NH_2, and ACTH 11–24 also delayed avoidance extinction if given at ten times the necessary ACTH 4–10 dose level. Therefore, similar to Eberle and Schwyzer's report [7] of two active sites in MSH responsible for MSH activity, it was concluded there is a redundancy of information within the ACTH molecule with respect to its behavioral activity [13].

It has been demonstrated that intracranial administration of fragments of the N-terminus of ACTH also induces excessive grooming prior to the stretching and yawning syndrome [8, 9, 14, 15, 20]. Again, ACTH 4–7 is capable of inducing the excessive grooming response, whereas, further structure activity studies revealed that, even at a high dose level, ACTH 4–10 is inactive in this test. Thus it seems the presently known ACTH-CNS structure-activity relationships are not identical to each other (avoidance extinction: [12,13]; excessive grooming: [19,20]; opiate receptor binding: [10,16]). However, the general principle of dormant activity and induction of such activity by chain elongation seems to apply in most cases [11].

Interestingly, it was found when the Phe^7 residue in ACTH 4–10 was replaced by its D-enantiomer, the peptide facilitated rather than delayed extinction of an active avoidance response [1,6]; or induced rather than was inactive when excessive grooming was studied [9, 15, 20]. The differential behavioral effect was only found for analogues with the Phe^7 residue in the D-configuration. Since [D-Phe^7] ACTH 4–10 also facilitated extinction of conditioned avoidance behavior in hypophysectomized rats, it was argued that the D-enantiomer most likely is not displaying its behavioral effect by counteraction of endogenous ACTH-like peptides [1]. Therefore, it has been suggested that [D-Phe^7] ACTH 4–10 contains a new intrinsic activity with regard to behavior and thus may be acting at a level different from that of ACTH 4–10 [1,2].

To test the above mentioned hypothesis, the present

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study was undertaken to study the effect of [D-Phe^7] ACTH 4–10 on grooming behavior with or without the presence of exogenously administered ACTH 4–10. Furthermore, an additional experiment measured the effect of simultaneous administration of the two peptides on extinction of conditioned avoidance behavior.

GENERAL METHOD

Surgery and Injections

For both the grooming and avoidance behavior experiments, male rats of an inbred Wistar strain (TNO, Zeist, The Netherlands) weighing approximately 140–160 g were used. One week prior to the observation session the rats were implanted with a polyethylene cannula in the foramen intraventriculare. Both surgery and intraventricular (IVC) injection into the conscious rat have been described before [9]. The volume of all IVC injections was 1 μl and substances were dissolved and diluted in saline. Synthetic ACTH 4–10 and [D-Phe^7]ACTH 4–10 were donated by Organon Int. B.V. (Oss, The Netherlands) and contained over 90% pure peptide.

GROOMING BEHAVIOR EXPERIMENT

Method

Immediately after the IVC injection, the rats were placed individually into glass boxes (24 × 12.5 × 14 cm) in a low noise room and 15 min later the behavioral analysis began. Using a 15 sec sampling technique [9], the observer recorded every 15th sec whether or not the rat displayed an element of its maintenance repertoire (vibrating, washing, grooming, scratching, locking paws, licking tail, head/body shake). If one of these elements was observed at the 15th sec, a positive score was given. This sampling technique has previously been proven to accurately reflect the time spent at maintenance behavior during the observation period [9]. Since the predominant element was grooming, we prefer to refer to grooming in keeping with previous reports.

Results

In the grooming test, saline treated rats during the first ten min of the observation period, usually display exploratory and maintenance behavior but then invariably fall asleep. Intraventricular injection of ACTH 4–10, even in a dose of 40 μg/μl, does not alter the behavioral response in the rats (see Fig. 1). As expected, the injection of 2 or 3 μg[D-Phe^7]ACTH 4–10 induces excessive grooming (Fig. 1) but, as noted before, the variability of the response among [D-Phe^7]ACTH 4–10 treated rats is considerable and larger than that seen after treatment with ACTH 1–24 [9,10]. Rats treated with combinations of ACTH 4–10 and [D-Phe^7]ACTH 4–10 showed a behavioral response as if they had been treated with [D-Phe^7] ACTH 4–10 alone. Thus, the access of ten times as much ACTH 4–10 at two different dose levels did not interfere with the effectiveness of [D-Phe^7]ACTH 4–10.

AVOIDANCE BEHAVIOR EXPERIMENT

There are differences between the grooming and the pole jump test with respect to the behavioral activity of L- and D-enantiomers of ACTH 4–10. Only [D-Phe^7] is able to induce the grooming response while ACTH 4–10 is inactive

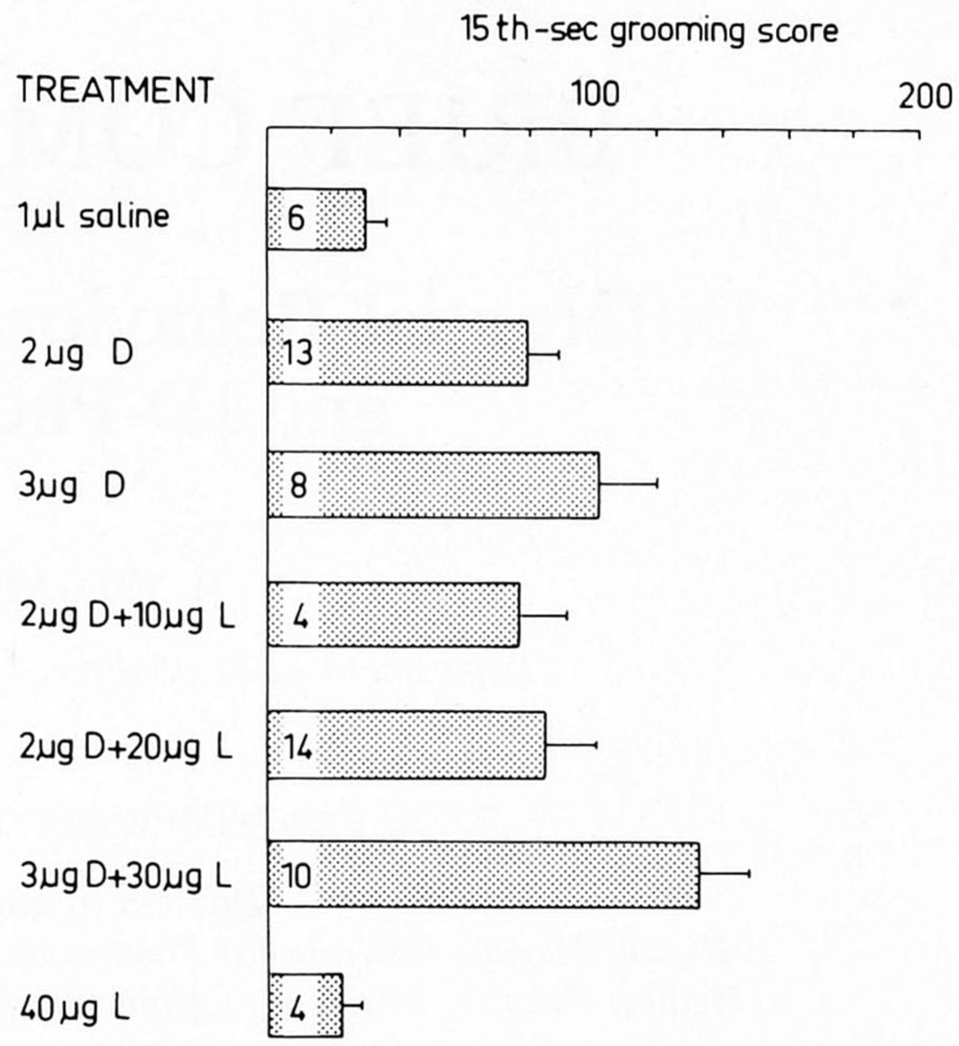

FIG. 1. ACTH 4–10 and excessive grooming in the rat. D = [D-Phe^7]ACTH 4–10; L = ACTH 4–10. Bars represent mean grooming scores ± SEM and the number of rats per group is indicated in bars. All groups treated with D or L + D display significant more grooming than saline treated rats (two-tailed Student *t*-test).

even at high doses. However, regarding the pole jump test, these two peptide configurations have opposite effects; [D-Phe^7]ACTH 4–10 facilitates extinction of the conditioned avoidance response whereas ACTH 4–10 delays the extinction. Hence, a study on the effect of a combination of the two peptides on avoidance extinction is more complicated and may lead to less clearcut results than in the grooming test. Therefore, the experimental design included two training conditions which allowed for both the facilitation of extinction by the D-enantiomer peptide and the delay of extinction by the peptide in the L-configuration. In condition A the delay of avoidance extinction by ACTH 4–10 can hardly be seen whereas the facilitation of extinction by the D-enantiomer clearly shows up [6]. In condition B, the rats received less acquisition training, resulting in a gradual extinction of the response during the extinction session. Under the circumstances of condition B, the delay of extinction by ACTH 4–10 becomes apparent.

Method

Condition A. Three days after the implantation of the brain cannula, the conditioning of an avoidance response in the pole jump box began. As described previously [17], each rat received 10 trials during one 10 min session per day for four consecutive days. The extinction session, consisting of 10 presentations of the conditioned stimulus not followed by the unconditioned stimulus, took place on the fifth day.

Condition B. Three days after the implantation of the cannula, pole jump box conditioning began. In this condition, each rat received only eight trials per one 10 min session per day for three consecutive days. The extinction session was run on day four and was identical to that of condition A.

Under both conditions A and B, immediately following the first extinction session (E1), rats received one of the following treatments: (a) 1 μl saline IVC; (b) 0.05 μg/μl ACTH 4–10, IVC; (c) 0.05 μg/μl [D-Phe^7] ACTH 4–10 IVC; and (d) 0.05 μg ACTH 4–10 + 0.05 μg [D-Phe^7] ACTH 4–10 per 1 μl IVC.

The doses used in the pole jump test were considerably lower than those used in the grooming test since they were based on the dose-response relationship previously established for ACTH effects on avoidance conditioning after IVC administration [5]. Four hr after injection, the rats were subjected to a second extinction session (E2) (10 trials, 10 min) and the performance during the second session was compared to that during the first session.

Results

In condition A there was no difference among treatment groups with respect to the performance during the fourth acquisition session and the E1 session (Fig. 2A). All rats made 8 or more conditioned avoidance responses (CAR's) during the E1 session. If saline or ACTH 4–10 was injected immediately following the E1 session, the performance during the E2 session four hr later was not different from that seen during the E1 session. However, after treatment with [D-Phe^7] ACTH 4–10 or with the equimolar combination of the L- and D-Phe^7-enantiomer of ACTH 4-10, the number of CAR's during the E2 session was significantly lower than that seen in the E1 session (Fig. 2A).

In condition B, no significant differences with respect to avoidance performance were observed (Fig. 2B) during the third acquisition and first extinction session. As expected, in this condition saline treated rats tended to perform less conditioned avoidance responses during extinction session E2. This tendency was clearly counteracted by prior treatment with ACTH 4–10 and enhanced by prior treatment with [D-Phe^7] ACTH 4–10. The combination of L and D peptide resulted in a performance during the E2 session which was not different from that displayed by saline treated rats.

Due to overtraining of the avoidance response in condition A, the effect of ACTH 4–10 on avoidance extinction is difficult to measure. However, in condition A it can be seen that the presence of ACTH 4–10 does not interfere with the facilitatory action of the [D-Phe^7] ACTH 4–10. Yet, in condition B where a delay of extinction by ACTH 4–10 is observed, the combination of the L and D peptides results in a behavior not different from that of controls.

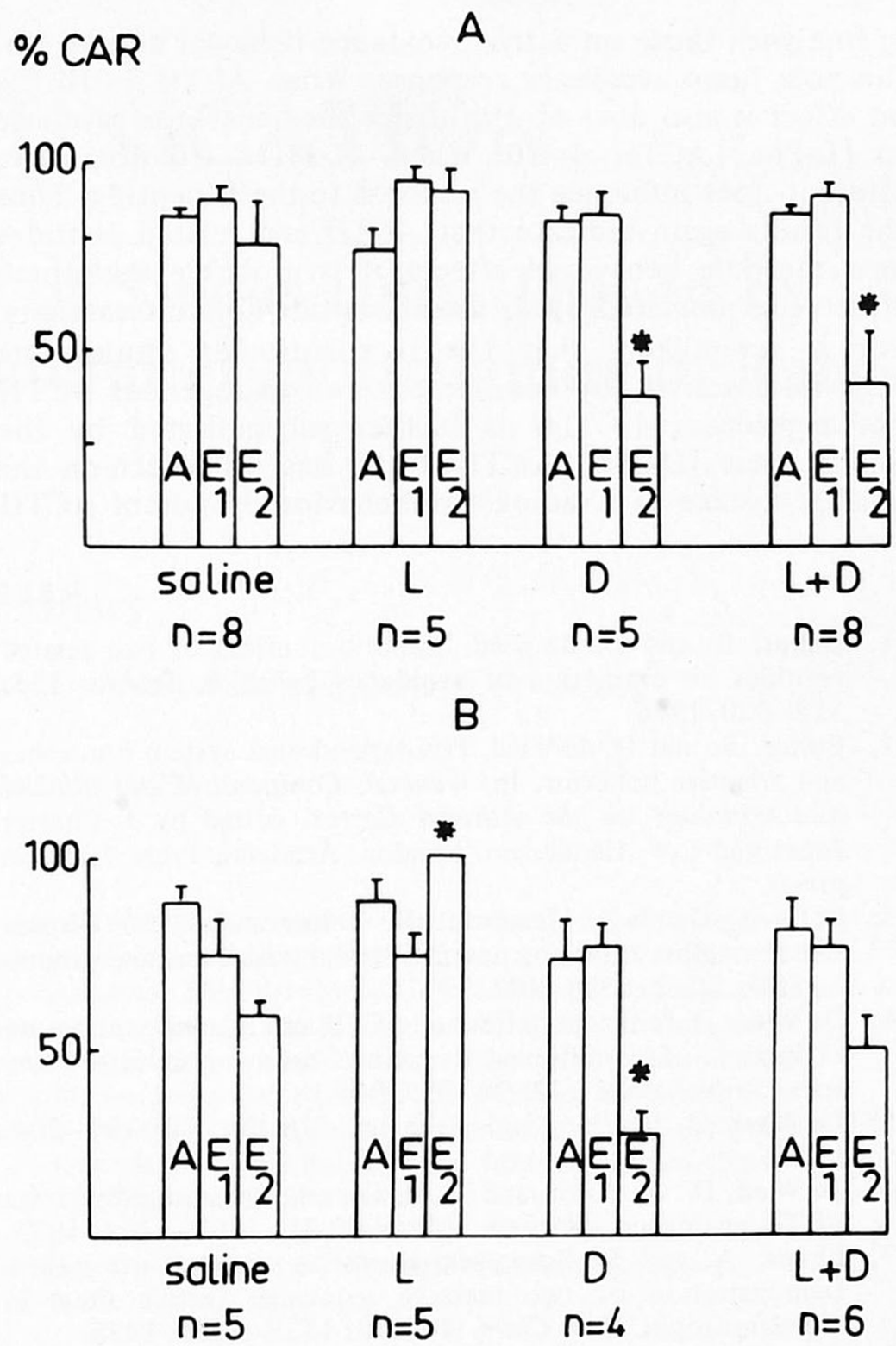

FIG. 2. ACTH 4–10 and extinction of a conditioned avoidance response (CAR). Upper part (2A): condition A (see text), lower part (2B): condition B (see text). Bars represent mean avoidance performance ± SEM. A = last acquisition session, E1 = first extinction session, E2 = second extinction session, L = ACTH 4–10, D = [D-Phe^7] ACTH 4–10, n = number of rats. *Significantly different from saline (two-tailed Student *t*-test).

DISCUSSION

The present study underscores the notion that ACTH 4–10 and [D-Phe^7] ACTH 4–10 affect brain function in a multiple manner. As reviewed in the introductory paragraphs, different structure-activity relationships seem to hold for various ACTH-CNS interactions. Furthermore, there is good evidence to assume that the effects of ACTH on the delay of avoidance extinction and excessive grooming behavior are mediated through different neural substrates [3]. Thus it was postulated that there is more than one brain receptor involved in the various ACTH-CNS interactions [21].

ACTH 4–10 seems to have dormant grooming inducing activity since shortening of the heptapeptide to ACTH 4–7 results in a peptide with an activity similar to that of [D-Phe^7] ACTH 4–10 [20]. With respect to grooming, it seems that ACTH 4–10 is not recognized by the ACTH-sensitive site. Chain shortening or D-substitution results in effective receptor activation which is likely to be due to the alteration in the proposed stereoconformation of ACTH 4–10 [13]. As discussed elsewhere, differences in metabolic stability resulting from D-substitution are unlikely to be an important determinant since other D-substitution in ACTH 4–10 are not effective in inducing grooming [11]. Also, the effectiveness of [D-Phe^7] ACTH 4–10 despite the presence of excess ACTH 4–10 is taken to imply that the L-peptide does not interfere with the transport of the D-peptide to its receptor or with D-peptide receptor interaction itself. In the avoidance task the two peptides exert an opposite influence on extinction of active avoidance behavior. In condition A the L peptide has no effect when given alone or in combination with the D peptide. In condition B both peptides exert their specific effect and when the combination is given the resulting behavior is not different from saline treated animals. These findings are taken to indicate that the effects on grooming behavior are

in line with those on active avoidance behavior as tested in the pole jump avoidance response: When ACTH 4–10 has no effect it also does not influence the behavioral response to [D-Phe^7]ACTH 4–10. When ACTH 4–10 does have effect it does influence the response to the D peptide. Thus the results again indicate that ACTH and related peptides have multiple behavioral effects; it is probable that these effects are mediated by different peptide-CNS interactions. For it is unlikely that the D enantiomer displays its behavioral activity by counteraction of endogenous ACTH like peptides [1]. This is further substantiated by the finding that [D-Phe^7]ACTH 4–10 has no effect on the specific uptake of a radioactive behaviorally potent ACTH 4–9 analogon in the septal complex [19] one of the regions crucial for the effect of ACTH on avoidance extinction [18]. Thus, by proper use of the behavioral paradigm it is possible to demonstrate the differential behavioral effects of these ACTH fragments and to get clues as to the mechanism of action. Generalisations concerning these effects, however, cannot be made at present.

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