Physiology & Behavior, Vol. 25, pp. 959–962. Pergamon Press and Brain Research Publ., 1980. Printed in the U.S.A.

Unusual Dose-Related Effect of an Endorphin Analog in a Complex Maze

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Received 12 September 1980

KASTIN, A. J., M. D. MAUK, A. V. SCHALLY AND D. H. COY. *Unusual dose-related effect of an endorphin analog in a complex maze*. PHYSIOL. BEHAV. **25**(6) 959–962, 1980.—Hungry adult rats were tested in a 12-choice maze for their ability to find a reward of food 15 minutes after being injected peripherally with (D-Ala²)-β-endorphin. Injection of the endorphin analog in a dose of 80 μg/kg body weight resulted in rats running the maze significantly slower and with more errors than rats injected with diluent. Animals receiving a dose ten times larger (800 μg/kg) were indistinguishable from controls in both running speed and errors, thus making a toxic effect unlikely. Possible changes in appetite, thirst, olfaction, emotionality, and general motor activity did not seem to explain the results. The inverted U-shaped dose-related response seemed to represent a variant from previous observations with CNS-active peptides in that the smaller dose impaired rather than enhanced performance.

Peptide Behavior Maze Learning Dose-response Opiate CNS Activity

AT the time that evidence for the "extra-endocrine" actions of hypothalamic peptides upon the central nervous system (CNS) was first presented, it was noted that the effect of MIF-I (Pro-Leu-Gly-NH_2) was absent at very low doses but was greater at smaller than at larger doses [13]. Since then, this inverted U- or bell-shaped curve also has been found for MIF-I in mice [1, 7, 15], rats [2,9], monkeys [4,12], and human beings [5,6]. In each case, the smaller dose was more effective in improving performance.

The present study extends the non-linear dose-related response of peptides in a slightly different direction. Using a different task and type of peptide, we now show impaired performance after administration of the smaller dose in comparison with a larger dose or diluent.

METHOD

Adult male rats weighing about 150 g (Blue Spruce Farms, Inc., Altamont, NY) were tested in a 12-choice Warden maze (Lafayette Instruments Co., Lafayette, IN) as described previously [10]. The time required for a hungry rat (90% body weight) to run the maze for a reward of food was measured. The turns in the maze were in the following order: L(left), R(right), L,L,R,L,L,R,L,R,L,R. An error was defined as entry into one of the 12 branched cul-de-sacs.

Upon arrival, the rats were placed for three days in a partitioned section of the sound-attenuated room in which they were to be tested and then handled during the next two days. On each of the subsequent four days, rats were put into the goalbox, containing food, for two minutes. During the final four days, the rats were injected intraperitioneally (IP) with peptide or diluent 15 minutes before being placed in the maze. A rat was allowed to eat the food for one minute after reaching the goal box and then transferred to the start box for the second trial. Calculations were based on the mean of two trials for each of the last three days designated as the days of acquisition. Any mean greater than 500 seconds excluded that rat from all calculations. The raw data were evaluated by a two-way analysis of variance with repeated measures on the factor of days followed by Duncan's Multiple Range Test where appropriate.

(D-Ala²)-β-endorphin was synthesized by solid-phase methods and dissolved in a solution of saline acidified with 0.01 M acetic acid. In the main experiments, it was administered IP at a dose of 80 μg/kg to one group of rats and 800 μg/kg body weight to a second group. A third group received the diluent vehicle as a control. The injection volume for all solutions was 1 ml/kg. Squads of animals from each group were tested daily in randomized order. In the other experiments, naloxone was injected IP at a dose of 5 mg/kg or 0.1 mg/kg at the same time as 80 μg/kg (D-Ala²)-β-endorphin; each group received two injections in these studies, even if one or both was diluent. All solutions were made fresh each week, coded, and kept at 4°C.

RESULTS

Running times evaluated by analysis of variance revealed a significant main effect for treatment, $F(2,49)=7.97$, $p<0.01$, and days, $F(2,98)=63.65$, $p<0.01$; the treatment×day interaction was not significant ($p=0.08$). On both the first and second day of acquisition, rats injected with 80 μg/kg (D-Ala²)-β-endorphin were found by Duncan's Multiple Range Test to have run the maze significantly ($p<0.05$) slower than rats injected with 800 μg/kg of the peptide or controls injected with the diluent. No significant differences were found between the group receiving the larger dose of the endorphin analog or controls on either of these days or among any groups on the last day. The results for running times are shown in Fig. 1.

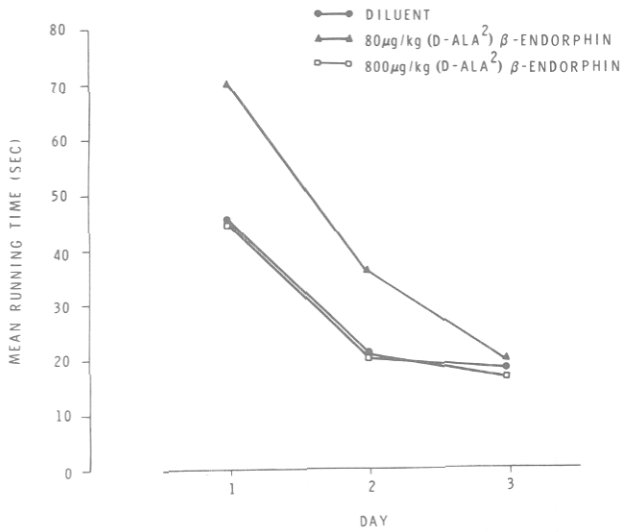

FIG. 1. Mean running times for rats injected with 80 μg/kg (D-Ala²)-β-endorphin (n=16), 800 μg/kg (D-Ala²)-β-endorphin (n=18), or diluent (n=16) in the 12-choice maze.

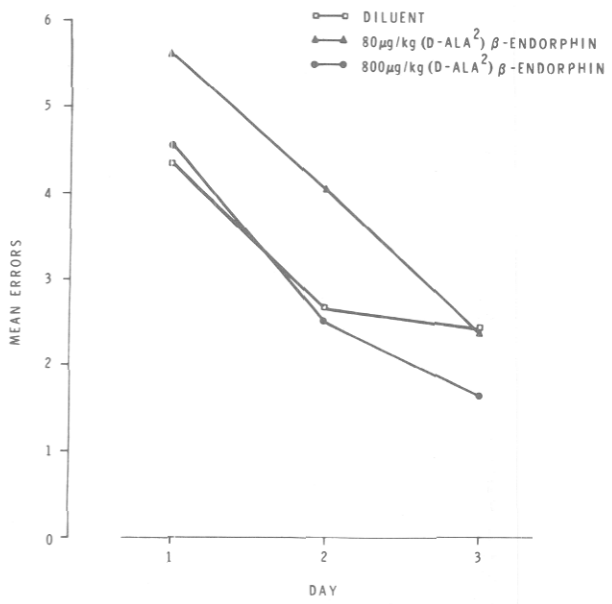

FIG. 2. Mean errors for rats injected with 80 μg/kg (D-Ala²)-β-endorphin (n=16), 800 μg/kg (D-Ala²)-β-endorphin (n=18), or diluent (n=16) in the 12-choice maze.

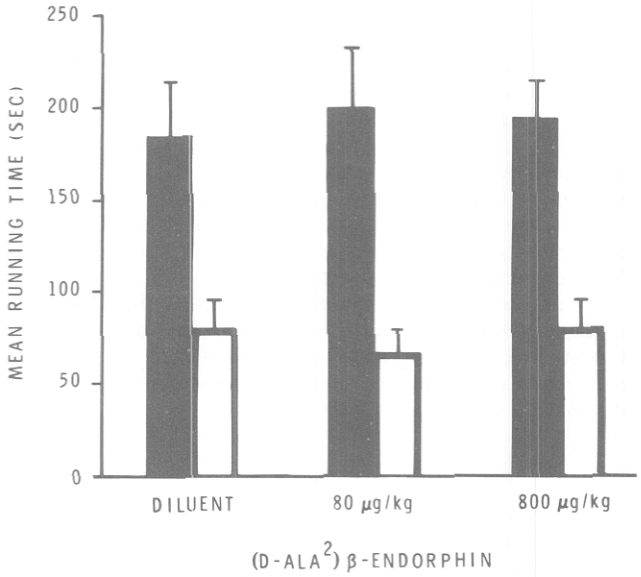

FIG. 3. Running times for the first two trials after injection of the endorphin analogue or diluent on the day of exploration. No effect of the peptide on either trial was apparent.

The findings for errors were similar. Analysis of variance revealed a significant main effect of treatment, $F(2,49)=4.93$, $p<0.02$, and days, $F(2,98)=54.28$, $p<0.01$, but no significant interaction ($p<0.2$). Duncan's Multiple Range Test showed that the effect of the endorphin analog was significant ($p<0.05$) on the second and third days of acquisition, and almost so on the first day ($p<0.1$). Rats injected with 80 μg/kg (D-Ala²)-β-endorphin made significantly more errors than rats injected with 800 μg/kg or diluent (Fig. 2). No reliable differences for errors were found between rats receiving the larger dose of the endorphin analog or controls on any of the three days of testing.

Both trials from the day of exploration on which the rats first received injections with peptide or diluent were analyzed separately. As can be seen in Fig. 3, there were essentially no differences among groups in the first trial. Differences only appeared on the subsequent days of testing.

Another set of experiments was performed to test the effects of naloxone on rats receiving 80 μg/kg (D-Ala²)-β-endorphin. The larger dose of naloxone (5 mg/kg) by itself tended to slow running times on the first two days of acquisition in comparison to diluent (81.1 ± 15.3 vs. 55.6 ± 5.6 sec on the first day, 34.3 ± 8.4 vs. 22.0 ± 2.8 sec on the second day). On all three days, rats receiving the smaller dose of naloxone (0.1 mg/kg) ran the maze with essentially the same speed as rats receiving only diluent. This smaller dose of naloxone tended to reverse the slowing caused by 80 μg/kg (D-Ala²)-β-endorphin to a greater extent than did the larger dose of naloxone. However, none of these effects were statistically significant.

The number of errors made by rats in the set of experiments involving naloxone did not seem to parallel the running times as closely as has been seen in other experiments involving statistically significant effects with these two measures. On the second day, both the 0.1 and 5 mg/kg doses of naloxone tended to produce fewer errors (2.6 ± 0.3 and 2.9 ± 0.5) than did the diluent (3.6 ± 0.4). As might be expected from the effects of naloxone alone on number of errors, both doses of naloxone tended to reduce the errors made on the first two days by rats injected with the endorphin analog.

DISCUSSION

Administration of 80 μg/kg of (D-Ala²)-β-endorphin resulted in rats running the complex maze significantly slower (Fig. 1) and with more errors (Fig. 2) than rats injected with 800 μg/kg or with diluent. This constitutes another example of the inverted U-shaped dose-response relationship observed with other CNS-active peptides like MIF-I in which a smaller dose exerts a greater effect than that seen with a

larger dose or with the zero dose (diluent vehicle). The present study, however, represents a variant of this situation. Although the dose-response relationship is characterized by the same general U-shape, the observed effect is slightly different. Instead of the smaller dose enhancing performance in the test situation, it impaired performance.

Preliminary results with other peptides suggest that almost every possible pattern of response may be encountered. Using the same complex maze and procedure, we observed the following tendencies among rats injected with 80 μg/kg and 800 μg/kg of several peptides as compared with controls: both doses may result in slightly slower running speeds (Tyr-D-Arg); both doses may result in no change in running speeds (dynorphin); both doses may result in slightly faster running speeds (neo-MSH); the higher dose may result in slower running speeds, but the lower dose may have no effect (Tyr-D-Ala-Gly); the lower dose may slightly increase running speed and the higher dose be without effect (Tyr-D-Phe); and, as also found in the present study, the lower dose may result in slightly slower running speeds and the higher dose may be without effect (Tyr-D-Phe-Gly). None of these trends were statistically significant in these pilot-type experiments. MIF-I, the peptide which in a variety of other test systems has exemplified the inverted U-shaped dose-response relationship, did not show that pattern in the complex maze used in this study. Preliminary results indicated that 80 μg/kg MIF-I resulted in a slightly slower running speed and 800 μg/kg MIF-I had a slightly greater, non-significant, tendency in the same direction; this contrasts with previous results obtained with MIF-I in the same task under somewhat different conditions [14].

In a previous study involving the use of opiate peptides in the same task, rats injected with 80 μg/kg Met-enkephalin ran the maze faster than controls [10]. This effect apparently was not mediated by typical opiate receptors. A minimal change in the stereochemistry of only one amino acid of Met-enkephalin results in an analog, (D-Phe⁴)-Met-enkephalin, with essentially no opiate binding [3]. Yet this analog seemed to be as effective as the parent Met-enkephalin in improving running times of rats in the maze [10]. In addition, this dose (80 μg/kg) of Met-enkephalin given IP was known not to be sufficient to cause analgesia even when injected directly into the brain.

(D-Ala²)-β-endorphin, at the same dose-range, caused marked analgesia when administered centrally [16] but not when given peripherally [8]. Its greater analgesic potency than Met-enkephalin suggested the possibility that its effect in slowing running times in the present experiment might be more closely related to its classical opiate properties. The non-significant tendency for reversal of this effect on latency by naloxone may have been partially obscured by a direct slowing of running times with the higher dose (5 mg/kg) of the opiate antagonist. This slowing did not appear to occur with the smaller dose (0.1 mg/kg) of naloxone itself which seemed to cause a greater, but still non-significant, reversal. Nevertheless, the apparent behavioral activity of naloxone may make the use of analgesically inactive analogs a superior method of testing for the dissociation of behavioral and narcotic effects.

Evaluation of decreased rather than increased performance after administration of a substance presents the potential problem of differentiating the observed effect from a toxic one. The results obtained in the present study with (D-Ala²)-β-endorphin obviate this difficulty since, unlike the linear dose-response seen in toxic reactions, only the smaller but not the larger dose of the endorphin analog resulted in impaired performance in the complex maze.

Although the observed effect of (D-Ala²)-β-endorphin is unlikely to represent a toxic reaction, it has not been established that the effect only involves learning. Slower running speed could reflect an action of the peptide in decreasing olfaction, appetite, thirst, emotionality or general activity, in increasing grooming, or in changing other variables. Decreased olfactory ability tended to be ruled out by direct test in the present study, grooming was observed to be negligible in all groups, and no effects of the identical dose (80 μg/kg) of this endorphin analog on food intake, water ingestion, body weight, or emotionality as determined by frequency of defecation were found in a previous study [11].

In that study, activity was measured by wheel-running for four complete days at one and 24 hours after injection of the same peptide while the rat was in its home cage as well as in a different situation. Of these four measures of general activity examined after injection of (D-Ala²)-β-endorphin, a slowing was reported in the two dependent variables which seemed less applicable to the present study than the two in which no effect was observed. Significant slowing of activity was found for wheel-running 24 hours after injection while the rats remained in their home cages, but no significant effect of the endorphin analog was observed on wheel-running one hour after injection or when the rat was removed from the home cage and placed in a different situation. Since the maze used here constituted a different situation from the home cage and since all testing in the present experiment occurred less than an hour after injection, the results of the previous study [11] reduce, but do not eliminate, the likelihood that the effects on running time could be explained only by an action on locomotion, even though biphasic effects of morphine on activity are well known.

Most of these explanations for slower running time, with the possible exception of learning, would be expected to affect the very first trial in the maze. Yet, the decreased running speed (Fig. 3) and increased errors did not begin during the day of exploration. Moreover, the rats receiving 80 μg/kg of the endorphin analog seemed to be more active in retracing their path into previously entered cul-de-sacs. The tendency for the differences among groups to disappear after the first few days of the task, a phenomenon we have noticed for the peptides α-MSH and enkephalin which enhance performance in the maze [10,14], would not be unexpected in a learning task involving a ceiling effect. In addition, any explanation of the results with the smaller dose must account for the lack of a significant effect of the larger dose.

ACKNOWLEDGEMENTS

This study was supported in part by the Medical Research Service of the Veterans Administration. The authors thank Dr. James Zadina for reviewing the manuscript and Ms. Debra Bayhi for typing it.

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