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Effects of an Enkephalin Analog on Complex Learning in the Rhesus Monkey[1]

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OLSON, G. A., R. D. OLSON, A. J. KASTIN, M. T. GREEN, R. ROIG-SMITH, C. W. HILL AND D. H. COY. *Effects of an enkephalin analog on complex learning in the rhesus monkey.* PHARMAC. BIOCHEM. BEHAV. 11(3) 341–345, 1979.—Facilitation of the learning of a discrimination reversal task for a reward of food was found in rhesus monkeys after subcutaneous administration of a potent pentafluorinated enkephalin analog, (D-Ala^2)-F$_5$,Phe^4-enkephalin-NH$_2$. General activity, short-term memory, startle, and analgesia, however, were not significantly affected. In a within-subject design, each of 6 monkeys (3 males and 3 females) received each of 5 doses of the enkephalin analog (0.1, 1, 10, 100, and 1,000 μg/kg). One daily injection was made for 7 consecutive days, including pre- and posttests on the first and last days with the diluent control. The enkephalin doses, with the exception of the 0.1 μg/kg level, produced significantly faster learning than the diluent. Some sex differences were suggested by the data, but these effects are difficult to interpret. The results suggest that relatively small amounts of this analog given systematically can exert a reliable effect on a complex behavior such as reversal learning at doses devoid of opiate effects, due perhaps to enhanced cognitive flexibility rather than improvement in short-term memory or association formation.

Enkephalin analog Rhesus monkeys Discrimination reversal learning

RECENTLY there has been a great amount of interest in discovering the properties of the opiate peptides after central and peripheral administration. Potent effects have been observed with central administration of the peptides [1, 8, 9, 12], but effects after peripheral injections are less clear. The ability of the peptides to withstand degradation in the bloodstream and to cross the blood-brain barrier has been questioned. However evidence has now been presented which suggests that endorphins and enkephalins have both of these qualities, since behavioral changes after their peripheral administration are being reported with increasing frequency.

Intraperitoneal (IP) injections of Met-enkephalin and (D-Ala^2)-Met-enkephalin were found to produce marked potentiation of the behavioral effects of DOPA and reduction of footshock-induced fighting and audiogenic seizures in rats [17]. Even an hour after peripheral injections of Met-enkephalin, of its (D-Phe^4)- or (D-Ala^2)-analog, or of β-endorphin, decreased immobility and helplessness were evident when rats were placed in water from which they could not escape [10]. Other studies have also shown decreased activity levels after intravenous (IV) administration of β-endorphin or its (D-Ala^2)-analog in rats [8], cats [2], and squirrel monkeys [14].

In goldfish, intracranial (IC) and IP injections of enkephalin, endorphin, and several of their analogs also produced decreased activity, with no significant difference in potency of the effects based on route of administration, even though the latency of the effects was greater for the IV than for the IC injections [16]. Furthermore, in a fear habituation paradigm in goldfish, Olson *et al.* [15] found that (D-Ala^2)-β-endorphin produced significantly longer response latencies after both IC and IP injections than did the diluent control, perhaps indicative of immobilization of the fish.

A rare finding of analgesia after peripheral administration of a potent opioid peptide was reported by Roemer *et al.* [19]. Analgesic activity, as measured by the tail flick test, the hot plate test, the inflamed paw test, or the shock titration test, was noted in mice, rats, and rhesus monkeys after IV, subcutaneous (SC), oral, or rectal administration of a powerful enkephalin analog (FK-33-824). Cross-tolerance of this analog with morphine in morphine-dependent monkeys and addiction of previously drug-naive monkeys with self-administration of the analog were also observed when FK-33-824 was injected IV. In a subsequent study, Mello and Mendelson [13] found that IV self-administration of the

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same analog maintained operant responding for morphine reinforcement when the analog was substituted for morphine in morphine-dependent rhesus monkeys. FK-33-824 also prevented the appearance of morphine withdrawal symptoms after IV injections.

The first effects of peripheral injections of opioid peptides on higher cognitive processes such as the acquisition of an instrumental task were reported in 1976. Learning of a complex maze by rats was facilitated by IP injections of Met-enkephalin and two analogs, (D-Ala^2)-Met-enkephalin-amide and (D-Phe^4)-Met-enkephalin [11]. The present study reports the effects of systemic administration of an enkephalin analog on the learning of a discrimination reversal short-term memory, general activity, startle, and analgesia in rhesus monkeys after varying doses of the analog.

METHOD

Animals

Six juvenile rhesus monkeys *(Macaca mulatta)*, three males and three females, were used in the study. The monkeys were born and raised in the colony of the Department of Psychology, Louisiana State University at Baton Rouge. They ranged from 3 to 5 years of age and from 2.9 to 3.9 kg of body weight at the start of the study and were on an ad lib schedule for food and water during the entire study.

Drugs

A pentaflourinated enkephalin analog, (D-Ala^2,F$_5$Phe^4)-Met-enkephalin-NH$_2$, was synthesized by solid phase methods[4]) and dissolved in a vehicle consisting of 0.9% saline acidified with acetic acid to 0.01 M, with a pH of 4.1. The vehicle solution also served as the diluent control condition. In addition to the diluent condition, concentrations of 0.1, 1, 10, 100, and 1000 μg/kg were used as coded solutions. Injections were administered SC while the monkeys were immobilized in a squeeze cage.

Apparatus

The monkeys were housed singly in standard cages situated closely together in the same large room, allowing interactions between them; they did, indeed, at times grab at one another, take another's food, and take part in grooming each other. Intervals of general activity were measured with a stop watch while the animals were in their home cages. Standard transfer cages were used to move the monkeys from one cage to another for injections and for testing in the remainder of the tasks.

A Wisconsin General Test Apparatus (WGTA) which allowed the monkey to perform from the transfer cage was used to test discrimination reversal and delayed response tasks. Food reinforcement was given during these two tasks and consisted of Fruit Loops, which were well liked and which were reinforcing in spite of the ad lib food schedule. Different stimulus items were used each day in the discrimination reversal task, but a pair of identical stimuli were used throughout the study for the delayed response task. While the animals were still in the transfer cages, they were tested for responsiveness to noxious stimuli which included a light (Mallory Big Bruiser L57) shone at the face, an air puff produced by a can of pressurized air (Omit Plus) directed at the face, and a pin (attached to a dowel rod) with which analgesia was tested at several points of the body.

Design

Each monkey was tested at each of the dose levels with a different dose every day for 7 consecutive days, including pre- and posttests of the diluent control on the first and last days. Except for the diluent, the doses were presented using a double-blind procedure and a randomized block design, so that each dose level was used at least once each day. This controlled for daily fluctuations in activity and responsiveness among the animals in the housing quarters. Times of injections of each dose level were also counterbalanced to control for diurnal variations.

Procedure

After the injection, the monkey was returned to the home cage for the measure of general activity. Activity was recorded every 5 sec for a total of 10 min, with a notation of the behavior and posture of the animal at each time of observation. For purposes of analysis, these observations were later converted to a numerical scale, with 0 indicating no activity and 6.5 the most activity (simultaneous locomotion and vocalization).

After the activity measure, the WGTA was used to study the delayed response and discrimination reversal problems. During previous training sessions, all monkeys had practiced solving both kinds of tasks, so that no shaping was necessary. The delayed response task was presented first, with each animal receiving a total of 30 trials; the delay between placement of the food under the stimulus and the opportunity for the monkey to make a choice was either 0, 30, or 60 sec, with 10 trials at each interval. The right-left placement of the food was counterbalanced to control for preference in position. The number of correct responses at each delay interval was recorded.

The discrimination reversal problem was presented immediately after completion of the delayed response task. The monkey practiced choosing between two easily discriminable stimuli until a criterion of 14 of 15 correct responses had been reached. The reversal then took place, and the trials were again continued until the criterion of 14 of 15 correct responses was attained. The number of trials required to reach each criterion was recorded.

Finally, the monkey was exposed to each of the three noxious stimuli to determine reactivity. Three presentations of the light were made, followed by three air puffs, followed by three attempts to elicit a response of pain with the pin prick. For each trial, the same experimenter indicated whether or not the monkey responded to the stimulus; thus a maximum score of 3 and a minimum of 0 were possible for each of the three stimuli.

RESULTS

Discrimination Reversals

The *t*-tests comparing the performances of the monkeys on the pre- and posttest days (i.e., on the diluent controls) yielded no significance, so the data were collapsed, and in subsequent analyses the means of these two days were used as the values of the diluent controls. Even though there was no significant difference in the two measures, this procedure provided a conservative approach to eliminate practice effects.

The discrimination reversal task yielded three different scores: trials to reach criterion on the original discrimina-

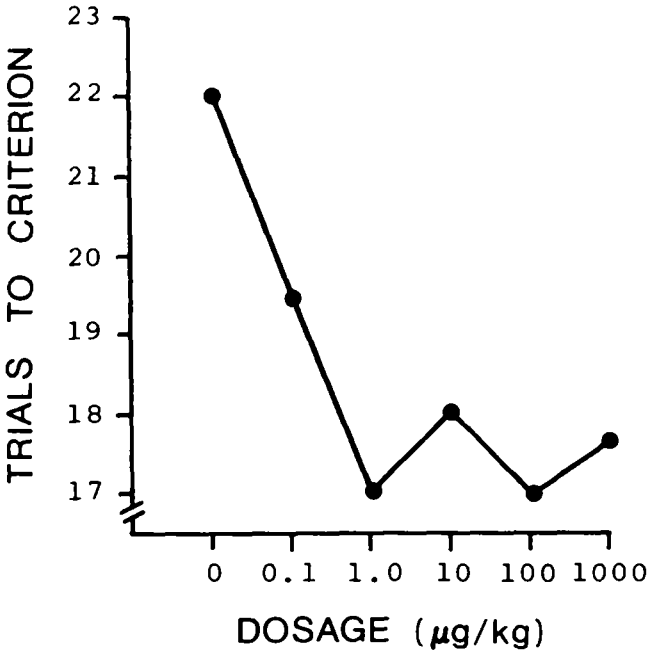

FIG. 1. Dose-response curve for mean trials to criterion on the reversal phase of the discrimination reversal task.

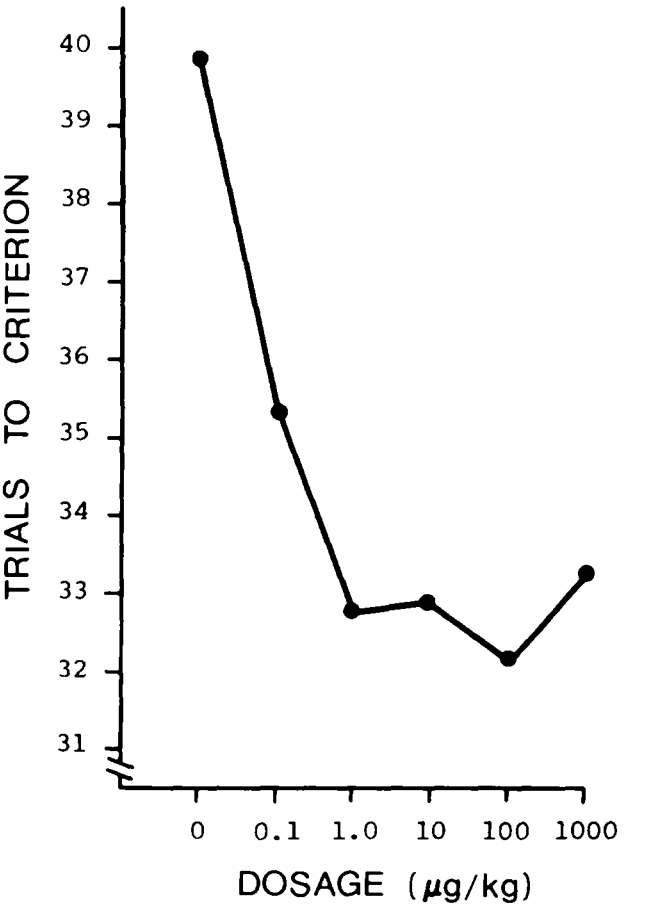

FIG. 2. Dose-response curve for mean trials to criterion for total trials on original discrimination and discrimination reversal.

tion, trials to reach criterion on the reversal phase, and total trials to criterion for both phases. A mixed analysis of variance was performed on each of these measures. There were no significant effects for the measure of trials to criterion on the original discrimination. For the scores of the reversal phase, however, a reliable main effect for dosage level was found, $F(5,20)=2.68$, $p=0.05$, with the injections of the peptide facilitating performance. The dose-response curve for trials to criterion for the reversal phase is presented in Fig. 1. Subsequent Sheffe's tests for multiple comparisons indicated that, although there were no significant comparisons for any one of the doses against any other single dose, the diluent was reliably different from the combined scores of the 1, 100, and 1000 μg/kg doses, $F(5,30)=12.31$, $p=0.05$, and it just missed being significantly different from the combined scores of the 1, 10, 100, and 1000 μg/kg doses, $F(5,30)=12.15$, $p<0.06$.

For the total trials to criterion for both phases of the task, the analysis of variance also yielded a significant main effect for dosage, with the peptide injections again producing improved performance, $F(5,20)=2.95$, $p=0.04$. The dose-response curve for this measure is presented in Fig. 2. As with the previous measure, there were no reliable comparisons between any two individual doses, but the diluent was significantly different from several combinations of the other doses: for diluent vs 1 and 100 μg/kg, $F(5,30)=12.88$, $p<0.05$; for diluent vs 1, 10, and 100 μg/kg, $F(5,30)=13.95$, $p<0.05$; for diluent vs 1, 10, 100 and 1000 μg/kg, $F(5,30)=14.07$, $p<0.05$. The comparison of the diluent against all other doses combined just missed significance, $F(5,30)=12.54$, $p<0.06$.

The scores were also analyzed using a reversal/acquisition ratio, as suggested by Rumbaugh and Jeeves [20] to help compensate for differential acquisition of the original discrimination. However, since this analysis did not produce any different or additional information, in agreement with Warren [24], the results are not reported in detail here.

Delayed Response

On the delayed response task, there was no reliable effect for dosage, but there was a significant main effect for time delay, $F(2,8)=55.68$, $p<0.01$, with increasing delays between the placement of the food reinforcement and the opportunity to respond producing poorer performance. Subsequent Sheffe's tests revealed that the 0 sec delay produced more correct responses that either the 30 sec delay, $F(2,70)=169.00$, $p<0.01$, or the 60 sec delay, $F(2,70)=134.18$, $p<0.01$, but the 30 sec and 60 sec conditions did not differ from each other.

In additon, the sex×dosage interaction approached significance, $F(5,20)=2.57$, $p=0.06$. In general, the performance of the females with the injections of peptide was better than their performance with injections of the diluent, but for the males, the reverse was true, with the diluent producing better performance than the peptide.

Noxious Stimuli and Activity

The analysis of variance on the measure of reactivity to the light yielded a reliable sex×dosage interaction,

$F(5,20)=2.83$, $p=0.04$. Subsequent F tests for simple effects revealed that the females responded more to the light than the males did at the 1 μg/kg dose, $F(1,24)=11.54$, $p<0.01$. No other comparisons were significant. There were no reliable findings with the other two measures of reactivity to noxious stimuli (air puff and pin prick), nor were there any significant results for the measure of general activity.

DISCUSSION

On the discrimination reversal task, the significantly better performance produced by the enkephalin analog suggests that peripheral administration of some doses of the enkephalin analog facilitated learning of the reversal. This finding cannot be explained by an effect of practice for which performance would improve over days, since there was no decrease in the number of trials required for learning across days and since measures of the effects of the diluent both before and after the injections of the enkephalin did not differ from each other. This finding is in agreement with a previous report [11] of improvement in the performance of a different kind of learning task, maze running, after IP injection of other enkephalin analogs.

Since there is no significant effect for the enkephalin analog on the measure of general activity in this study, it appears that the improved performance on the discrimination reversal task cannot be accounted for simply as a function of increased arousal or an increased activation state, but is probably more specific to the learning task. Further support for this view came from the finding that there was no significant change in reaction to noxious stimulation, so that the animals did not simply show altered responsiveness to external stimuli.

It appears that most of the facilitated behavior in our study was in the reversal phase of the problem, since there was no reliable effect for the enkephalin doses as ascertained by the measure of trials to reach criterion on the original discrimination. But there was significant difference between the enkephalin and the diluent for trials to accomplish the reversal and for total trials to master the whole task. Thus, it appears that ability to form associations was not significantly influenced by the enkephalin but that the monkeys were better able to recognize the change after the reversal took place and were better able to adjust to it after administration of enkephalin.

On the delayed response task which involves mostly short-term memory and attention [25], there was no significant main effect for the injections of enkephalin. This suggested that the enhanced performance on the discrimination reversal task was not highly related to either of these two processes, but left open the possibility of an increased flexibility in handling new situations. There was a reliable effect for the length of the delay interval, with performance after the 0 sec delay not surprisingly being much superior to that after 30 or 60 sec delay, confirming the importance of short-term memory for this task.

It has been suggested [7,18] that successful reversal learning in general can be attributed to (a) motivational or other effects of general activity, (b) specific memory or attentional effects, (c) ability to form associations, and (d) differential extinction. Since effects of the first three factors were not found in this study, the primary influence of the injections of enkephalin might be on the extinction of the original discrimination. Differential extinction has frequently been used as an explanation for effects found in reversal tasks (e.g., [21,23]). Although impaired extinction of a pole-jumping avoidance response after injection of a similar peptide has been reported [5], we are suggesting the possibility of improved extinction after injection of the opiate peptide in this appetitive learning task.

Although two recent studies have shown that morphine [22] or an enkephalin analog [3] suppressed rather than enhanced a previously learned bar pressing response, they should not be considered as presenting evidence contradictory to that found here. In both studies the dose levels were much higher than those used in the current study, 5 mg/kg [3] and 1–10 mg/kg [22], perhaps producing a sedative effect in the animals that resulted in decreased activity overall. A smaller dose of morphine, 0.3 mg/kg, which is more comparable to some of our doses, was found to increase bar pressing rate [20], although this effect could have been due to a general excitation rather than being specific to the bar press itself. Furthermore, these studies were concerned with the maintenance of an operant response, not its acquisition, as in the reversal phase of our study.

Mello and Mendelson [13] did find a decrease in the number of food pellets earned with a bar press after administration of FK-33-824 in their monkeys, but there was no decrease in bar pressing for administration of the enkephalin analog as a reward. They attributed the decreased responding for food to an increase in gastrointestinal motility accompanied by decreased appetite. We, however, did not find any decreased attractiveness of food to our monkeys after injection of the pentaflourinated analog; instead, the monkeys eagerly consumed all food offered to them and looked for more.

A simple linear relationship between increased dosage and improved performance was not found in this study. There were trends indicating that the greatest changes in performance occurred after the 1, 10, and 100 μg/kg doses, with the much larger dose of 1000 μg/kg being somewhat less effective. The possible biphasic nature of the opioid peptides has been previously noted in several studies in which other measures of responding were used (e.g. [10,17]).

There was, however, a strong trend toward a sex×dose interaction on the delayed response task, indicating that in females the enkephalin produced improved performance whereas in males performance declined after the enkephalin. Caution should be used in interpreting this finding since it lacked statistical significance, but it suggests that sex variables should be considered in future studies involving these peptides.

In conclusion, it appears that some small but reliable facilitating effects can be found after peripheral administration of relatively small doses of an enkephalin analog in rhesus monkeys tested in a discrimination reversal task. This effect seems to be independent of any analgesic properties of the peptide.

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