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Systemic Administration of Met-Enkephalin, (D-Ala^2)-Met-Enkephalin, β-Endorphin, and (D-Ala^2)-β-Endorphin: Effects on Eating, Drinking and Activity Measures in Rats

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KING, M. G., A. J. KASTIN, R. D. OLSON AND D. H. COY. *Systemic administration of Met-enkephalin, (D-Ala^2)-Met-enkephalin, β-endorphin, and (D-Ala^2)-β-endorphin: Effects on eating, drinking and activity measures in rats.* PHARMAC. BIOCHEM. BEHAV. 11(4) 407–411, 1979.—Rats were given four daily, interperitoneal injections (80 μg/kg) of Met-enkephalin, (D-Ala^2)-Met-enkephalin-NH_2, β-endorphin, (D-Ala^2)-β-endorphin or the diluent (0.9% NaCl acidified to, 0.01 M with acetic acid). Animals were subsequently tested for food and water intake and activity. Met-enkephalin injections did not affect any of the measures but its (D-Ala^2) analog reduced food intake and some of the activity measures in a complicated way. β-Endorphin injections did not affect food or water intake; in familiar situations these animals were less active while novel situations seemed to potentiate activity. The (D-Ala^2) analog reduced wheel running over 24 hours.

Enkephalin *Peptides* *Endorphin* *Activity* *Eating* *Drinking*

SOME polypeptide fragments of the pituitary protein, β-lipotropin, in addition to opiate-like effects have been implicated in a variety of other behaviors after central administration [4]. Among the more potent of these naturally occurring amino acid sequences are the 61–65 sequence known as enkephalin [3] and the C-terminal fragment [61–91] known as β-endorphin [2]. Central administration of enkephalins and endorphins has been shown to influence markedly a variety of behaviors in several species [5].

Evidence is also accumulating which indicates that some fragments and analogues of β-lipotropin seem to cross the blood-brain barrier and exert similar, though usually less profound effects [5,7]. In this respect, enkephalins have been reported to modulate behaviors not related to analgesia, e.g., Kastin *et al.* [6] first reported that systemic administration of Met-enkephalin and a potent analog, (D-Ala^2)-Met-enkephalin-NH_2, facilitated acquisition of a complex maze task. In the same context they also carried out preliminary studies on other behaviors which may have influenced the reported outcome: food consumption, water intake, activity, and olfactory acuity, but found no significant effect which would account for the facilitation of learning. The present experiments extend these control studies with Met-enkephalin and (D-Ala^2)-Met-enkephalin-NH_2.

Systemic administration of endorphins has also been reported to modulate behaviors not related to analgesia. Veith *et al.* [9] first reported that IP administration of α, β or γ-endorphin or their (D-Ala^2) analogs appear to affect various components of openfield behavior.

The above findings demonstrate that enkephalins and endorphins may affect complex behaviors even when systemically applied. However, interpretation of these demonstrations is complicated by the scarcity or absence of data relative to more basic behaviors. The main aim of the present study was to determine whether Met-enkephalin, (D-Ala^2)-Met-enkephalin-NH_2, β-endorphin and (D-Ala^2)-β-endorphin affected food consumption, water intake, and several measures of activity and emotionality.

METHOD

Animals

Eighty male albino Wistar rats were used. In most cases, they were obtained at a mean weight of about 160 g from Charles River Breeding Laboratories, 7–14 days before experimentation. The rats were housed individually until the beginning of testing with free access to laboratory chow and water in their home cages.

Apparatus

During testing, each animal was housed in a Wahmann Activity Wheel and was tested on a Stoelting Elecronic Ac-

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tivity Monitor (No. 31400). The test area of the monitor consisted of a platform (47.8×40.3 cm) marked into four equal rectangles. A masonite screen 28 cm high surrounded the platform. Animals were maintained at 23 ± 1°C on a 12/12 hr light/dark cycle with the ambient noise level of 67 dB SPL. Light level in the running wheels was 10 nits and 60 nits in the attached cages.

Procedure

The rats were transferred in batches of 10 from the holding area and each confined in the cage attached to a Wahmann Activity Wheel. For each of the 3 days before testing, every animal was handled for 15 min using a gloved hand. During this time, they were provided with free access in the cage to water in a graduated bottle and ground cubes in a feeding dish. In each replicate, rats were randomly assigned alternatively to either the experimental or control group.

On the first test day (Day 1), at 1400 hr, the wheel-running score was noted and the food cup and water bottle were removed. The food was weighted and made up to 60 g with freshly ground chow. Water levels were noted and the bottles refilled. At 1435, the first rat was weighed and injected IP with 80 μg/kg of one of the following coded solutions: Met-enkephalin, (D-Ala²)-Met-enkephalin-NH_2, β-endorphin, (D-Ala²)-β-endorphin, and diluent (0.9% NaCl acidified to 0.01 M with acetic acid). The peptides were synthesized by solid-phase methods [1]. Fifteen minutes after injection, each rat was lifted in its cage from the wheel and tested on two activity measures. These were: free activity on the platform (PAF) and platform activity in the living cage (PAC). Each day the order of these activity measures was counterbalanced with half the animals taking one test first. For PAF, the rat was placed on the platform of the activity monitor for 2 min and the following recorded: activity count from the monitor, number of squares entered by the rat, and number of boluses deposited. For PAC, the cage containing the rat was placed on the platform for 2 min and the following recorded: activity count from the monitor and number of boluses deposited. Between tests, boluses were removed and urine absorbed with clean gauze sponges. Between rats, the platform was sponged with water and wiped with clean gauze moistened with 70% alcohol in water. After activity tests on the platform each cage was reattached to its wheel and the replenished food and water containers were secured. The door connecting the cage and wheel was opened thus allowing the rat access to the wheel for the first time. Sixty minutes later, the number of revolutions run was read (WR1). At 1400 hr the following day, a second reading of wheel running was made (WR24).

On Days 2, 3 and 4 the above procedure was repeated. On Day 5, only wheel activity, food intake and water levels were recorded so that there were four complete 24 hr records of activity measures, and food and water intake.

RESULTS

For each peptide, β-endorphin, (D-Ala²)-β-endorphin, Met-enkephalin, (D-Ala²)-Met-enkephalin-NH_2, the following dependent variables were analyzed: (1) nutritional parameters—body weight (BW), food intake (Fd), water (H_2O) consumption; (2) platform activity—PAC, boluses bol. during PAC, PAF, squares entered during PAF, boluses during PAF; (3) wheel running activity—WR1, WR24. Only statistically significant effects are reported.

β-Endorphin

Data from the several dependent variable measures in the rats injected with β-endorphin were analyzed separately using a 2 (peptide/diluent)×4 (days) analysis of variance (ANOVA) where appropriate. Data from the replicate had to be discarded because the peptide became contaminated.

The measure of food intake had a significant effect of days, $F(3,24)=25.95$, $p<0.01$, with the intake in both groups rising from a mean of 18.48 g on Day 1 to 20.10 g on Day 4. By contrast, body weight showed only a strong trend towards an effect of days, $F(3,24)=2.84$, $p=0.06$. Water intake exhibited only a significant effect of peptide×days, $F(3,24)=3.1$, $p<0.05$. This interaction arose mainly from one animal in the control group which inexplicably drank only 3 ml on Day 2.

The platform activity measures were: PAC, PAF, number of boluses deposited during PAC and during PAF, number of squares entered during PAF. PAC had a significant effect due to peptide, $F(1,8)=2.97$, $p=0.02$ with a depressed mean after treatment with β-endorphin of 46.4 activity units and a mean of 107.1 units after injection of the diluent.

PAF showed a significant decrease in activity counts over days, $F(3,24)=20.36$, $p<0.001$, from Day 1 (mean=290 counts) to Day 4 (mean=89 counts). For the associated measure of squares entered, the ANOVA was inappropriate; these frequencies were summed over days and analysed by t-test, but no significant difference emerged. The number of boluses was too low to satisfy the assumptions for the 3-way ANOVA and was also analyzed by t-test ($t=8.99$, $p<0.01$). The peptide treated group has a mean of 7 boluses and the control mean was 3.6.

WR1 showed a significant fall over days, $F(3,24)=6.94$, $p=0.002$ from Day 1 (mean=98 revs) to Day 4 (mean=36 revs) but no significant effect due to treatment. The effect of days was also significant for WR24, $F(3,24)=7.49$, $p=0.001$, decreasing from Day 1 (mean=769 revs) to Day 4 (mean=366 revs).

(D-Ala)²-β-Endorphin

Data from each of the several dependent variable measures in the two replicate groups of rats receiving (D-Ala)²-β-endorphin were analyzed by a 3-way ANOVA: 2 (replication 1/replication 2)×2 (peptide/diluent)×4 (days), where appropriate.

In the 3-way ANOVA for water intake, no significant differences emerged. The ANOVA for food intake showed a significant rise over days, $F(3,48)=4.93$, $p=0.005$, from Day 1 (mean=15.1 g) but no effect of peptide. The only significant difference to emerge from the ANOVA for body weight was due to the replications, $F(1,16)=41.33$, $p<0.001$, with the rats in the heavier replicate being 6 days older.

The 3-way ANOVA for PAC measures revealed a significant fall in activity counts over days, $F(3,48)=8.27$, $p<0.001$, from Day 1 (mean=108) to Day 4 (mean=30 counts). In addition there was a significant effect of peptide, $F(1,16)=4.43$, $p=0.049$, rising from a mean of 42.5 activity-counts after the peptide to a mean of 89.35 counts after the diluent (Table 1).

In the 3-way ANOVA for PAF, there was a significant fall in activity counts over days, $F(3,48)=28.10$, $p<0.001$, from Day 1 (mean=329 counts) to Day 4 (mean=137 counts). The associated measure, squares entered, also showed a significant fall over days, $F(3,48)=28.43$, $p<0.001$, on the ANOVA from Day 1 (mean=12 squares) to Day 4 (mean=3 squares).

TABLE 1
SUMMARY OF SIGNIFICANT EFFECTS INVOLVING PEPTIDE TREATMENT

Dependent Variable	β-Endorphin	(D-Ala)² β-Endorphin	Met-Enkephalin	(D-Ala)² Met-Enkephalin
BW	—	—	—	peptide
Fd	—	—	—	peptide
H_2O	peptide×days	—	—	—
PAC	peptide	peptide	—	—
Bol.	na	na	na	na
PAF	—	—	—	reps×peptide×days
Bol.	peptide×days	—	—	—
Squares	—	—	—	—
WR1	—	—	—	—
WR24	—	peptide	—	—

na=analysis not appropriate because of small numbers.

The other associated measure, number of boluses, was inappropriate for analysis by 3-way ANOVA. The bolus frequency for each rat was summed over days and analyzed by 2-way ANOVA. No significant differences were found.

In the 3-way ANOVA for WR1, there was a significant fall over days, $F(3,24)=10.95$, $p<0.001$, from Day 1 (mean=176 revs) to Day 4 (mean=102 revs). Results of the 3-way ANOVA for WR24 were more complicated; in addition to a significant effect of days, $F(3,48)=7.87$, $p<0.001$, a significant effect of peptide, $F(1,16)=12.42$, $p<0.01$, was also found (Table 1). The mean number of revolutions for the peptide-treated group was 431 compared with 670 for the diluent-treated group. The effect of days arose from a decrease in running from Day 1 (mean=695 revs) to Day 4 (mean=468 revs).

Met-Enkephalin

Data from each of the several dependent variable measures in the rats injected with Met-enkephalin were analyzed by a 3-way ANOVA: 2 (replication 1/replication 2)×2 (peptide/diluent)×4 (days), where appropriate.

The 3-way ANOVA for body weight revealed a significant effect of replication, $F(1,16)=170.56$, $p<0.0001$, which was expected since the age of the rats during testing replicate 1 was 110–114 days and in replicate 2 was 70–74 days. (This discrepancy was unavoidable due to an unanticipated shortage of supply during the experiment.) In addition, there was an effect of days, $F(3,48)=3.09$, $p=0.035$ due to a slight but consistent fall in body weight in all groups: in replicate 1, a mean drop of 3.2 g relative to a mean body weight of 333.5 g and in replicate 2, a mean drop of 3.7 g relative to a mean body weight of 267.8.

The ANOVA for food intake revealed a significant effect of days, $F(3,48)=2.88$, $p<0.05$, and a significant effect of replicates×days, $F(3,48)=5.89$, $p<0.01$. The interaction arose from a significant reduction in food intake on Days 1 and 2 in the peptide group of replicate 2 ($p<0.01$ on the Scheffè test) and on Day 1 in the peptide group of replicate 2 ($p<0.05$).

The ANOVA for water consumption revealed only a significant effect of days×replicates, $F(3,48)=8.76$, $p<0.001$. This interaction arose mainly from reverse trends in the diluent groups.

Analyses of variance of the variables associated with the activity platform (PAC, PAF, boluses, squares entered) exhibited some consistency. Only the effect of days was significant for PAC, $F(3,48)=20.96$, $p<0.001$, which arose from a consistent fall in activity over days. This was reflected in the associated measure of squares entered during PAF for which the effect of days alone was significant, $F(3,48)=25.21$, $p<0.001$. Analysis of frequencies of boluses was difficult to interpret due to the small numbers. Boluses during PAF were summed for each rat over the four test days and the individual totals analyzed using a 2-way ANOVA. No significant effects emerged.

ANOVA for the WR1 measure revealed a significant effect of replication, $F(1,16)=8.96$, $p<0.01$, which arouse from consistently more wheel running (mean=90 revs) in the younger, lighter group (replicate 2) than in replicate 1 (mean=51 revs). In addition, there emerged a significant effect of days, $F(3,48)=33.50$, $p<0.001$, due to the decrease in all groups of wheel running over the four test days from Day 1 (mean=139 revs) to Day 4 (mean=44 revs).

ANOVA of the WR24 data showed a significant effect of days, $F(3,48)=9.15$, $p<0.001$) due mainly to decrease from Day 1 (mean=620 revs) to Day 4 (mean=500 revs). However, a significant effect of replicates×days also emerged, $F(3,48)=4.47$, $p<0.01$, due to the group receiving enkephalin in replicate 2 approaching its level for habituation faster.

(D-Ala²)-Met-Enkephalin-NH_2

The data from each of the several dependent variable measures in the study with (D-Ala²)-Met-Enkephalin-NH_2 were analyzed where appropriate by a 3-way ANOVA: (replication 1/replication 2)×2 (peptide/diluent)×4 (days).

The 3-way ANOVA for body weight revealed a significant effect of replications, $F(1,16)=17.40$, $p<0.001$, which was expected since replicate 2 was 10 days older than replicate 1. In addition, a significant effect of peptide emerged, $F(1,16)=6.10$, $p<0.05$, which arose from lower mean body weights in groups receiving peptide relative to their respective control groups (mean peptide groups=292.2 g and mean diluent groups=307.7 g).

The ANOVA for food intake showed the following significant effects: for replicats, $F(1,16)=5.85$, $p<0.05$; for

peptide, $F(1,16)=5.85$, $p<0.05$, and for replicates×days, $F(3,48)=3.75$, $p<0.05$. The effect of replication arose from greater food consumption by the older, heavier replicate (mean=15.2 g/day). The effect of peptide arose from lower food intake in peptide-treated animals (mean=15.2 g/day compared with mean=17.8 g/day). The effect of replicates×days arose from a decrease over days in replicate 1 and an increase over days in replicate 2. The ANOVA for water intake revealed no significant effects.

The variables associated with the activity platform were: PAC, PAF, squares entered and boluses deposited. ANOVA for PAC revealed the following significant effects: for replicates, $F(1,16)=4.78$, $p<0.05$, and for days, $F(1,16)=p<0.01$. The effect of replicates arose from greater activity in the cage in the older replicate (mean=107.13 counts) compared with the younger group (mean=55.65 counts). The effect of days arose from the decrease of activity in all groups from Day 1 (mean=129.95 counts) to Day 4 (mean=50.25 counts). The frequencies of boluses were too low for statistical analysis.

ANOVA for PAF revealed the following significant effects: for replicates, $F(1,16)=7.64$, $p<0.05$, for days, $F(1,16)=46.13$, $p<0.001$, and for replicates×peptides×days, $F(3,48)=3.30$, $p=0.028$. The effect of replicates arose from greater platform activity (mean=263.0 counts) in the older replicate compared with the younger replicate (mean=191.78 counts). The effect of days arose from a consistent habituation from Day 1 (mean =365.0 counts) to Day 4 (mean=139.0 counts). The effect of replicates × peptide × days arose from Day 3 when a different change of trend occurred in peptide and diluent groups in the replicates.

The ANOVA for number of squares entered revealed the following significant effects: for replicates, $F(1,16)=7.56$, $p=0.014$, for days, $F(1,16)=40.30$, $p<0.001$, and for replicates×days, $F(1,16)=5.04$, $p=0.004$.

The effect of replication arose from a greater mean of squares entered in the younger group (mean=10.0 squares) than in the older group (mean=6.5 squares). The effect of days arose from an habituation from Day 1 (mean=14.9 squares) to Day 4 (mean=4.7 squares). The effect of replicates×days arose from different rates of habituation within replicates. ANOVA for the number of boluses revealed no significant effects.

ANOVAs were carried out on wheel running activity one hour and 24 hr after injection. The ANOVA for WR1 revealed only a significant effect of days, $F(3,48)=20.50$, $p<0.001$. This arose from a consistent decrease in the wheel running response from Day 1 (mean=184 revs) to Day 4 (mean=75 revs). The ANOVA for WR24 revealed only a significant effect of days, $F(3,48)=9.78$, $p<0.001$. This arose from a consistent decrease in the wheel running response from Day 1 (mean=749 revs) to Day 4 (mean=492 counts).

DISCUSSION

Table 1 summarizes significant effects from the ANOVA's involving treatment with the peptides. The most apparent aspect of Table 1 is that Met-enkephalin did not affect any of the dependent variables. This outcome is consistent with the control studies reported by Kastin *et al.* [6] in which Met-enkephalin applied systematically had no effect on food consumption, water intake, body weight or activity. However, they also reported preliminary results showing that the potent analog (D-Ala^2)-Met-enkephalin-NH_2, also had no effect on any of the above dependent variables. Table 1 shows that this analog reduced food intake and body weight and also affected the activity measures, free platform activity and number of squares entered.

The differences in the effects of the two peptides on nutritional and activity variables noted in Table 1 are most readily ascribed to the difference in potency, e.g. (D-Ala^2)-Met-enkephalin-NH_2 appeared to be much more potent than Met-enkephalin on the dopa potentiation test [8,10]. However, this explanation should be accepted with caution since Kastin *et al.* [6] reported that increasing the IP dose of Met-enkephalin from 80 to 800 μg/kg did not appear to change food consumption in the learning situation.

β-Endorphin had its main effect on home cage activity (Table 1). Performance of the peptide-treated group was reduced significantly on platform activity while in the home cage. In the PAC situation, frequencies of defecation in experimental and control groups were so low that statistical tests could not be applied. The inference supported by the data, however, is that low levels of emotionality prevailed in both groups. The activity scores are consistent with the findings of Goldstein *et al.* [2] and Olson [6].

In the PAF situation, by contrast, the measures of free platform activity and squares entered were not affected but the group receiving the peptide defecated more frequently over the 4 test days indicating greater emotionality. Thus, in familiar situations (home cage and wheel) the peptide-treated animals were less active but the novel situation (free platform) seemed to promote activity while also potentiating emotionality. Veith *et al.* [9] reported a consistent rise in motor activity during open field testing of rats after 100 μg IP of β-endorphin but the difference was not significant. The determinants of this discrepancy in trend are difficult to specify due to methodological differences between the studies, e.g., the open field had a surface area of 0.37 m^2 compared with 0.19 m^2 for the activity platform. Veith *et al.* also reported a significant increase in grooming but comparison is not possible since this variable was not measured during free platform activity in the present experiments.

In general, the analog (D-Ala^2)-β-endorphin, affected activity measures in a similar but not identical way to β-endorphin. Both activity in the home cage and wheel running over 24 hr were depressed in the peptide group. As was the case for β-endorphin, free platform activity and squares entered were not affected but, unlike the β-endorphin treatment, neither was the emotionality measure of defecation. This result is at variance with those of Veith *et al.* [9] who reported a significant rise in frequency of defecation during open field testing of rats after 100 μg IP of the analog.

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