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Enkephalin and a Potent Analog Facilitate Maze Performance after Intraperitoneal Administration in Rats

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KASTIN, A. J., E. L. SCOLLAN, M. G. KING, A. V. SCHALLY AND D. H. COY. *Enkephalin and a potent analog facilitate maze performance after intraperitoneal administration in rats.* PHARMAC. BIOCHEM. BEHAV. 5(6) 691–695, 1976. – Met-enkephalin and its analog [D-Ala^2]-Met-enkephalin-NH$_2$ were administered intraperitoneally (IP) at a dose of 80 μg/kg body weight to hungry rats which were tested over 3 days for their ability to run a complex, 12 choice Warden maze for a reward of food. Animals receiving either peptide negotiated the maze significantly ($p < 0.01$) faster (74.1 and 73.5 vs. 128.8 sec) and made significantly ($p < 0.01$) fewer errors (5.5 and 5.4 vs. 9.1) than animals receiving the diluent vehicle. These findings did not seem to be explained by differences in appetite, thirst, olfaction, or general activity. Rats injected in a preliminary study with an analog, [D-Phe^4]- Met-enkephalin, which has essentially no opiate activity appeared to run the maze as fast as rats injected with [D-Ala^2]-Met-enkephalin-NH$_2$ and with just as few errors. Injection of morphine seemed to result in slower running times and more errors in the maze. These results demonstrate that enkephalin and some of its analogs can exert significant behavioral changes after IP administration and that these behavioral effects probably can be dissociated from the opiate effects.

Enkephalin Maze Acquisition Appetitive Behavior Peptide Opiate Brain

OUR CONCEPT [8,12] that hypothalamic peptides may have a dual effect, acting on the pituitary as well as directly on the brain, led us to expect that the brain peptide enkephalin [5] might have other effects in addition to its opiate activity. Early work indicated that the opiate activity of enkephalin could be demonstrated only after direct injection into the brain. However, we found direct evidence suggesting that enkephalin crosses the blood-brain barrier [7]. Moreover, the great potency of enkephalin in the DOPA-potentiation test after intraperitoneal (IP) injection [13] was consistent with the idea of its passage into the brain. Since morphine also has some activity in this neuropharmacological test [13], it was not completely clear whether the DOPA-potentiating activity of enkephalin given IP involved opiate receptors.

Accordingly, hungry rats were tested in the appetitive task of running to a reward of food in a complex 12 choice maze. If the rats ran the maze either significantly faster or slower after IP enkephalin than after diluent, then this would be the first demonstration of a behavioral action of enkephalin in a learning situation after systemic administration. If the rats ran the maze faster after enkephalin, as we have found for α-melanocyte-stimulating hormone (α-MSH) [14], then this also would be the first demonstration of an action of enkephalin opposite in direction to that which would be expected with opiates.

METHOD

Apparatus

A series of galvanized metal straightaways and branched cul-de-sacs obtained from Lafayette Instruments Co., Lafayette, IN were assembled into a complex maze composed of 12 choice points leading toward a single goal box (12.5 × 12.5 × 46 cm) or into a blind alley. Each unit, about 10 × 12.5 × 36 cm in size, was arranged in a general U-shape with the following pattern of turns: right (R), left (L), L, R, L, L, R, L, L, R, L, R. The reward consisted of a wet mash prepared daily from ground food pellets (Purina Laboratory Chow), powdered milk, and dextrose in a 4:4:1 ratio mixed with water. The maze was located in an isolated, sound-attenuated room at the VA Hospital connected to a separate room in which the rats were housed and injected. Constant, indirect illumination and background white noise were present in both rooms.

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Animals

More than a hundred male, albino Sprague-Dawley rats purchased from Blue Spruce Farms, Altamont, NY were used in these experiments. The rats were obtained separately in groups of 18 every week or two and weighed 150–200 g before deprivation of food. Upon arrival, each rat was assigned a number from a computerized table of random numbers. The numbers were then arranged in order and consecutively assigned to one of 3 treatments.

Treatment

For the 4 main experiments, A, B, C, and D, treatment consisted of the IP injection of Met^5-enkephalin (80 μg/kg body weight), [D-Ala^2]-Met^5-enkephalin-NH$_2$ (80 μg/kg), and diluent vehicle as a control (0.9% saline acidified with acetic acid to 0.01 M, pH 4.1). Separate studies involved two different concentrations of Met^5-enkephalin (80 μg/kg and 800 μg/kg), [D-Phe^4]-Met^5-enkephalin (80 μg/kg), or morphine sulfate (80, 800, and 8000 μg/kg), as well as diluent. In addition, several control studies were performed. The pentapeptides were synthesized by solid phase methods as described previously [2,13]. Solutions were coded so that the experimenter, the only one handling the rats, did not know the contents of the solutions which were made fresh each week and kept at 4°C at all times.

Procedure

The main stages of training involved gentling, deprivation of food, formation of an association between food and the goal box, exploration of the novel maze, and testing of acquisition. Upon arrival from the vendor, animals were housed individually with free access to food pellets and water for 7 days, being weighed and handled to achieve gentling on the last two days of the first week. Deprivation of food began on the seventh day when the rats were given only 6 g of food pellets. This ration was found to result in a drop of body weight of the rats to about 90% of their previous weight by the following day. Thereafter they were given 12 g of food pellets daily to maintain their weight at about this level of mild deprivation. On the first, second, third and sixth days of the second week after arrival, the rats were placed in the goal box for 1 min during which time they could eat the mash.

Each rat was injected IP once a day 15 min before being placed in the start box on the first 4 days of the third week. Exploration of the maze for 2 trials took place on Day 14 and testing was performed for 2 trials on each of the next 3 days designated as Days 1–3 of acquisition. The time elapsed between the rat leaving the start box and reaching the food with the door of the goal box closed was considered running time. An entry into a cul-de-sac was designated as an error. During the day of exploration as well as the 3 days of acquisition, the rat was allowed to eat the mash for 1 min upon reaching the goal box and was then transferred immediately to the start box for the second trial. After eating for 1 min in the goal box at the end of the second trial, the rat was returned to the home cage where he was given his daily ration of 12 g food pellets. If a rat did not enter the goal box by 900 sec, he was removed from the maze and placed in the goal box; the remainder of the test then proceeded as usual. A rat with two 900 sec scores on the day of exploration or a 900 sec score on any of the 3 days of acquisition was eliminated from the experiment. The calculations for running time and number of errors were based on the mean of 2 trials for each day.

Statistics

Differences among groups were determined from the raw data by analysis of variance with repeated measures on the factor of days, followed for the interaction terms by F tests for simple effects and Scheffé tests, and for the main effect of treatment by Duncan's new multiple range test. The occasional exclusion of a rat from the study because of illness or failure to reach the criteria just described was decided before the code was broken. Because the interaction between treatment and replication was not significant, data from the 4 replications (Experiments A, B, C, and D - Tables 1 and 2) were pooled so that the groups consisted of 18 rats receiving Met-enkephalin, 17 rats receiving [D-Ala^2]-Met-enkephalin-NH$_2$, and 19 rats receiving diluent.

RESULTS

Analysis of variance of running times revealed a significant main effect of treatment, $F(2,51) = 4.02$, $p<0.025$, and days, $F(2,102) = 22.29$, $p<0.01$. The interaction of treatment × days also was significant, $F(4,102) = 3.44$, $p<0.025$. Analysis of this interaction showed that the running times after either Met-enkephalin (98.2 sec) or [D-Ala^2]-Met-enkephalin-NH$_2$ (110.1 sec) were significantly, ($p<0.01$) faster than after the diluent (216.2 sec) on Day 1, almost significantly, $F(2,51) = 3.01$, $p = 0.058$, faster on Day 2 (64.9 and 60.4 vs. 98.0 sec), but not significantly faster on Day 3 (59.1 and 50.2 vs. 72.4 sec). Further analysis of the main effect of treatment revealed

TABLE 1

MEAN RUNNING TIME (SEC) OF RATS IN A COMPLEX APPETITIVE MAZE DURING 3 DAYS OF ACQUISITION AFTER IP INJECTION OF ENKEPHALIN OR ITS ANALOG

	Met-enkephalin	[D-Ala^2] Met-enkephalin-NH$_2$	Diluent
Experiment A:			
Day 1	118.8	96.7	229.3
Day 2	86.6	69.3	137.0
Day 3	77.0	50.6	87.4
Experiment B:			
Day 1	88.0	221.2	260.5
Day 2	78.0	69.3	122.8
Day 3	121.2	55.1	145.0
Experiment C:			
Day 1	105.6	124.5	262.1
Day 2	73.1	84.7	101.9
Day 3	45.3	69.5	49.9
Experiment D:			
Day 1	83.8	58.4	145.3
Day 2	36.9	36.2	46.2
Day 3	27.7	37.7	26.5
Pooled (A, B, C, D):			
Day 1	98.2	110.1	216.2
Day 2	64.9	60.3	98.0
Day 3	59.1	50.2	72.4

TABLE 2

MEAN NUMBER OF ERRORS OF RATS IN A COMPLEX APPETITIVE MAZE DURING 3 DAYS OF ACQUISITION AFTER IP INJECTION OF ENKEPHALIN OR ITS ANALOG

	Met-enkephalin	[D-Ala²] Met-enkephalin-NH_2	Diluent
Experiment A:			
Day 1	6.1	6.2	14.8
Day 2	5.2	6.3	9.7
Day 3	5.6	4.0	7.2
Experiment B:			
Day 1	6.3	11.5	15.6
Day 2	7.3	6.3	10.1
Day 3	11.5	4.7	9.1
Experiment C:			
Day 1	5.4	6.5	16.2
Day 2	4.3	5.0	6.4
Day 3	3.4	3.8	4.1
Experiment D:			
Day 1	7.2	5.6	11.0
Day 2	3.8	3.6	4.7
Day 3	3.2	3.9	3.7
Pooled (A, B, C, D):			
Day 1	6.3	7.0	14.1
Day 2	4.8	5.1	7.5
Day 3	5.2	4.1	5.8

that the mean running time over 3 days for the group receiving Met-enkephalin (222.2 sec) as well as that for the group receiving [D-Ala²]-Met-enkephalin-NH_2 [220.6 sec) was significantly ($p<0.01$) different from that for diluent (386.6 sec) but not from each other. The rate of acquisition as determined by F tests for simple effects over days differed among the groups ($p<0.05$). Rats receiving the enkephalins initially acquired the task faster ($p<0.05$, Scheffé test with specific error variances for days) than rats receiving diluent (Table 1 and Fig. 1). A possible loss in potency of the peptides kept in solution for 4 days was not found in a preliminary experiment but the development of tolerance or the presence of an asymptotic minimum for running time was not ruled out.

A similar analysis of variance was performed on the number of errors made by the rats while running the maze. There was a main effect of treatment, $F(2,51) = 5.83$, $p < 0.01$, and days, $F(2,102) = 16.32$, $p < 0.01$, as well as the interaction between them, $F(4,102) = 4.33$, $p < 0.05$. Each of the groups receiving Met-enkephalin and [D-Ala²]-Met-enkephalin-NH² made significantly fewer errors than the group receiving diluent on the first day (6.3 and 7.0 vs. 14.1 $p<0.01$) as well as on the second day of acquisition (4.8 and 5.1 vs. 7.5, $p < 0.05$). As with the running times, the differences on Day 3 were not statistically significant (Table 2 and Fig. 2). Analysis of the main effect of treatment for 3 days showed that significantly ($p < 0.01$) fewer errors were made by rats receiving either Met-enkephalin (16.4) or [D-Ala²]-Met-enkephalin-NH² (16.2) as compared with rats receiving diluent (27.4).

Another study was performed in the same way to examine the effects of a larger dose of Met-enkephalin. Rats injected IP with 80 μg/kg and 800 μg/kg Met-enkephalin appeared to acquire the task quicker than rats injected with

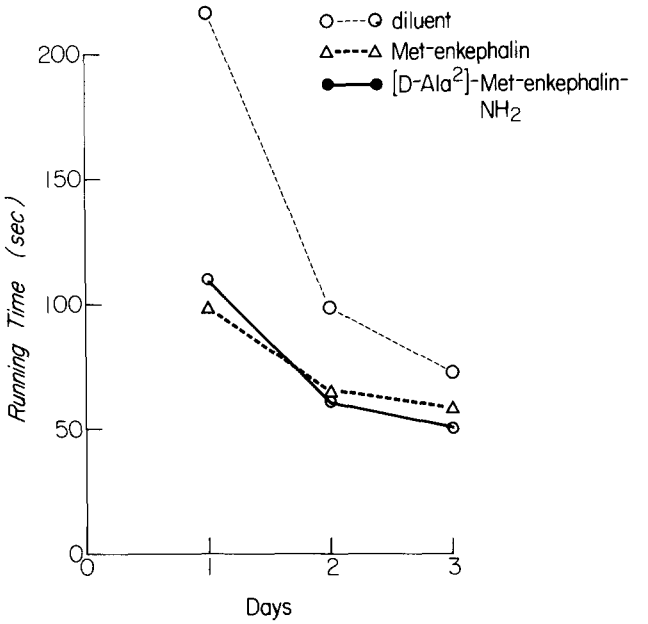

FIG. 1. Daily pooled mean number of sec to run a 12 choice Warden maze after injection of Met-enkephalin, [D-Ala²]-Met-enkephalin-NH², or diluent.

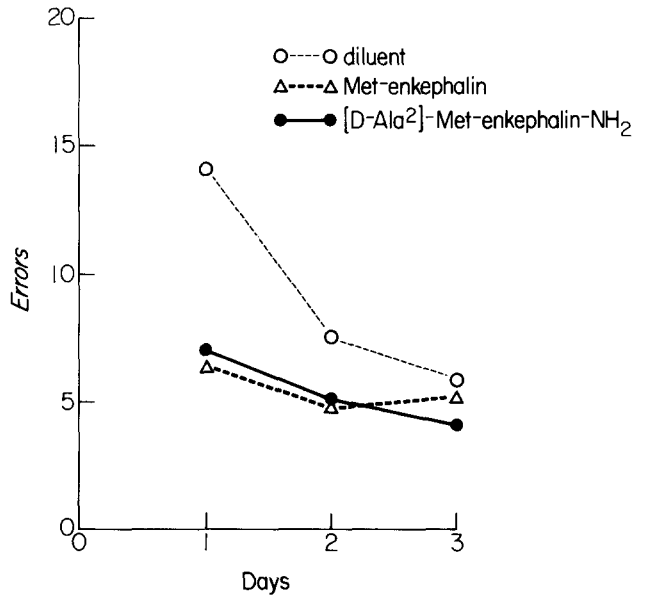

FIG. 2. Daily pooled mean number of errors made in running a 12 choice Warden maze after injection of Met-enkephalin, [D-Ala²]-Met-enkephalin-NH_2, or diluent.

diluent. The animals receiving the 800 μg/kg dose of Met-enkephalin ran slightly but not significantly faster (60.6 sec) than the rats receiving the usual dose (80 μg/kg) of the same material (71.5 sec).

[D-Phe⁴]-Met-enkephalin, an analog with essentially no opiate activity in the systems studied [2], was tested with [D-Ala²]-Met-enkephalin-NH² and diluent in the same paradigm used for the experiments already described. Although analysis of variance with repeated measures on days failed to reveal a statistically significant effect on the overall data from the small number (5/group) of rats

involved, on the first day of acquisition the 2 analogs seemed equally effective in causing faster running speeds (105.1 and 106.4 vs. 126.5 sec) and fewer errors (6.0 and 6.7 vs. 9.1) than diluent; neither analog caused greater speed or reduced errors on the next 2 days. There was no substantial difference in the amount of mash eaten by the hungry rats injected with [D-Phe^4]-Met-enkephalin, [D-Ala^2]-Met-enkephalin-NH_2 or diluent (2.3 and 2.6 vs. 2.5 g) during the 2 min in the goal box on Day 1 or in the overall consumption of food for the entire 3 days.

In order to test for an effect of the enkephalins on olfaction, the hungry rats from the experiment just discussed were placed overnight in individual containers in which 3 pellets (3 g each) of aromatic food (Tekland Hypophysectomized Rat Test Diet) were partially buried but visible in 1–2 cm of processed fine chips of essentially odorless wood. The next day 15 min after injection the rats were placed in the start box, the floor of which was covered with the same material. Fifteen sec later, the guillotine door separating the start box from the goal box was lifted. Accustomed to the novelty of the wood chips from the previous 24 hr, the rats spent little time in exploration. The time taken for the hungry rat to find the single pellet of food hidden just beneath the surface of the processed wood chips lining the floor of the goal box was recorded for 1 trial. Considerable sniffing was evident. There was no statistically significant difference in latencies among the groups; the times for the rats injected with [D-Ala^2]-Met-enkephalin-NH_2 and diluent were almost identical (40.6. vs. 40.4 sec) and slightly longer for the [D-Phe^4]-analog.

After the last of the 4 main experiments (D), its 17 rats were allowed free access to food for a week while the daily injections continued. Pellets of food were ground into a fine powder and access to the powder was restricted to a small opening in the container of food. This greatly reduced the amount of food dropped through the mesh floors of the cages and thus facilitated accurate measurements. During the last 4 days, food and water intakes were measured. No significant differences were found in the ingestion of food (23.8 vs. 23.4 vs. 24.4 g), intake of water (31.6 vs. 32.8 vs. 31.4 ml), or the body weights (194 vs. 193 vs. 199 g) of the rats injected with Met-enkephalin, [D-Ala^2]-Met-enkephalin-NH_2, or diluent respectively.

A preliminary assessment of the effect of [D-Ala^2]-Met-enkephalin-NH_2 on general activity was made for 4 days in another small group of rats. Body weights, ingestion of food, and intake of water were again similar as was fecal excretion in a familiar as well as novel environment. No consistent changes in activity sufficient to explain the faster running speed in the maze were found for animals in their home cages, activity wheels, or on the platform of an electronic activity monitor (Stoelting Co.).

The effects of the administration of morphine sulfate to rats were tested in a separate study in which a total of 16 rats were injected IP with diluent, 80 μg/kg morphine, 800 μg/kg morphine, or 8 mg/kg morphine. The rats receiving diluent ran the maze faster (71.9 sec) than did the rats receiving 80 μg/kg morphine (133.6 sec), 800 μg/kg morphine (231.0 sec), or 8 mg/kg morphine (158.7 sec). Rats receiving diluent also made fewer errors (5.7) than did the rats receiving 80 μg/kg morphine (7.8), 800 μg/kg morphine (11.4), or 8 mg/kg morphine (7.0). These overall means reflected relatively constant differences during the 3 days of acquisition. A maximum of 500 sec was allowed each rat in the maze; 1 rat in each of the morphine groups had not reached the goal box by that time but was included in the results. The variability and small number of animals made interpretation difficult, particularly of the greatest effect at the 800 μg/kg dose. Regardless, it is clear that rats injected IP with morphine did not run the maze faster than controls, in contrast to the findings after injection of enkephalin.

DISCUSSION

The results demonstrate a highly significant behavioral effect of enkephalin after administration by the IP route. This is consistent with the activity of enkephalin in the DOPA-potentiation test after IP injection [13]. It may also be considered partial support for our findings suggesting that enkephalin crosses the blood-brain barrier [7], in contrast to the presumption of others that enkephalin and even [D-Ala^2]-Met-enkephalin-NH_2 do not penetrate the brain [1,10].

In addition to our demonstration of a behavioral effect of enkephalin after IP injection, the results indicate that enkephalin exerts effects which may be separate from its opiate activity. It generally has been observed that opiates produce drowsiness and reduced motor activity. This would lead to a slower running rate in the maze, as we found with morphine, rather than the significantly faster rate observed in the rats injected with enkephalin. Since the rats were hungry, it seemed possible that the main action of enkephalin was to increase appetite even though morphine exerts the opposite effect. This was ruled out by our findings in several experiments that rats injected with enkephalin or its potent analog ate no more than control rats. Similarly, the possibility that enkephalin increases the ability of the rat to follow olfactory cues seemed to be eliminated by a direct test of the ability of a few rats to find buried aromatic food. A general augmentation of activity also was unlikely to explain the faster running times of the rats injected with the enkephalins since these rats made fewer errors in the maze and in preliminary studies were no more active than controls in several measures of activity. This lack of change in activity and in fecal excretion of rats receiving enkephalin also reduced the likelihood that altered arousal could explain why the greatest differences among groups occurred during the first days of acquisition.

[D-Ala^2]-Met-enkephalin is apparently more potent than Met-enkephalin in the DOPA-potentiation test after IP injection [13] and in the tail-flick test of analgesia after intracerebroventricular injection [15]. This analog also has been found to have a greater affinity for brain opiate receptors and to be more active than Met-enkephalin in inhibiting electrically evoked contractions of the mouse vas deferens [2]. The activity of [D-Ala^2]-Met-enkephalin-NH_2 in the analgesic tests is similar to that of [D-Ala^2]-Met-enkephalin [2,10]. Rats injected IP with 80 μg/kg [D-Ala^2]-Met-enkephalin-NH_2 ran the maze faster for a reward of food than did the rats injected IP with diluent.

No reliable differences in mean running times for 3 days (220.6 vs. 222.2 sec) or number of errors (16.2 vs. 16.4) between the groups receiving [D-Ala^2]-Met-enkephalin-NH_2 and Met-enkephalin itself were found. Yet it has been observed that [D-Ala^2]-Met-enkephalin-NH_2 is about 900% more potent than Met-enkephalin in the mouse vas deferens bioassay for morphine-like activity [2], has a 50% greater affinity for opiate receptors from the particulate fractions of homogenates of rat brain [2], and exerts a considerably

greater analgesic action when micro-injected into the rat brain [10]. Although it is tempting to construe these finding as additional evidence for a dissociation of behavioral and opiate activites, a 10-times greater dose of enkephalin improved running time in a single experiment only by about 15%. However, the [D-Phe^4] analog of enkephalin, which is practically devoid of opiate activity [2], seemed in a preliminary study to be just as active as the [D-Ala^2-NH_2]-analog in acquisition of the Warden maze by rats. Furthermore, even a large dose of [D-Ala^2]-Met-enkephalin-NH_2 failed to elicit analgesia after systemic injection [10]. In addition, IP injection of morphine apparently caused the rats to run the maze slower and with more errors than rats injected with diluent, findings exactly opposite to those observed after injection of the natural opiate Met-enkephalin or some of its analogs. Taken together, all these results indicate that the behavioral and opiate effects of enkephalin can be dissociated. A role of enkephalin in neurotransmission and a reevaluation of opiate actions could be considered.

The half-time disappearance of radioactivity after the injection of labeled Met-enkephalin appears to be about a minute [7]. The half life of [D-Ala^2]-Met-enkephalin in rat plasma in vitro has been preliminarily reported to be at least 20 times longer [4]. The rapid breakdown of Met-enkephalin in plasma and brain has contributed to the skepticism that any action of Met-enkephalin would be found after systemic injection. Just as the behavioral and EEG effects of α-MSH persist far longer than its half-time disappearance from plasma [6], so it seems that the behavioral effects of Met-enkephalin do not require persistence of the peptide in blood. Decreased resistance to enzymatic degradation in rat blood contributes to the greater potency of at least one analog of α-MSH but may not fully explain it [9]. Similarly, it is possible that the activity of [D-Ala^2]-Met-enkephalin-NH_2 cannot be fully explained only by its continued presence in the circulation, particularly in view of the actions of Met- enkephalin more than an hour after IP administration in the DOPA-potentiation test [13] and more than 15 min later in the complex maze used in these experiments.

There is some direct evidence that enkephalin [7], like α-MSH [6] and MSH-release inhibiting factor-I (Pro-Leu-Gly-NH_2 = MIF-I) [3], can cross the blood-brain barrier. These 3 compounds represent peptides mainly found in the cerebrum, pituitary, and hypothalamus. The brain peptide enkephalin resembles the hypothalamic peptides and perhaps pituitary peptides in that all these compounds may be able to exert dual effects.

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REFERENCES

1. Chang, J. K., B. T. W. Fong, A. Pert and C. B. Pert. Opiate receptor affinities and behavioral effects of enkephalin: structure-activity relationship of ten synthetic peptide analogues. *Life Sci.* **18**: 1473–1482, 1976.
2. Coy, D. H., A. J. Kastin, A. V. Schally, O. Morin, N. G. Caron, F. Labrie, J. M. Walker, R. Fertel, G. G. Berntson and C. A. Sandman. Synthesis and opioid activities of stereoisomers and other D-amino acid analogs of methionine-enkephalin. *Biochem. Biophys. Res. Commun.* **73**: 632–638, 1976.
3. Greenberg, R., C. E. Whalley, F. Jourdikian, I. S. Mendelson, K. Nikolics, D. H. Coy, A. V. Schally and A. J. Kastin. Peptides readily penetrate the blood-brain barrier. Uptake of peptides by synaptosomes is passive. *Pharmac. Biochem. Behav.* Suppl. 1, Vol. 5: 151–158, 1976.
4. Hambrook, J. M., B. A. Morgan, M. J. Rance and C. F. C. Smith. Mode of deactivation of the enkephalins by rat and human plasma and rat brain homogenates. *Nature* **262**: 782–783, 1976.
5. Hughes, J., T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan and H. R. Morris. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258**: 577–579, 1975.
6. Kastin, A. J., C. Nissen, K. Nikolics, K. Medzihradszky, D. H. Coy, I. Teplan and A. V. Schally. Distribution of ^{3}H-α-MSH in rat brain. *Brain Res. Bull.* **1**: 19–26, 1976.
7. Kastin, A. J., C. Nissen, A. V. Schally and D. H. Coy. Blood-brain barrier, half-time disappearance, and brain distribution for labeled enkephalin and a potent analog. *Brain Res. Bull.*, **1**: 583–589, 1976.
8. Kastin, A. J., N. P. Plotnikoff, A. V. Schally and C. A. Sandman. Endocrine and CNS effects of hypothalamic peptides and MSH. *Reviews of Neuroscience.* New York: Raven Press, 1976, p. 111–148.
9. Marks, N., F. Stern and A. J. Kastin. Biodegradation of α-MSH and derived peptides by rat brain extracts, and by rat and human serum. *Brain Res. Bull.*, **1**: 591–593, 1976.
10. Pert, C. B., A. Pert, J. K. Chang and B. T. W. Fong. [D-Ala^2]-Met-enkephalin-amide: a potent, long-lasting synthetic pentapeptide analgesic. *Science* **194**: 330–332, 1976.
11. Plotnikoff, N. P. and A. J. Kastin. Neuropharmacological tests with α-melanocyte stimulating hormone. *Life Sci.* **18**: 1217–1222, 1976.
12. Plotnikoff, N. P., A. J. Kastin, M. S. Anderson and A. V. Schally. DOPA potentiation by a hypothalamic factor, MSH release-inhibiting hormone (MIF). *Life Sci.* **10**: 1279–1283, 1971.
13. Plotnikoff, N. P., A. J. Kastin, D. H. Coy, C. W. Christensen, A. V. Schally and M. A. Spirtes. Neuropharmacological actions of enkephalin after systemic administration. *Life Sci.* **19**: 1283–1288, 1976.
14. Stratton, L. O. and A. J. Kastin. Increased acquisition of a complex appetitive task after MSH and MIF. *Pharmac. Biochem. Behav.* **3**: 901–904, 1975.
15. Walker, M. J., G. Berntson, C. A. Sandman, D. H. Coy, A. V. Schally and A. J. Kastin. An analogue of enkephalin having a prolonged opiate-like effect in vivo. *Science*, in press.