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Effects of Endomorphin-1 on Open-Field Behavior and on the Hypothalamic-Pituitary-Adrenal System

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The effects of endomorphin-1 (EM1) on behavioral responses and on the hypothalamic-pituitary-adrenal system were investigated in mice. Locomotor activity was measured in an "open-field" apparatus, with parallel recording of the numbers of rearings and groomings. Different doses of the peptide (250 ng to 5 µg) were administered to the animals intracerebroventricularly 30 min before the tests. EM1 caused significant increases in the locomotor activity and the number of rearings. The effect of EM1 on the basal corticosterone secretion was also investigated. At a dose of 5 µg, the peptide significantly increased plasma corticosterone level. The corticotropin-releasing hormone (CRH) antagonist α-helical CRH_{9-41}, applied 30 min prior to EM1 administration, completely abolished the increases in both locomotion and the number of rearings and attenuated the corticosterone release evoked by EM1. These results suggest that the EM1-induced increases in locomotion and rearing activity as well as the pituitary-adrenal activation are mediated by CRH.

Key Words: Endomorphin-1; open-field behavior; hypothalamic-pituitary-adrenal axis.

Introduction

The endomorphins are opioid peptides recently isolated from the bovine brain. Radioreceptor binding assays have revealed that these peptides possess high affinity and selectivity for µ-opioid receptors *(1)*. It has been concluded that they might be endogenous ligands for the morphine receptors, and they were therefore named endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2; EM1) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2; EM2) *(2)*.

EM1-like immunoreactivity is widely and densely distributed throughout the rat brain (posterior hypothalamic nuclei, locus coeruleus, and amygdala), as demonstrated by immunocytochemical studies *(3)*. This phenomenon suggests that EM1 might be an endogenous peptide mediator of nociceptive, behavioral, and autonomous processes, in which opioid regulation plays a well-established and critical role *(4)*.

Some physiologic effects of EM1 have already been described. These results reflect that this opioid peptide has a distinct pharmacologic profile, which is in some cases markedly different from that of morphine. It exerts a profound spinal analgesic effect *(2,5)*, similarly to morphine, and it even antagonizes neuropathic pain, whereas morphine seems ineffective *(6)*. Furthermore, EM1, like morphine *(7,8)*, has anxiolytic and orexigenic properties *(9)*, but its cardiovascular activity *(10)* appears to differ from that of morphine *(11)*. These functional differences may result from the differences in selectivity of EM1 and morphine regarding the opioid receptor subtypes: EM1 displays high selectivity to the µ-opioid receptor, and morphine binds to both the µ-and the δ-receptors *(1,2,12–15)*. In addition, EM1 and morphine prefer different molecular forms of the µ-opioid receptor *(16)*, and only EM1 exhibits an ability to internalize its receptor *(17)*.

It is clear from previous studies that morphine has a noteworthy impact on locomotion *(18)* and is a pronounced activator of the hypothalamic-pituitary-adrenal (HPA) system *(19,20)*. The neuroanatomic distribution of EM1 *(3)* suggests that as an endogenous ligand of the μ_1- and μ_2-opioid receptors, it may participate in the behavioral and endocrine processes.

The purpose of the present study was to examine the effects of EM1 on behavioral responses, and to compare the actions of this endogenous opioid ligand to those of morphine. Because previous studies *(21)* had suggested that corticotropin-releasing hormone (CRH), the central regulator of the HPA system, may be involved in stress-related behavior, the effects of EM1 on the HPA system and the possible roles of CRH in locomotion, rearing, grooming, and endocrine processes were also investigated.

Results

The administration of EM1 (0.25–5 µg) into the right lateral brain ventricle significantly increased locomotor activity (Table 1). A dose of 0.5 µg increased the number

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Table 1
Effects of Intracerebroventricular Administration of EM1 on Behavioral Responses[a]

	Control (16)	EM1 (0.25 μg) (10)	EM1 (0.5 μg) (10)	EM1 (1 μg) (12)	EM1 (2 μg) (10)	EM1 (5 μg) (6)
Locomotion	108.13 ± 9.60	134.50 ± 11.74	170.70 ± 12.32[b]	186.50 ± 11.62[b]	154.20 ± 9.39	166.50 ± 13.76
Rearing	11.44 ± 1.51	14.10 ± 1.26	15.20 ± 1.92	21.08 ± 1.50[b]	12.50 ± 1.56	11.83 ± 2.01
Grooming	0.31 ± 0.12	0.60 ± 0.16	0.70 ± 0.15	0.50 ± 0.15	0.3 ± 0.15	0.33 ± 0.33

[a]Behavioral activity was characterized by the total number of explored squares, rearings, or groomings/3-min test sessions. Numbers in parentheses are the number of animals used.
[b]$p < 0.05$ vs control.

Table 2
Effects of Intracerebroventricular Administration of CRH Antagonist α-Helical CRH_{9-41} on EM1-Induced Behavioral Responses[a]

	Control (15)	CRH antagonist (1 μg) (12)	EM1 (1 μg) (15)	CRH antagonist + EM1 (8)
Locomotion	136.27 ± 6.61	145.5 ± 6.40	188.0 ± 8.91	128.25 ± 10.06[b]
Rearing	15.13 ± 1.50	14.42 ± 1.28	20.60 ± 1.27	13.63 ± 2.00[b]
Grooming	0.87 ± 0.17	0.50 ± 0.150	0.47 ± 0.13	0.88 ± 0.13

[a]Behavioral activity was characterized by the total number of explored squares, rearings, or groomings/3-min test sessions. Numbers in parentheses are the number of animals used.
[b]$p < 0.05$ vs EM1.

of squares explored (F[5, 58], $p < 0.01$ vs control) but the most effective dose was 1 μg ($p < 0.001$ vs the control). The higher doses of the peptide (2 and 5 μg) did not give rise to further increases in locomotion ($p = 0.075$ and 0.08 vs control, respectively). The tetrapeptide dose (1 μg) that led to the most significant response in locomotor activity also elevated the number of rearings ($p < 0.001$ vs control) (Table 1). Regarding grooming, EM1 elicited only a tendency to an increase, which did not prove to be statisticallly significant. Preliminary administration of the CRH antagonist α-helical CRH_{9-41} (1 μg) completely abolished the increases in both locomotion (F[3, 46], $p < 0.001$ vs EM1) and the number of rearings ($p < 0.05$ vs EM1) (Table 2).

EM1 likewise induced a significant increase in plasma corticosterone level. A dose of 5 μg elevated the corticosterone level by 157% as compared with control (F[3, 35] = 4.48, $p < 0.05$) (Table 3). The corticosterone response induced by EM1 was also inhibited by α-helical CRH_{9-41} (F[3, 24]) = 7.96, $p < 0.05$ vs EM1) (Table 4).

Discussion

Even though EM1 differs considerably in structure from previously known endogenous opioids *(2)*, it displays marked selectivity toward μ_1- and μ_2-opioid receptors *(1,2)*. The widespread distribution of EM1 in the central nervous system *(3)* and the high activity of this peptide on the μ-opioid receptors raise the possibility that EM1 acts as an endogenous equivalent of morphine in numerous physiologic processes.

The role of the opioid system in the regulation of locomotion *(22)* is well characterized. Earlier studies demonstrated that opioid peptides influence the locomotor activity through the μ-, δ-, and κ-opioid receptors: the μ- and δ-opioid receptors mediate locomotor hyperactivity *(23,24)*, whereas selective κ-receptor agonists decrease linear locomotion *(25)*. Further behavioral phenomena are also under opioid control. The effects of morphine on rearing and grooming are strongly dose and time related presumably as a consequence of different involvements of opioid receptor subtypes *(26–28)*. Participation of an opiate mechanism in the corticosterone response previously has been demonstrated as well: acute administration of morphine activated the HPA axis *(29)*.

We have established that intracerebroventricular administration of EM1 modulates the behavioral response: it facilitates the locomotor activity and elevates the number of rearings. Concerning locomotion, EM1 exhibited a bell-shaped dose-response curve, with a downturn phase at higher doses, a phenomenon already described regarding the effects of other neuropeptides *(30,31)* and discussed by Pliska *(32)*. EM1 also modified the rearing activity, displaying a similar dose-response curve, but this effect was less marked. When the receptor specificity of EM1 is taken into consideration, these results indicate that opioids mainly stimulate locomotion and rearing through the μ-opioid receptor. That α-helical CRH_{9-41} completely abolished the increases in both locomotion and the number of rearings lends strong support to the hypothesis that the EM1-induced behavioral responses are mediated by CRH.

We found that EM1 did not significantly alter grooming activity. Morphine seems to exert quite ambiguous effects on grooming, depending on the dose and the testing sched-

Table 3
Effects of Intracerebroventricular Administration of EM1 on Corticosterone Level[a]

	Control (14)	EM1 (1 μg) (8)	EM1 (2 μg) (9)	EM1 (5 μg) (8)
Corticosterone level (mean ± SEM)	15.16 ± 0.94	14.33 ± 0.68	17.97 ± 1.18	23.88 ± 4.17[b]

[a]Corticosterone concentrations are given in micrograms/100 milliliters. Numbers in parentheses are the number of animals used.
[b]$p < 0.05$ vs control.

Table 4
Effects of Intracerebroventricular Administration of CRH Antagonist α-Helical CRH_{9-41} on EM1-Induced Corticosterone Level[a]

	Control (7)	CRH antagonist (1 μg) (5)	CRH antagonist + EM1 (8)	EM1 (5 μg) (8)
Corticosterone level (mean ± SEM)	13.33 ± 0.85	13.98 ± 1.15	15.10 ± 0.66[b]	18.40 ± 0.76

[a]Corticosterone concentrations are given in micrograms/100 milliliters. Numbers in parentheses are the number of animals used.
[b]$p < 0.05$ vs EMI.

ule *(27,28)*. Previous studies revealed that some actions of morphine are mediated by the δ-opioid receptor *(33)*, and δ-opioid agonist activates grooming *(23)*. These data, taken together with our findings, indicate that μ-opioid mediation may not play a crucial role in the regulation of grooming.

Because increase in locomotion may reflect not only exploration but also fear, our results might reflect that EM1-induced anxiety (mediated by CRH release) can evoke an increase in locomotion and acts as a stress paradigm, too. However, in our experiments the differences between the effective EM1 dose on behavior (1 μg) and on plasma corticosterone (5 μg) might suggest that different neural mechanisms are involved in these actions even though both effects can be attenuated or blocked by the CRH antagonist. EM1 presumably induces CRH release at a hypothalamic level, and this action can be inhibited by the antagonist at a pituitary level. On the other hand, the behavioral effect of EM1 may be elicited in extrahypothalamic structures. Nevertheless, the behavioral response also appears to be mediated by CRH, because it could be inhibited by the CRH antagonist. In accordance with previous findings *(34,35)*, our results suggest that CRH plays a marked role in the regulation not only of the HPA system, but also of locomotion and rearing, and the EM1-induced behavioral and endocrine responses strongly depend on CRH release.

Materials and Methods

Reagents and Drugs

Ethyl alcohol, methylene chloride, and sulfuric acid of analytical grade (Reanal, Budapest) were used for the corticosterone assay. EM1 was obtained from the Institute of Biochemistry (Biological Research Center, Hungarian Academy of Sciences, Szeged) and was synthesized as described by Tömböly et al. *(36)*. The CRH antagonist α-helical $CRH_{9–41}$ was purchased from Bachem (Switzerland).

Animals

Male CFLP mice of an outbred strain (LATI, Gödöllö, Hungary) weighing 25–35 g were used at the age of 5 wk. The animals were kept on a standard diet and on a 12-h light and 12-h dark cycle. The animals were maintained and handled during the experiments in accordance with the instructions of the University of Szeged Ethical Committee for the Protection of Animals in Research.

Surgery

For the intracerebroventricular administration of EM1 or saline alone (control animals), the mice were subjected to the following surgical procedure. They were operated on under pentobarbital (55 mg/kg of nembutal) anesthesia 1 wk before the experiment. A polyethylene cannula was inserted into the right lateral brain ventricle. At the end of the experiment, to verify the correct position of the cannulae, each mouse was sacrificed after pentobarbital anesthesia, methylene blue was injected into the head, and the brains were then dissected. Only data on animals in which the methylene blue diffused to all the ventricles were included in the statistical analyses.

Treatment

Intracerebroventricular peptide administration and behavioral testing was carried out according to previously published data *(31)*.

Protocol 1

Different doses of EM1 dissolved in 0.9% saline (from 250 ng to 5 μg) was administered intracerebroventricularly in a volume of 2 μL. Control mice received saline alone. Thirty min after EM1 administration the animals were subjected to behavioral tests or were sacrificed to obtain blood samples for the corticosterone assay.

Protocol 2

For this experimental setting, we selected the doses of EM1 that proved to be the most effective in protocol 1 (1 μg for the behavioral test and 5 μg for the corticosterone assay), and animals were divided into four treatment groups. Groups I and III received intracerebroventricular saline alone, and α-helical CRF_{9-41} was administered to groups II and IV. Thirty min later groups III and IV were treated with EM1, and vehicle was injected into groups I and II. Thirty min after EM1 administration, the animals were subjected to behavioral tests or were sacrificed to obtain blood samples for the corticosterone assay.

Behavioral Testing

Locomotor activity was measured 30 min after EM1 treatment. The mice were removed from their home cages and placed in the center of an open-field box consisting of 49 squares (5 × 5 cm each). The locomotor activity was characterized by the total number of squares explored during 3-min test sessions. The numbers of rearings (the animals stood on their hind legs) and groomings (face washing, forepaw licking, and head stroking) were also recorded.

Corticosterone Assay

To determine plasma corticosterone concentrations, the animals were decapitated 30 min after EM1 treatment. Trunk blood was collected in heparinized tubes. The plasma corticosterone concentration was measured by the fluorescence assay described by Zenker and Bernstein *(37)* as modified by Purves and Sirett *(38)*.

Statistical Analyses

Values are presented as means ± SEM. Data were evaluated statistically with an analysis of variance procedure followed by Tukey's post hoc test. A probability level of $p < 0.05$ was considered to be statistically significant.

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