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Antidepressant-Like Effect of Endomorphin-1 and Endomorphin-2 in Mice

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Endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) are two recently isolated μ-opioid selective peptides with a potent antinociceptive activity, involved in a number of physiological processes, including food intake, vasomotricity, sexual behavior, as well as neuroendocrine and cardiorespiratory functions. The neuroanatomical distribution of endomorphins prompted us to study their antidepressant activity in two animal behavioral models of depression: forced-swimming and tail-suspension tests. In both tests, the intracerebroventricular (i.c.v.) injection of either endomorphin-1 or endomorphin-2 significantly decreased the duration of immobility, interpreted as an expression of 'behavioral despair', which could be related to the depression syndrome. These effects of endomorphins did not result from the stimulation of the animal motor activity. We have also demonstrated that the antidepressant-like effect of endomorphins was antagonized by the universal opioid antagonist, naloxone and the μ-opioid receptor selective antagonist, β-funaltrexamine. In contrast, this effect was not antagonized by δ- and κ-opioid receptor selective antagonists, naltrindole and nor-binaltorphimine, respectively. The results of the present study demonstrate that endomorphin-1 and endomorphin-2 produce potent antidepressant-like effects after i.c.v. injection in mice. We may suggest that endomorphins and the μ-opioid receptors might be involved in the physiopathology of depressive disorders, and that the endomorphinergic system could serve as a novel target for the development of antidepressant drugs.

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INTRODUCTION

The discovery of the three types of opioid receptors (μ, δ, and κ) over 30 years ago initiated an extensive search for their selective endogenous ligands. The δ-selective enkephalins were the first (Hughes *et al*, 1975), followed soon afterwards by the κ-selective dynorphins (Chavkin *et al*, 1982) and nonselective β-endorphin (Li and Chung, 1976). The μ-receptor was the first opioid receptor identified in binding assays and its importance in mediating the antinociceptive action of morphine and other clinically used analgesics has been well documented (Pasternak, 1993). Yet, the search for the endogenous ligand for the μ-receptor has lagged far behind the other opioid receptor types.

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In 1997 Zadina *et al* (1997) synthesized and later isolated from mammalian brain two novel endogenous peptides, endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2), which bind to the μ-opioid receptor with extremely high affinity and selectivity. Radioimmunological and immunocytochemical analyses revealed that both endomorphins are densely distributed throughout the central nervous system, near neurons expressing the μ-opioid receptors (Martin-Schild *et al*, 1999; Zadina *et al*, 1999; Zadina, 2002). Endomorphins were found in the limbic system (septum, nucleus accumbens, amygdala), thalamic nuclei, locus coeruleus, and in the brain stem (Schreff *et al*, 1998; Martin-Schild *et al*, 1999; Zadina, 2002). Some of these regions are known to be involved in mood disorders, such as anxiety and depression (Drevets and Raichle, 1992; Drevets *et al*, 1992; Drevets, 1998; Sheline, 2000). It has also been demonstrated that endomorphins and the μ-opioid receptors are present in the brain regions containing monoamine neurotransmitters (serotonin, dopamine, and noradrenaline), which play a key role in the physiopathology of depressive disorders. In fact, endomorphins have been shown to modulate serotoninergic (Chen *et al*, 2001; Tao and Auerbach, 2002; Hung *et al*, 2003), dopaminergic (Chen *et al*, 2001; Ukai and Lin, 2002;

Bujdoso *et al*, 2003; Huang *et al*, 2004), and noradrenergic (Chen *et al*, 2001; Al-Khrasani *et al*, 2003; Hung *et al*, 2003) transmissions.

The possible role of endogenous opioid peptides in depression is supported by neurochemical and neurobehavioral findings (Belluzi and Stein, 1977; De Witte *et al*, 1989). However, little is known about the antidepressant activity of endomorphins. The aim of the present study was to investigate the antidepressant effects of endomorphin-1 and endomorphin-2 after intracerebroventricular (i.c.v.) administration in mice in two behavioral models: forced-swimming and the tail-suspension tests (TST). We have also examined the effect of both endomorphins on the locomotor activity, which might influence nonspecifically the animal responses in these tests.

MATERIALS AND METHODS

Animals

Male Swiss albino CD1 mice (IFFA-CREDO/Charles River, Saint-Germain sur L'Arbresle, France), weighing 20–22 g, were used throughout the study. The animals were housed 20 per Makrolon cage (L: 40, W: 25, H: 18 cm), with free access to standard semisynthetic laboratory diet (UAR, Villemoisson sur Orge, France) and tap water *ad libitum*, under controlled environmental conditions (ventilated room, temperature $22 \pm 1^{\circ}C$, 12 h light/12 h dark cycle, lights on between 07:00 and 19:00 hours). All experiments were carried out between 09:00 and 18:00 hours, unless otherwise stated, in test rooms adjacent to the animal rooms. The experiments were performed according to the European Communities Council Directive from 24 November 1986 (86/609/EEC) and were conducted by authorized investigators. Mice were tested only once and killed immediately thereafter by decapitation.

Chemicals

Peptides were synthesized by standard solid–phase procedures as described before (Fichna *et al*, 2004), using techniques for Fmoc-protected amino acids on MBHA Rink peptide resin (100–200 mesh, 0.8 mM/g, Novabiochem). 20% Piperidine in dimethylformamide was used for deprotection of Fmoc-groups and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) was employed to facilitate coupling. Simultaneous deprotection and cleavage from the resin was accomplished by treatment with trifluoroacetic acid: triisopropylsilane: water (95: 2.5: 2.5) for 3 h at room temperature. Crude peptides were purified by RP HPLC on a Vydac C_{18} column (1×25 cm) using the solvent system of 0.1% TFA in water (A)/80% acetonitrile in water containing 0.1% TFA (B) and a linear gradient. Calculated values for protonated molecular ions were in agreement with those obtained using FAB mass spectrometry.

Opioid receptor antagonists, naloxone hydrochloride, β-funaltrexamine hydrochloride, naltrindole dihydrochloride, and nor-binaltorphimine hydrochloride were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Peptides and opioid receptor antagonists were dissolved in sterile saline (0.9% NaCl) just before treatment.

Intracerebroventricular Injections

i.c.v. injections (10 µl/mouse) were performed in the left brain ventricle of manually immobilized mice, with a Hamilton microsyringe (50 µl, Hamilton, Bonaduz, Switzerland) connected to a needle (length: 3.5 mm, diameter: 0.5 mm), as described elsewhere (Fichna *et al*, 2004). I.c.v. injections were performed by an experienced investigator, who frequently controlled the regularity and the success of injections using a methylene blue dye and who observed, after killing and frontal brain sectioning that the injection was successful in more than 95% of trials. It was also verified in several animals, after killing, that the mark of the needle puncture on the parietal bone was located at least 1.5 mm behind the bregma and at least 2.5 mm before the lambda, with laterality between 1 and 2 mm relative to the brain median line. This corresponds to stereotaxic coordinates of the left lateral ventricle in Lehmann's atlas for mice (Lehmann, 1974): anteriority 2.95–4.15 mm, laterality 0.95–2 mm, and depth 3–4.5 mm. The i.c.v. injection method was approved by the Regional Ethical Committee for Animal Experimentation (Normandy; no. N/10-04-04-12).

Forced-Swimming Test

The forced-swimming test was essentially similar to that described by Porsolt *et al* (1977). The apparatus with larger Plexiglas cylinders (14 cm in diameter instead of 10 cm), similar to that employed by Semba and Takahashi (1988) and Do-Rego *et al* (2002, 2005) was used, since Sunal *et al* (1994) demonstrated that a cylinder with a larger diameter decreases the number of false positive responses. The apparatus consisted of two Plexiglas cylinders (20 cm height, 14 cm internal diameter), placed side-by-side in a Makrolon cage (L: 38, W: 24, H: 18 cm) and separated by an opaque screen. The Makrolon cage was filled with water ($22 \pm 1^{\circ}C$) to a height of 12 cm, instead of 6 cm suggested by Porsolt *et al* (1977), since, according to Petit-Demouliere *et al* (2005), the depth of water is an important parameter to be considered as mice should not sense a limit under the level of water. The behavior of mice would indeed be altered if their tails touch the bottom of the cylinder.

At 15 min before the test the animals were isolated in small individual cages (L: 25, W: 9, H: 8 cm) at an ambient temperature ($22 \pm 1^{\circ}C$). After i.c.v. injection two mice were tested simultaneously for a 6-min period. Total duration of immobility was measured during three consecutive periods of 2 min, each using an automated image analysis system (Videotrack MV 45). The method was approved by the Regional Ethical Committee for Animal Experimentation (Normandy; no. N/09-04-04-11).

Tail-Suspension Test

The TST was performed as described previously (Steru *et al*, 1987), using a computerized device (ITEMATIC-TST) developed by ITEMLabo (Le Kremlin-Bicêtre, France). At 15 min before the test the animals were isolated in small individual cages (L: 25, W: 9, H: 8 cm) at an ambient temperature ($22 \pm 1^{\circ}C$). After i.c.v. injection mice were suspended by tail, using an adhesive Scotch tape, to a hook connected to a strain gauge, which detected all movements

of an animal. The signals were transmitted to a central unit, which calculated the total duration of animal immobility over 6 min of the test. Six animals were tested simultaneously. The method was approved by the Regional Ethical Committee for Animal Experimentation (Normandy; no. N/14-04-04-16).

Measurement of Locomotor Activity

Locomotor activity was assessed automatically in a Digiscan actimeter (Omnitech Electronics Inc., Columbus, OH, USA), which monitored horizontal displacements and vertical movements. The animals were placed individually in $20 \times 20 \times 30$ cm compartments, in a dimly illuminated and quiet room. The responses were expressed as the number of crossed beams by mouse during four consecutive 15 min periods.

Statistical Analysis

The data are expressed as means $\pm$ SEM. Differences between groups were assessed by one-way analysis of variance (ANOVA) followed by a *post hoc* multiple comparison Student–Newman–Keuls test. Antagonist effects in the combination experiments were analyzed using two-way analysis of variance (ANOVA) and a *post hoc* multiple comparison Student–Newman–Keuls test was used for multiple comparisons between groups. A probability level of 0.05 or lower was considered as statistically significant.

RESULTS

Dose–Response Effect of Endomorphins on the Forced-Swimming and TST

In the present study, the activity of endomorphin-1 and endomorphin-2 was examined in two animal behavioral models of depression: forced-swimming and TST, as well as in locomotor activity assay. The results of the forced-swimming test, performed 10 min after i.c.v. administration of endomorphin-1 and endomorphin-2, in a dose range 0.3–30 μg/mouse, are shown in Figure 1. Both peptides decreased, in a dose-dependent manner, the duration of immobility in all three time periods of the test and during the total time of experiment (0–360 s). In the total time of experiment, endomorphins produced a significant effect ($p < 0.05$–0.001) in all tested doses.

In the TST, the i.c.v. injection of endomorphin-1 or endomorphin-2 (0.3–30 μg/mouse) 10 min before the assay reduced, in a dose-dependent manner, the duration of animal immobility (Figure 2). For both peptides, the signi-

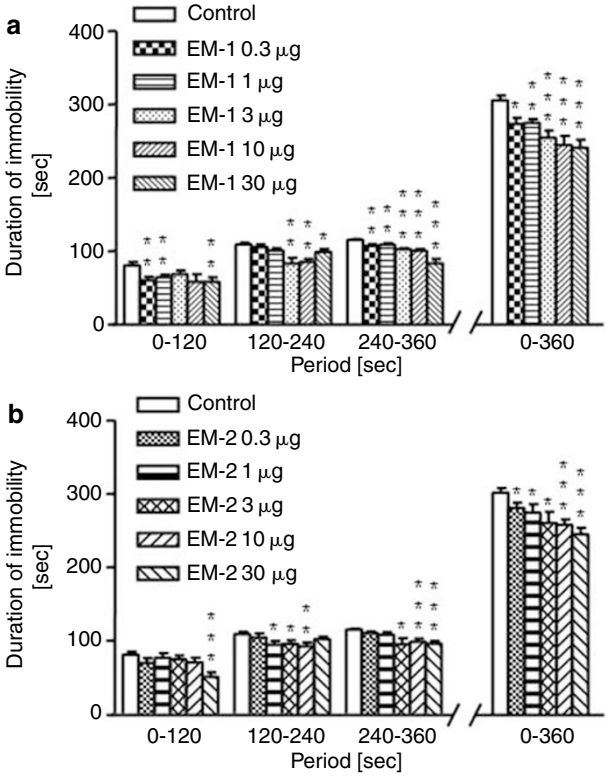

Figure 1 Dose–response effect of endomorphin-1 and endomorphin-2 on the forced-swimming test. Mice were injected i.c.v. with saline or peptides (0.3–30 μg/animal) and submitted to the swimming test 10 min after the injection. The duration of immobility was measured during a 6-min period (three consecutive periods of 2 min each). Data represent mean $\pm$ SEM of 10 mice per group. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, as compared to respective control by using one-way ANOVA followed by the Student–Newman–Keuls' test.

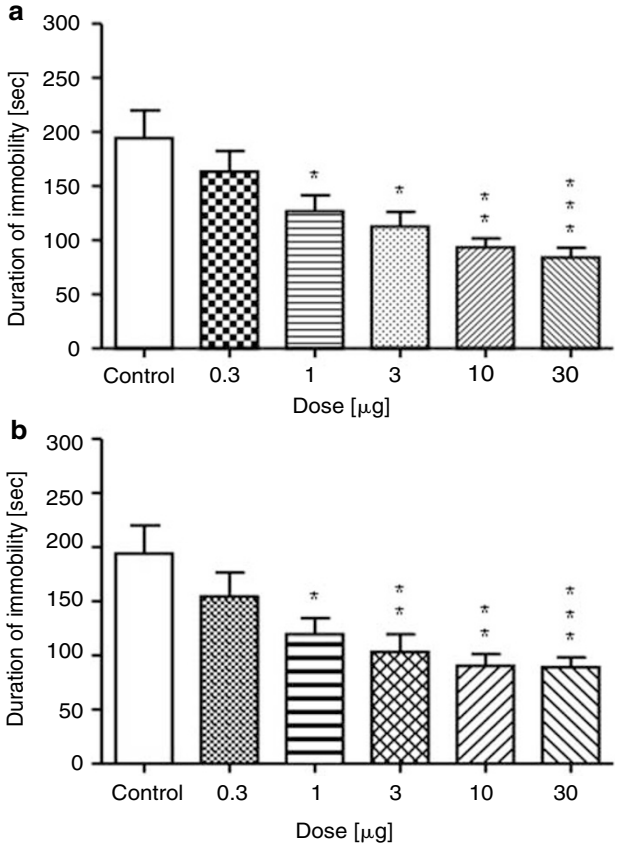

Figure 2 Dose–response effect of endomorphin-1 and endomorphin-2 on the TST. Mice were injected i.c.v. with saline or peptides (0.3–30 μg/animal) and submitted to the test 10 min after the injection. The duration of immobility was measured during a 6-min period. Data represent mean $\pm$ SEM of 10 mice per group. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, as compared to respective control by using one-way ANOVA followed by the Student–Newman–Keuls' test.

ficant effect ($p<0.05$–0.001) was observed at the doses ranging from 1 to 30 μg/mouse.

Time–Course of the Effect of Endomorphins on the Forced-Swimming Test

The time–response curves for endomorphin-1 and endomorphin-2 (10 μg/animal, i.c.v.) in the forced-swimming test are shown in Figure 3. The effects of endomorphins were measured 10, 15, 20, 30, 45, or 60 min after administration. The inhibitory effect of endomorphin-1 was only observed 10 and 15 min after injection. It was significant ($p<0.05$–0.001) in the second (120–240 s) and third (240–360 s) period of the test and during the total time of experiment. Endomorphin-2 produced a significant effect ($p<0.05$–0.01) only 10 min after administration.

Dose–Response Effect of Endomorphins on Locomotor Activity

The influence of the i.c.v. administration of endomorphin-1 and endomorphin-2 on locomotor activity was measured over four consecutive periods of 15 min each (Figure 4). The peptides did not modify the horizontal locomotor activity in any of the time periods of the test (Figure 4a and b for endomorphin-1 and endomorphin-2, respectively). Endomorphin-1, at the highest dose tested (30 μg/mouse), produced a significant inhibition ($p<0.05$) of the vertical locomotor activity in the first time period of the assay (0–15 min, Figure 4c). On the contrary, endomorphin-2, at the lowest dose (0.3 μg/animal), significantly ($p<0.05$) diminished vertical locomotor activity in the first (0–15 min) and third (45–60 min) time periods of the test, and at the dose of

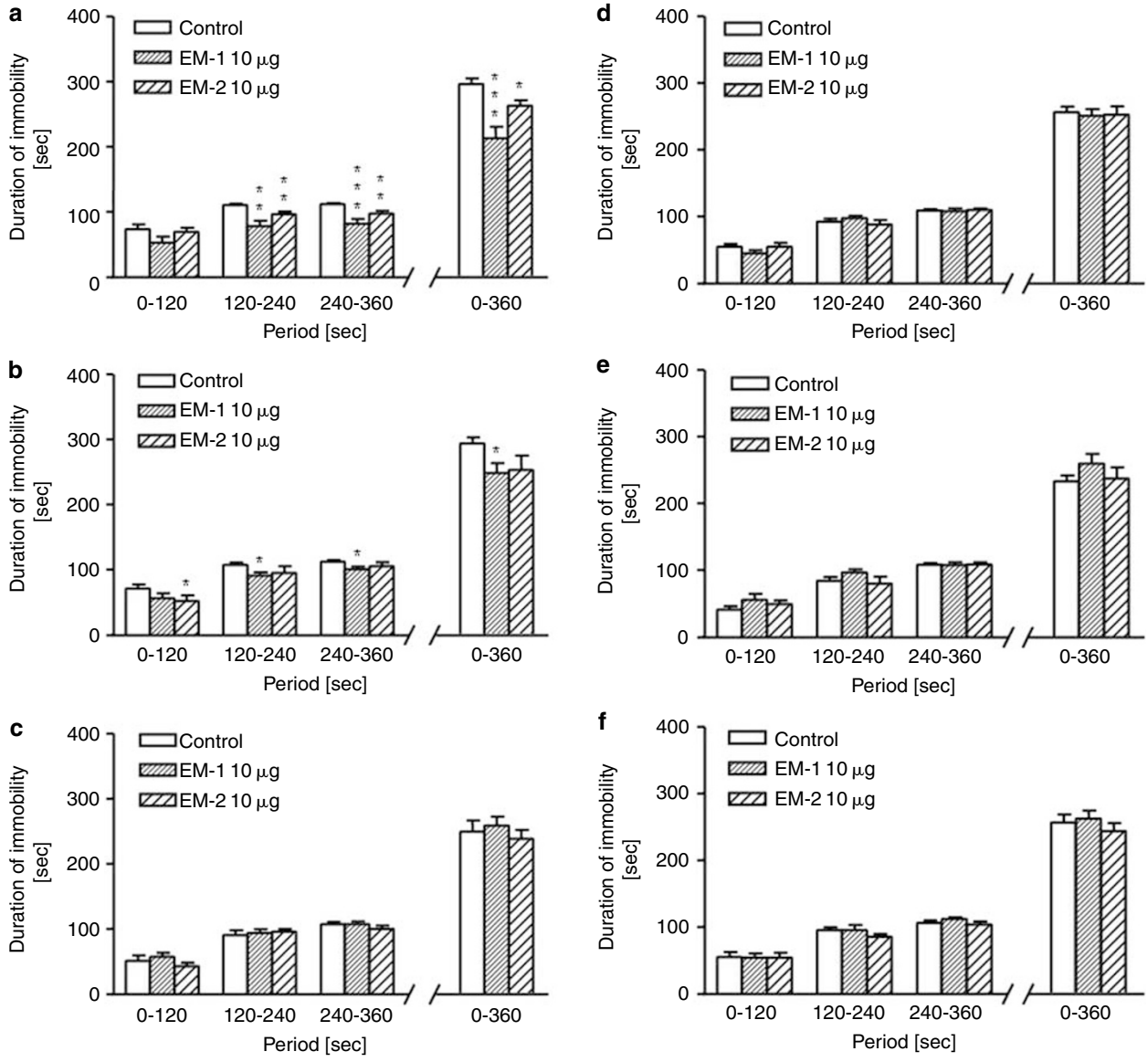

Figure 3 Time–course of the effect of endomorphin-1 and endomorphin-2 on the forced-swimming test. Mice were injected i.c.v. with saline or peptides (10 μg/animal) and submitted to the swimming test 10 (a), 15 (b), 20 (c), 30 (d), 45 (e), or 60 min (f) after the injection. The duration of immobility was measured during a 6-min period (three consecutive periods of 2 min each). Data represent mean ± SEM of 10 mice per group. *$p<0.05$; **$p<0.01$; ***$p<0.001$, as compared to respective control by using one-way ANOVA followed by the Student–Newman–Keuls' test.

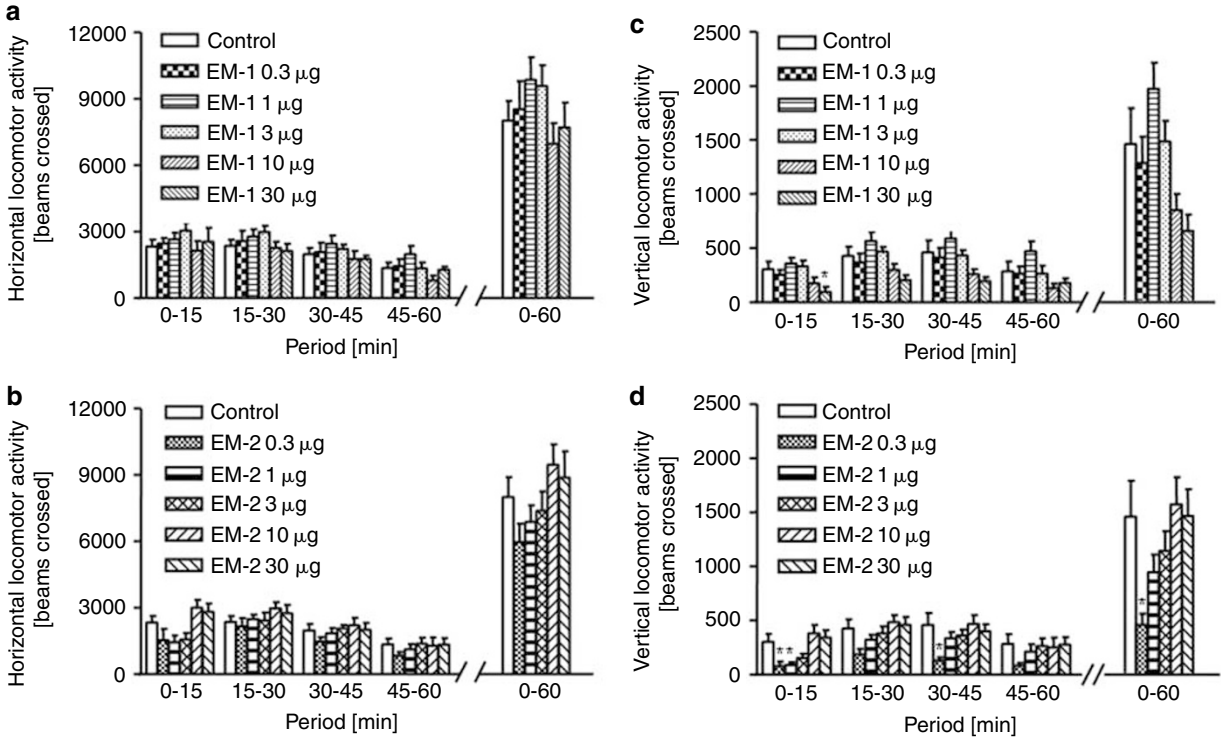

Figure 4 Dose–response effect of endomorphin-1 and endomorphin-2 on horizontal (a and b, respectively) and vertical (c and d, respectively) locomotor activity. Mice were injected i.c.v. with saline or peptides (0.3–30 μg/animal) and placed in the actimeters immediately after the injection. Horizontal displacements and vertical movements were measured for four consecutive periods of 15 min. Data represent mean ± SEM of 10 mice per group. $^*p < 0.05$, as compared to respective control by using one-way ANOVA followed by the Student–Newman–Keuls' test.

1 μg/animal in the first (0–15 min) time period of the test (Figure 4d).

Comparison of the Antagonist Properties of Naloxone on the Endomorphin-Induced Effect on the Forced-Swimming Test

The hypothesis that the inhibitory effect of endomorphins on the forced-swimming test is mediated through the opioid receptors was also examined. The duration of animal immobility was measured after a concomitant i.c.v. administration of endomorphin-1 or endomorphin-2 (3 μg/animal) and the universal opioid receptor antagonist naloxone (5 μg/animal) and compared to that produced by endomorphins injected alone. We observed a significant inhibitory effect of i.c.v. administered naloxone ($p < 0.05$–0.01) on endomorphins-induced behavior in all three time periods of the assay (Figure 5a and b). The pretreatment with naloxone, injected intraperitoneally (i.p.) at the dose of 1 mg/kg animal 30 min before the i.c.v. injection of endomorphins, also produced a significant antagonist effect ($p < 0.05$–0.001, Figure 5c and d). Naloxone administered alone had no effect on the time of animal immobility in the forced-swimming test in mice.

Selective Opioid Receptor Antagonists on the Endomorphin-Induced Effect on the Forced-Swimming Test

In order to examine which of the receptor types mediates the endomorphin-induced effect on the forced-swimming test, we have measured the time of animal immobility after a concomitant i.c.v. administration of endomorphin-1 or endomorphin-2 (3 μg/animal) and the selective opioid receptor antagonists. We observed a significant inhibitory effect ($p < 0.05$–0.001) of a selective μ-opioid receptor antagonist β-funaltrexamine (1 μg/animal, i.c.v.) on endomorphin-induced behavior in all three time periods of the assay (Figure 6). The coadministration of δ-selective antagonist naltrindole (1 μg/animal) and κ-selective antagonist nor-binaltorphimine (5 μg/animal) with endomorphins (3 μg/animal) produced no significant effect (Figures 7 and 8, respectively). The opioid antagonists administered alone had no effect on the time of animal immobility. These results clearly show that the endomorphin-induced effect on the forced-swimming test is mediated by the μ-opioid receptors.

DISCUSSION

The results of the present study suggest that endomorphin-1 and endomorphin-2 produce an antidepressant-like effect in the forced-swimming and the TST after i.c.v. injection in mice. This is the first report on the involvement of endomorphinergic system in physiopathology of depression. In most of the behavioral models of depression animals are exposed to mildly aversive situations, from which there is no possibility to escape and which induce recognizable behavioral changes. In both, the forced-swimming test and the TST, a prolonged exposure to aversive situations induces immobility, interpreted as an expression of behavioral despair, which could be related to

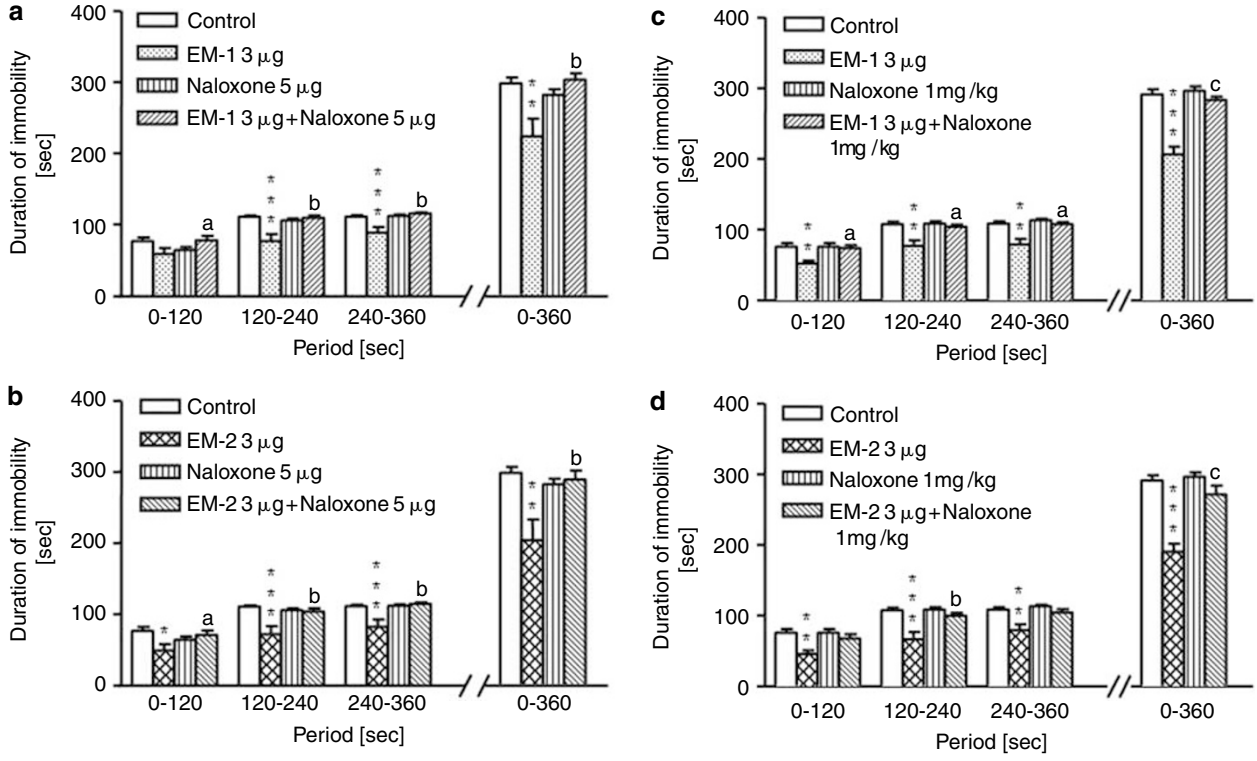

Figure 5 Comparison of the antagonist properties of naloxone (5 μg/animal, i.c.v. and 1 mg/kg, i.p.) on the endomorphin-1 (3 μg/animal, A and C, respectively) and endomorphin-2 (3 μg/animal, B and D, respectively)-induced effect on the forced-swimming test. The duration of immobility was measured during a 6-min period (three consecutive periods of 2 min each). Data represent mean ± SEM of 10 mice per group. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$, as compared to respective control by using two-way ANOVA followed by the Student–Newman–Keuls' test. A two-way ANOVA analysis revealed a significant interaction between: naloxone (5 μg/animal, i.c.v.) and endomorphin-1: $F(1,44)=6.327$; $^{a}p<0.05$ (0–120 s), $F(1,44)=11.775$; $^{b}p<0.01$ (120–240 s), $F(1,44)=9.102$; $^{b}p<0.01$ (240–360 s), $F(1,44)=10.850$; $^{b}p<0.01$ (0–360 s); between naloxone (5 μg/animal, i.c.v.) and endomorphin-2: $F(1,44)=6.800$; $^{a}p<0.05$ (0–120 s), $F(1,44)=7.836$; $^{b}p<0.01$ (120–240 s), $F(1,44)=8.063$; $^{b}p<0.01$ (240–360 s), $F(1,44)=8.962$; $^{b}p<0.01$ (0–360 s); between naloxone (1 mg/kg, i.p.) and endomorphin-1: $F(1,44)=4.629$; $^{a}p<0.05$ (0–120 s), $F(1,44)=6.559$; $^{a}p<0.05$ (120–240 s), $F(1,44)=6.474$; $^{a}p<0.05$ (240–360 s), $F(1,44)=21.724$; $^{c}p<0.001$ (0–360 s); between naloxone (1 mg/kg, i.p.) and endomorphin-2: $F(1,44)=7.922$; $^{b}p<0.01$ (120–240 s), $F(1,44)=15.038$; $^{c}p<0.001$ (0–360 s).

the depression syndrome (Porsolt *et al*, 1977; Steru *et al*, 1987). These tests are quite sensitive and relatively specific to all major classes of antidepressant agents, including tricyclics, serotonin-specific reuptake inhibitors, and monoamine oxidase inhibitors.

In our study, endomorphin-1 and endomorphin-2 significantly reduced the time of animal immobility in the forced-swimming test and the TST. These effects were dose-dependent and short-lasting; a significant response was observed only 10 and 15 min after i.c.v. administration. The magnitude of the effect induced by endomorphins was comparable to that observed after the treatment with well-known antidepressants and the compounds with potential antidepressant-like activity, both in the forced-swimming test (Petit-Demouliere *et al*, 2005) and the TST (Cryan *et al*, 2005).

Contrarily to other μ-opioid receptor agonists (Babbini and Davis, 1972), the antidepressant-like effect of endomorphins did not result from the stimulation of the animal motor activity, since the peptides did not increase the horizontal locomotor activity evaluated in a new environment. We have even observed a minor tendency to decrease the vertical movements. This is in good agreement with the classical observations that antidepressants reduce the immobility in the forced-swimming test at the doses that do not cause the stimulation of locomotion (Porsolt *et al*, 1977) or even tend to decrease locomotor activity (Duterte-Boucher *et al*, 1988; Bourin, 1990). The decrease of vertical locomotor activity after i.c.v. administration of endomorphin-2 was independent of its antidepressant-like effect, since it was observed for much longer time after peptide injection (45 and 15 min, respectively). It should be pointed out that in the earlier studies endomorphin-2, at low doses (0.25 and 0.5 μg/animal, i.c.v.), significantly stimulated locomotor activity in mice (Bujdoso *et al*, 2001). However, a different modality of testing (open field box) was used. Furthermore, a shorter duration of the assay (3 min) and a much longer time period (30 min) between peptide administration and beginning of the assay were applied.

Here, we also demonstrated that both naloxone, a reference opioid receptor antagonist with a relatively high affinity towards the μ-opioid receptors and β-funaltrexamine, a selective μ-opioid receptor antagonist, significantly antagonized the antidepressant-like effect of endomorphins. In contrast, neither naltrindole, a selective δ-opioid receptor antagonist, nor nor-binaltorphimine, a selective κ-opioid receptor antagonist, were able to block the antidepressant-like effect of endomorphins. These results indicate that the

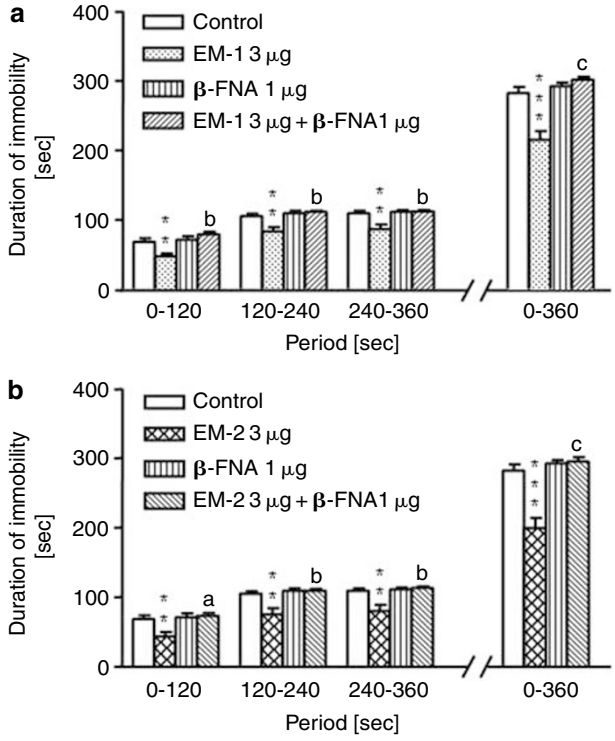

Figure 6 Comparison of the antagonist properties of β-funaltrexamine (β-FNA, 1 μg/animal) on the endomorphin-1 (3 μg/animal, a) and endomorphin-2 (3 μg/animal, b)-induced effect on the forced-swimming test. Mice were injected i.c.v. and submitted to the swimming test 10 min after the injection. The duration of immobility was measured during a 6-min period (three consecutive periods of 2 min each). Data represent mean ± SEM of 10 mice per group. $^{**}p<0.01$; $^{***}p<0.001$, as compared to respective control by using two-way ANOVA followed by the Student–Newman–Keuls' test. A two-way ANOVA analysis revealed a significant interaction between: β-funaltrexamine and endomorphin-1: $F(1,36)=8.999$; $^{b}p<0.01$ (0–120 s), $F(1,36)=7.978$; $^{b}p<0.01$ (120–240 s), $F(1,36)=8.139$; $^{b}p<0.01$ (240–360 s), $F(1,36)=20.759$; $^{c}p<0.001$ (0–360 s); between β-funaltrexamine and endomorphin-2: $F(1,36)=5.636$; $^{a}p<0.05$ (0–120 s), $F(1,36)=8.052$; $^{b}p<0.01$ (120–240 s), $F(1,36)=8.702$; $^{b}p<0.01$ (240–360 s), $F(1,36)=20.728$; $^{c}p<0.001$ (0–360 s).

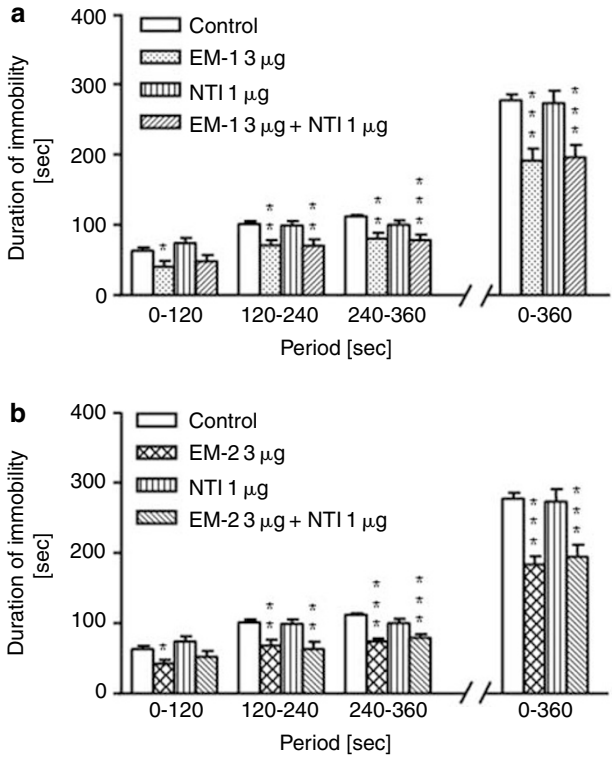

Figure 7 Comparison of the antagonist properties of naltrindole (NTI, 1 μg/animal) on the endomorphin-1 (3 μg/animal, a) and endomorphin-2 (3 μg/animal, b)-induced effect on the forced-swimming test. Mice were injected i.c.v. and submitted to the swimming test 10 min after the injection. The duration of immobility was measured during a 6-min period (three consecutive periods of 2 min each). Data represent mean ± SEM of 10 mice per group. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$, as compared to respective control by using two-way ANOVA followed by the Student–Newman–Keuls' test.

effect of endomorphins is mediated, in great part, through central μ-opioid receptors. Endomorphin-1 and endomorphin-2 were shown to possess a high affinity and an extreme selectivity for the μ-opioid receptors and most of their effects are mediated through the μ-binding sites (Champion *et al*, 1997; Asakawa *et al*, 1998; Czapla *et al*, 2000; Fichna *et al*, 2005). It has also been demonstrated that there is a significant association between the μ-opioid receptors and the etiology of depressive disorders. High concentration of the μ-opioid peptides and receptors was observed in the limbic areas involved in the regulation of mood (Mansour *et al*, 1988). Moreover, the μ-opioid receptor-knockout mice appear to have an altered emotional state that is consistent with a depressed mood (Filliol *et al*, 2000). Therefore, we could suggest that endomorphins produce their antidepressant-like effect by the activation of the μ-opioid receptors. This observation is in good agreement with the previous studies, showing that a wide variety of the μ-opioid receptor agonists, including β-endorphin and buprenorphine, possess antidepressant-like behavioral effects (Mansour *et al*, 1988; Darko *et al*, 1992; Bodkin *et al*, 1995; Tejedor-Real *et al*, 1995; Besson *et al*, 1996; Stoll and Rueter, 1999; Vilpoux *et al*, 2002) and that morphine, an alkaloid opiate with substantial affinity to the μ-opioid receptors, produces a significant antidepressant effect in the forced-swimming test after subcutaneous injections (Eschalier *et al*, 1987).

Endomorphins and the μ-opioid receptors are localized in the brain regions involved in the physiopathology of depression, such as the limbic system (septum, nucleus accumbens, amygdala), thalamic nuclei, locus coeruleus, and some regions of brain stem (Schreff *et al*, 1998; Martin-Schild *et al*, 1999; Zadina, 2002). These regions are known to contain neurotransmitter-like monoamines (serotonin, dopamine and noradrenaline), which play an important role in the etiology of depressive disorders. Numerous earlier studies have shown that endomorphins modulate serotoninergic (Chen *et al*, 2001; Tao and Auerbach, 2002; Hung *et al*, 2003), dopaminergic (Chen *et al*, 2001; Ukai and Lin, 2002; Bujdoso *et al*, 2003; Huang *et al*, 2004) and noradrenergic (Chen *et al*, 2001; Al-Khrasani *et al*, 2003; Hung *et al*, 2003) transmission. We could therefore suggest that the antidepressant-like effect of endomorphin-1 and endomorphin-2 might be mediated by the neurotransmitter-like monoamines.

In conclusion, the present study demonstrates a novel behavioral effect of two μ-receptor selective endogenous opioid peptides, endomorpin-1, and endomorpin-2. The

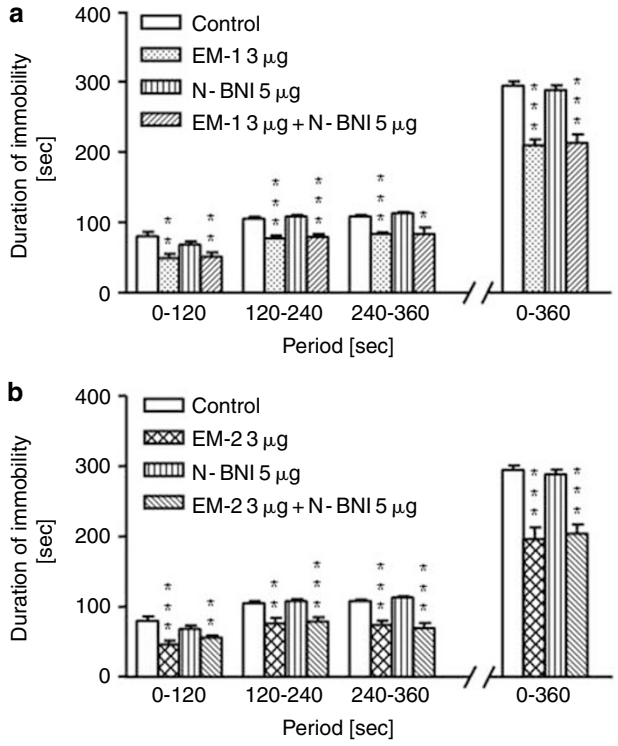

Figure 8 Comparison of the antagonist properties of nor-binaltorphimine (N-BNI, 5 μg/animal) on the endomorphin-1 (3 μg/animal, a) and endomorphin-2 (3 μg/animal, b)-induced effect on the forced-swimming test. Mice were injected i.c.v. and submitted to the swimming test 10 min after the injection. The duration of immobility was measured during a 6-min period (three consecutive periods of 2 min each). Data represent mean ± SEM of 10 mice per group. $*p<0.05$; $**p<0.01$; $***p<0.001$, as compared to respective control by using two-way ANOVA followed by the Student–Newman–Keuls' test.

data indicate that endomorphins produce a μ-opioid receptor-mediated antidepressant-like activity after i.c.v. administration in mice. Our results suggest that endomorphins and the μ-opioid receptors might play a key role in the physiopathology of depressive disorders, and that the endomorphinergic system could serve as a novel target for the development of antidepressant drugs.

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