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Multi-Dimensional Analyses of Behavior in Mice Treated with Morphine, Endorphins and [Des-Tyrosine^1]-γ-Endorphin

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KAMEYAMA, T. AND M. UKAI. *Multi-dimensional analyses of behavior in mice treated with morphine, endorphins and [des-tyrosine^1]-γ-endorphin.* PHARMACOL BIOCHEM BEHAV **19**(4) 671–677, 1983.—An investigation was made as to the effects of an intracerebral injection of morphine, endorphins and [des-tyrosine^1]-γ-endorphin (DTγE) on spontaneous locomotor activity in mice. This was done by exploiting a newly devised multi-dimensional behavioral analyser with a capacitance system. This apparatus simultaneously recorded nine different degrees of behavior (1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 and 1/256) according to the movement sizes in mice. Within 15 min after injection, γ-endorphin (5 and 10 μg), leucine-enkephalin (200 μg) and methionine-enkephalin (100 μg) produced a significant increase in the 1/1 size of movement. Fifteen to 30 min after injection, the movement patterns induced by morphine (40 μg) and DTγE (40 μg) became similar, although morphine (40 μg) caused a significant decrease in the 1/1 and 1/2 sizes of movement. β-Endorphin (2 μg) significantly decreased most of the movement sizes for 30 min compared with saline-treated group. [D-alanine^2]-methionine-enkephalinamide (20 μg) significantly decreased almost all the movement sizes within 15 min after injection. The significant alteration in the movement sizes induced by morphine and endorphins except for DTγE was antagonized by pretreatment with naloxone (1 mg/kg). These results strongly suggest the qualitative difference in the behavioral effects of each of the opioids and nonopioid.

Endorphins [Des-tyrosine^1]-γ-endorphin Locomotor activity Naloxone Mice

ENDOGENOUS opioid peptides have been found in specific brain areas and in the pituitary gland [9, 11, 16]. These peptides are known to have a morphine-like analgesic effect in animals [2,10]. On the other hand, leucine- and methionine-enkephalin have been reported to produce differential effects on morphine-induced analgesia [25] and an opposing effect on plasma corticosterone levels in ether stressed mice [8], suggesting a qualitative difference in the effects of morphine and endorphins. Although morphine presumably modifies spontaneous locomotor activity through a central site of action, the effects of this drug after central administration have not been intensively or systematically analysed. There is a similar paucity of data on the central effects of endorphins and [des-tyrosine^1]-γ-endorphin (DTγE) on spontaneous locomotor activity in animals [3, 7, 23], because most studies on mouse movement have not analysed locomotor activity as such, but rather such secondary expressions of locomotor as the total amount of whole body movements.

More recently the present authors have clearly shown that α-endorphin produces a significant increase in forward locomotion in the ethological behaviors such as activity (or patterns of activity) by using a newly devised multi-dimensional behavioral analyser (Animex II, Farad) with a capacitance system that classifies animals' movements into nine degrees according to amplitude measures [13]. This device has also been instrumental in demonstrating that α-endorphin exerts an enhancing effect on the apomorphine- or methamphetamine-induced hyperactivity in mice [13].

In our experiments, attempts have been made to correlate alterations of behavior with neurochemical changes in the brain. We have reported the effects of endorphins and enkephalins on the catecholamine and serotonin metabolism in the mouse brain with special reference to behavior [14, 15, 19, 20].

In the present experiment, Animex II was used to analyse the behavioral characteristics of mice injected with endorphins and DTγE as compared with morphine. It was also determined whether the behavioral patterns elicited by morphine, endorphins and DTγE are mediated via opiate receptors in the brain. In addition, the doses of endorphins and DTγE used in the experiment were compatible with the previously identified neurochemical evidence. A preliminary report of the study has been presented [24].

METHOD

Animals

Male ddY mice (Shizuoka Experimental Animal Co., Shizuoka, Japan) weighing between 18 and 25 g were used in these experiments. The animals arrived at least 3 days before the experiments. Separate groups of naive mice were used for each drug examined. Before the experiment, the mice

were given free access to food and water. Each mouse was housed in its own cage in a constantly illuminated room at a temperature of 23±1°C and a relative humidity 55±2.5%. The experiments were conducted between 10:00 a.m. and 6:00 p.m. in a quiet laboratory. The animals were injected intracerebrally with morphine, endorphins and DTγE and then placed in the center of the cage. All animals were used only once.

Intracerebral Injection

The uni-lateral injection site was 2 mm from either side of the midline on a line joining the anterior bases of the ears. The injection was made with 0.3 cm long needle attached to a 0.25 ml syringe. The needle was inserted perpendicularly through the skull and into the brain of the unanaesthetized mouse in a volume of 0.01 ml per mouse over a period of 15 sec as previously described [18]. The site was checked by injecting a 1 in 10 dilution of Indian ink in isotonic solution (0.9% NaCl, pH 7.5). Histological examinations revealed particles of the ink in the lateral and 3rd ventricles but not in the others. Neither insertion of the needle nor injection of 0.01 ml of isotonic saline solution had significant influence on the mice behavior.

Drugs

The following drugs were used: morphine hydrochloride (Shionogi and Co., Ltd., Osaka, Japan), naloxone hydrochloride (Sankyo Co., Ltd., Tokyo, Japan), β-endorphin, leucine(leu)- and methionine(met)-enkephalin (Protein Research Foundation, Osaka, Japan), γ-endorphin and [l-alanine^2]-methionine-enkephalinamide (D-Ala^2, Peninsula Laboratories, Inc., CA), [des-tyrosine^1]-γ-endorphin (DTγE, Takeda Chemical Industries Co., Ltd., Osaka, Japan). The drugs were dissolved in 0.9% saline. Their doses were expressed in terms of the base except for naloxone hydrochloride.

Procedure

Behavioral measurements were made for 30 min immediately after the drug administration. The Animex II equipped with an electronic microcomputer, was used for these measurements and permitted comparison of activities among the animals with different weight (Fig. 1). The sensor consisted of three pairs of electrodes and formed a capacitor bridge. Once an animal was placed in the space (15×21×14 cm) among the electrodes, the values of the capacitor then depended on the location of the mouse within that space. After converting the analog signal to digital, the DC-voltage movement spectrum analyser classified the movement into nine degrees (1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 and 1/256). Since the surface area of the cage in which mice could behave (ambulation, rearing and circling) was 490 mm, a 1/1 size of movement meant a series of 490 mm distance run. Therefore, the counters corresponded to the following movement sizes, 1/1=490 mm, 1/2=245 mm, 1/4=123 mm, 1/8=61 mm, 1/16=31 mm, 1/32=15 mm, 1/64=8 mm, 1/128=4 mm and 1/256=2 mm. The greatest movement was primarily registered on the 1/1 counter, and the smallest movement, such as tremor, on the 1/256 counter. Specific behavioral patterns induced by the drug were registered on the counters as follows, linear locomotion on 1/1 and 1/2, rearing and circling on 1/4, 1/8 and 1/16, grooming on 1/32 and 1/64 and convulsion on 1/128. The sensitivity (%) of the device was adjusted according to the mouse body weight (g) as follows, 18 g=29%, 19 g=28%, 20–21 g=27%, 22–23 g=26% and 24–25 g=25%.

The ordinates in the figures were labelled "Ratio (number of movements)." This ratio was derived using the formula: Ratio (number of movements) = (Value of drug-treated animals)/(Mean value of controls).

Statistical Analysis

The data were statistically analysed by a one-factor analysis of variance (ANOVA). The factor consisted of 5 different groups of drug treatment including saline-treated. In cases of significant overall F-scores, comparisons among means were made with Newman-Keuls tests. A p value of less than 0.05 was taken as the level of statistical significance. Data were expressed as means±S.E.

RESULTS

Intracerebral Injection

Insertion of the needle or injection of 0.01 ml of isotonic saline had a minimal effect on the mice. Immediately after removing the needle, the animals remained quiet for approximately 30 sec, and then resumed their normal activity. None of the control animals showed any residual or detrimental effects from the procedure. The typical saline-treated activity of mice was exemplified in Table 1. Although the several saline-treated mice were tested with each experiment, no significant difference in the behavior was present among the saline-treated groups according to the ANOVAs.

Effects of Morphine on the Movement Spectrum

Within 15 min after injection a 40 μg dose of morphine significantly decreased the 1/2 size of movement, $F(4,36)=3.27$, $p<0.05$, while a 1 mg/kg dose of naloxone significantly decreased the 1/1 size of movement, $F(4,36)=3.26$, $p<0.05$ (Fig. 2). Fifteen to 30 min after injection ANOVA indicated a significant relation in the following sizes: $F(4,36)=4.06$, $p<0.01$ in 1/1 size, $F(4,36)=3.73$, $p<0.05$ in 1/2 size, $F(4,36)=11.96$, $p<0.01$ in 1/4 size, $F(4,36)=17.15$, $p<0.01$ in 1/8 size, $F(4,36)=3.22$, $p<0.05$ in 1/32 size and $F(4,36)=3.0$, $p<0.05$ in 1/64 size. Fifteen to 30 min after injection a 40 μg dose of morphine significantly increased the 1/4, 1/8 and 1/32 sizes of movement. Within 15–30 min after a 20 μg dose of morphine was administered, the 1/1 size of movement was significantly decreased and the 1/4, 1/8 and 1/64 sizes of movement were conversely increased (Fig. 2). The effects of 40 μg of morphine were readily anagonized by 1 mg/kg of naloxone except for 1/1 and 1/2 sizes of movement.

Effects of β-Endorphin on the Movement Spectrum

Within 15 min after injection ANOVA revealed a significant relation in the following sizes of movement: $F(4,36)=4.36$, $p<0.01$ in 1/2 size, $F(4,36)=6.84$, $p<0.01$ in 1/4 size, $F(4,36)=6.09$, $p<0.01$ in 1/8 size, $F(4,36)=8.83$, $p<0.01$ in 1/16 size, $F(4,36)=5.12$, $p<0.01$ in 1/32 size, $F(4,36)=5.52$, $p<0.01$ in 1/64 size, $F(4,36)=5.27$, $p<0.01$ in 1/128 and $F(4,36)=4.49$, $p<0.01$ in 1/256 size. Within 15 min after injecting a 2 μg dose of β-endorphin, the sizes of movement other than 1/1 size were significantly decreased (Fig. 3). ANOVA also showed a significant relation in the following sizes of movement within 15–30 min after injec-

TABLE 1

ACTIVITY COUNTS OF SALINE-TREATED MICE

Session (min)	Size of movement								
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
0–15	4 ± 1	6 ± 1	10 ± 1	10 ± 1	20 ± 3	33 ± 5	31 ± 4	50 ± 5	99 ± 6
15–30	4 ± 1	4 ± 1	7 ± 2	12 ± 2	17 ± 3	29 ± 4	30 ± 4	55 ± 6	115 ± 7

Data represent mean ± S.E. for 10 mice. Mice were intracerebrally treated.

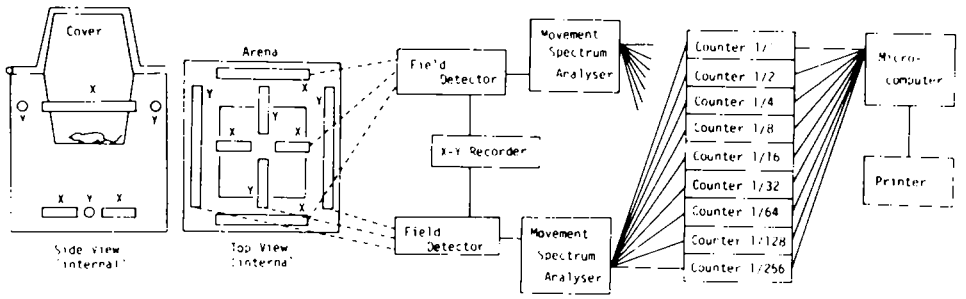

FIG. 1. Block diagram of Animex II. A field detector gives DC-voltage changes reflecting the shifted positions of an animal in a cage. A movement spectrum analyser can separate and classify the animals' movements into nine degrees. A microcomputer within the device provides instantaneous mean and S.E. calculations.

tion: $F(4,36)=4.37$, $p<0.01$ in 1/1 size, $F(4,36)=3.58$, $p<0.05$ in 1/2 size, $F(4,36)=3.1$, $p<0.05$ in 1/4 size, $F(4,36)=6.97$, $p<0.01$ in 1/16 size, $F(4,36)=5.31$, $p<0.01$ in 1/32 size and $F(4,36)=2.72$, $p<0.05$ in 1/64 size. The greater sizes of movement were inhibited by 1 and 2 μg doses of β-endorphin within 15–30 min after injection. The effects of 2 μg of β-endorphin were readily reversed by pretreatment with 1 mg/kg of naloxone.

Effects of γ-Endorphin on the Movement Spectrum

Within 15 min after injection ANOVA showed a significant relation in the following sizes of movement: $F(4,36)=5.66$, $p<0.01$ in 1/1 size and $F(4,36)=3.97$, $p<0.05$ in 1/2 size. At the 5 and 10 μg doses, γ-endorphin significantly increased the 1/1 size of movement within 15 min after injection (Fig. 4). Within 15–30 min ANOVA revealed a significant relation: $F(4,36)=4.27$, $p<0.01$ in 1/1 size of movement. γ-Endorphin significantly increased the 1/1 size of movement at the dose of 10 μg within 15–30 min. However, a 5 μg dose of γ-endorphin did not show any effect on the sizes of movement. The increase in 1/1 size of movement induced by γ-endorphin was significantly antagonized by 1 mg/kg of naloxone.

Effects of DTγE on the Movement Spectrum

Within 15 min after injection ANOVA indicated a significant relation in the following sizes of movement: $F(4,36)=3.89$, $p<0.05$ in 1/1 size, $F(4,36)=3.08$, $p<0.05$ in 1/2 size, $F(4,36)=4.04$, $p<0.01$ in 1/4 size, $F(4,36)=4.08$, $p<0.01$ in 1/8 size and $F(4,36)=3.6$, $p<0.05$ in 1/128 size. Within 15 min after injection, DTγE at the dose of 40 μg significantly decreased the 1/1, 1/2, 1/4, 1/8 and 1/128 sizes of movement (Fig. 5). Fifteen to 30 min after injection ANOVA

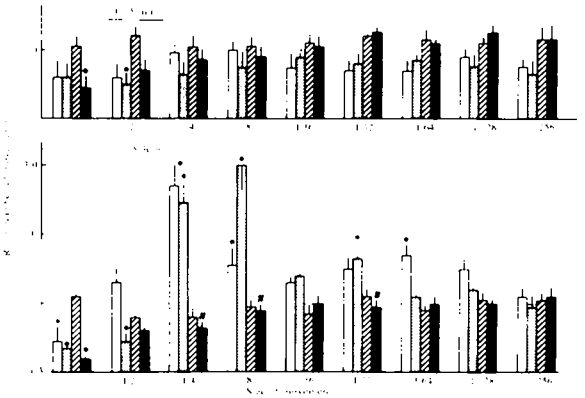

FIG. 2. Spontaneous movements in mice after morphine and the influence of naloxone. Values depict the mean+S.E. for 10 mice. Animals were pretreated with 1 mg/kg of naloxone at −10 min. *Denotes significant difference from saline control, $p<0.05$. #Denotes significant difference from corresponding morphine (40 μg) treated group, $p<0.05$. -----: Saline, open bar: morphine 20 μg/mouse, IC, stippled bar: 40 μg/mouse, IC, slanted line bar: naloxone 1 mg/kg, SC, solid bar: naloxone 1 mg/kg, SC + morphine 40 μg/mouse, IC.

revealed a significant relation in the following sizes of movement: $F(4,36)=4.17$, $p<0.01$ in 1/1 size, $F(4,36)=5.23$, $p<0.01$ in 1/2 size, $F(4,36)=6.05$, $p<0.01$ in 1/4 size, $F(4,36)=7.04$, $p<0.01$ in 1/8 size and $F(4,36)=3.64$, $p<0.05$ in 1/32 size. Fifteen to 30 min after injection the drug significantly increased the 1/1, 1/2, 1/4, 1/8 and 1/32 sizes of movement at the dose of 40 μg and the 1/4 size at a 20 μg dose. One mg/kg of naloxone did not significantly antagonize the DTγE-induced effects.

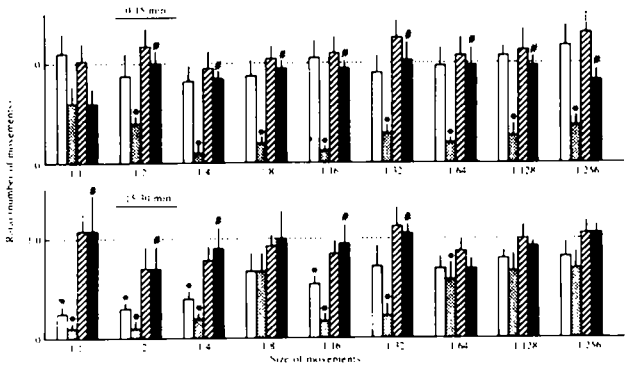

FIG. 3. Spontaneous movements in mice after β-endorphin and the influence of naloxone. Values depict the mean±S.E. for 10 mice. Animals were pretreated with 1 mg/kg of naloxone at −10 min. *Denotes significant difference from saline control, $p<0.05$. #Denotes significant difference from corresponding β-endorphin (2 μg) treated group, $p<0.05$. -----: Saline, open bar: β-endorphin 1 μg/mouse, IC, stippled bar: 2 μg/mouse, IC, slanted line bar: naloxone 1 mg/kg, SC, solid bar: naloxone 1 mg/kg, SC + β-endorphin 2 μg/mouse, IC.

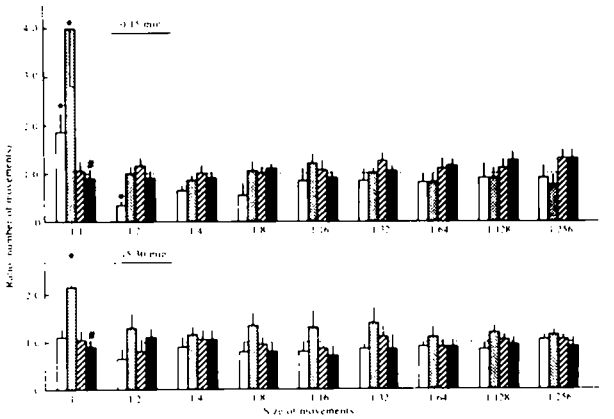

FIG. 4. Spontaneous movements in mice after γ-endorphin and the influence of naloxone. Values depict the mean±S.E. for 10 mice. Animals were pretreated with 1 mg/kg of naloxone at −10 min. *Denotes significant difference from saline control, $p<0.05$. #Denotes significant difference from corresponding γ-endorphin (10 μg) treated group, $p<0.05$, -----: Saline, open bar: γ-endorphin 5 μg/mouse, IC, stippled bar: 10 μg/mouse, IC, slanted line bar: naloxone 1 mg/kg, SC, solid bar: naloxone 1 mg/kg, SC + γ-endorphin 10 μg/mouse, IC.

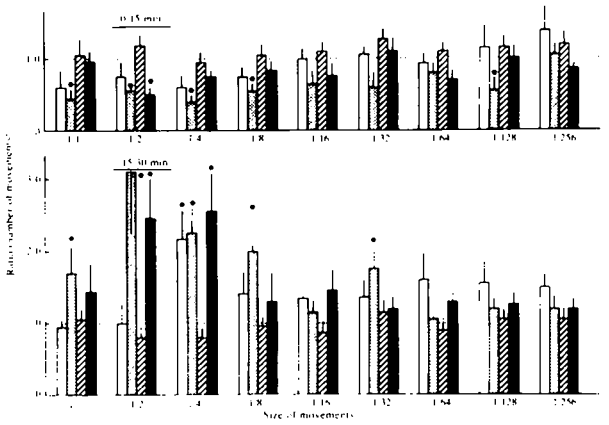

FIG. 5. Spontaneous movements in mice after [des-tyrosine^1]-γ-endorphin (DTγE) and the influence of naloxone. Values depict the mean±S.E. for 10 mice. Animals were pretreated with 1 mg/kg of naloxone at −10 min. *Denotes significant difference from saline control, $p<0.05$. -----: Saline, open bar: DTγE 20 μg/mouse, IC, stippled bar: 40 μg/mouse, IC, slanted line bar: naloxone 1 mg/kg, SC, solid bar: naloxone 1 mg/kg, SC + DTγE 40 μg/mouse, IC.

Effects of Leu-enkephalin on the Movement Spectrum

Within 15 min after injection ANOVA showed a significant effect in the following sizes of movement: $F(4,36)=10.34$, $p<0.01$ in 1/1 size, $F(4,36)=5.92$, $p<0.01$ in 1/2 size, $F(4,36)=3.41$, $p<0.05$ in 1/4 size, $F(4,36)=5.15$, $p<0.01$ in 1/8 size, $F(4,36)=4.39$, $p<0.01$ in 1/16 size, $F(4,36)=5.15$, $p<0.01$ in 1/32 size and $F(4,36)=2.99$, $p<0.05$ in 1/64 size. Leu-enkephalin at the dose of 100 μg caused a significant decrease in most movement sizes other than 1/128 and 1/256 sizes of movement within 15 min after injection (Fig. 6). This decrease also appeared within 15–30 min. ANOVA showed a significant effect in the following sizes of movement: $F(4,36)=3.32$, $p<0.05$ in 1/1 size, $F(4,36)=6.83$, $p<0.01$ in 1/2 size, $F(4,36)=5.09$, $p<0.01$ in 1/4 size, $F(4,36)=4.26$, $p<0.01$ in 1/16 size, $F(4,36)=2.88$, $p<0.05$ in 1/32 size and $F(4,36)=4.49$, $p<0.01$ in 1/64 size. The drug at a 200 μg dose resulted in a significant enhancement of the 1/1 size of movement within 15 min after injection. The significant increase (0–15 min) and the subsequent decrease (15–30 min) in the 1/1 size of movement induced by 200 μg of leu-enkephalin were significantly reversed by 1 mg/kg of naloxone.

Effects of Met-Enkephalin on the Movement Spectrum

Within 15 min after injection ANOVA indicated a significant effect in the following sizes of movement: $F(4,36)=16.3$, $p<0.01$ in 1/1 size, $F(4,36)=2.98$, $p<0.05$ in 1/2 size, $F(4,36)=3.35$, $p<0.05$ in 1/4 size, $F(4,36)=4.48$, $p<0.01$ in 1/8 size, $F(4,36)=3.3$, $p<0.05$ in 1/16 size, $F(4,36)=4.03$, $p<0.01$ in 1/32 size, $F(4,36)=2.99$, $p<0.05$ in 1/64 size, $F(4,36)=3.46$, $p<0.05$ in 1/128 size and, $F(4,36)=4.85$, $p<0.01$ in 1/256 size. Met-enkephalin markedly enhanced the 1/1 size of movement within 15 min after a 100 μg injection (Fig. 7). On the contrary, a 200 μg dose of the drug decreased most movement sizes, except for the significant increase in the 1/1 size within 15 min after injection. Within 15–30 min ANOVA also revealed a significant effect in the following sizes of movements: $F(4,36)=4.09$, $p<0.01$ in 1/1 size, $F(4,36)=3.63$, $p<0.05$ in 1/2 size, $F(4,36)=3.68$, $p<0.05$ in 1/4 size, $F(4,36)=3.98$, $p<0.05$ in 1/16 size, $F(4,36)=5.41$, $p<0.01$ in 1/32 size, $F(4,36)=3.89$, $p<0.05$ in 1/64 size, $F(4,36)=4.71$, $p<0.01$ in 1/128 size and $F(4,36)=4.72$, $p<0.01$ in 1/256 size. A 200 μg dose of the drug decreased most movement sizes within 15–30 min as compared with the saline-treated group. The increase in the 1/1 size of movement induced by met-enkephalin (100 μg) was significantly antagonized by naloxone (1 mg/kg).

Effects of D-Ala^2 on the Movement Spectrum

Within 15 min after injection ANOVA revealed a significant relation in the following sizes of movement:

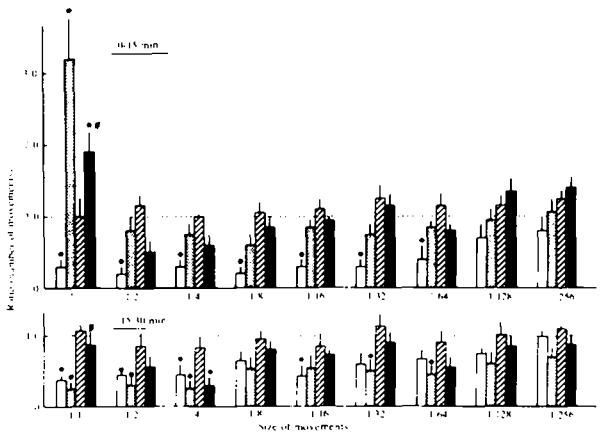

FIG. 6. Spontaneous movements in mice after leucine-enkephalin and the influence of naloxone. Values depict the mean±S.E. for 10 mice. Animals were pretreated with 1 mg/kg of naloxone at −10 min. *Denotes significant difference from saline control, $p<0.05$. #Denotes significant difference from corresponding leucine-enkephalin (200 μg) treated group, $p<0.05$. -----: Saline, open bar: leucine-enkephalin 100 μg/mouse, IC, stippled bar: 200 μg/mouse, IC, slanted line bar: naloxone 1 mg/kg, SC, solid bar: naloxone 1 mg/kg, SC + leucine-enkephalin 200 μg/mouse, IC.

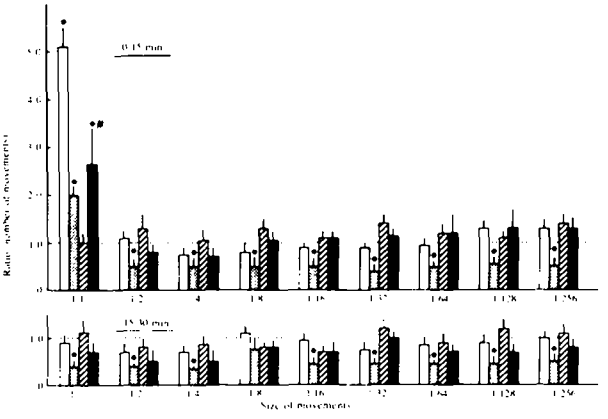

FIG. 7. Spontaneous movements in mice after methionine-enkephalin and the influence of naloxone. Values depict the mean±S.E. for 10 mice. Animals were pretreated with 1 mg/kg of naloxone at −10 min. *Denotes significant difference from saline control, $p<0.05$. #Denotes significant difference from corresponding methionine-enkephalin (100 μg) treated group, $p<0.05$. -----: Saline, open bar: methionine-enkephalin 100 μg/mouse, IC, stippled bar: 200 μg/mouse, IC, slanted line bar: naloxone 1 mg/kg, SC, solid bar: naloxone 1 mg/kg, SC + methionine-enkephalin 100 μg/mouse, IC.

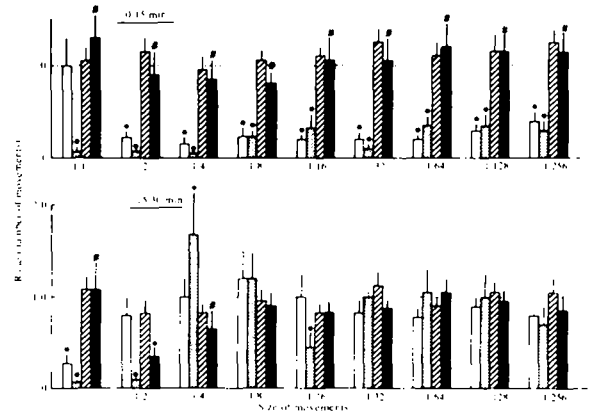

FIG. 8. Spontaneous movements in mice after [d-ala²]-methionine-enkephalinamide and the influence of naloxone. Values depict the mean±S.E. for 10 mice. Animals were pretreated with 1 mg/kg of naloxone at −10 min. *Denotes significant difference from saline control, $p<0.05$. #Denotes significant difference from corresponding [d-ala²]-methionine-enkephalinamide treated group, $p<0.05$. -----: Saline, open bar: [d-ala²]-methionine-enkephalinamide 10 μg/mouse, IC, stippled bar: 20 μg/mouse, IC, slanted line bar: naloxone 1 mg/kg, SC, solid bar: naloxone 1 mg/kg, SC + [d-ala²]-methionine-enkephalinamide 20 μg/mouse, IC.

$F(4,36)=3.12$, $p<0.05$ in 1/1 size, $F(4,36)=10.01$, $p<0.01$ in 1/2 size, $F(4,36)=10.49$, $p<0.01$ in 1/4 size, $F(4,36)=9.86$, $p<0.01$ in 1/8 size, $F(4,36)=6.3$, $p<0.01$ in 1/16 size, $F(4,36)=9.65$, $p<0.01$ in 1/32 size, $F(4,36)=6.87$, $p<0.01$ in 1/64 size, $F(4,36)=7.28$ in 1/128 size and $F(4,36)=7.11$, $p<0.01$ in 1/256 size. Within 15 min after a 10 μg dose injection, D-Ala² significantly decreased most movement sizes except for the 1/1 (Fig. 8). A 20 μg dose of the drug caused a marked decrease in all sizes of movement. Within 15–30 min ANOVA revealed a significant effect in the following sizes of movement: $F(4,36)=5.31$, $p<0.01$ in 1/1 size, $F(4,36)=3.99$, $p<0.05$ in 1/2 size, $F(4,36)=6.35$, $p<0.01$ in 1/4 size and $F(4,36)=3.8$, $p<0.05$ in 1/16 size. Restoration of the smaller movement sizes up to the control level resulted within 15–30 min after injecting 10 and 20 μg doses. On the contrary, when the drug was injected at a dose of 20 μg, a significant increase in the 1/4 size of movement was noted (Fig. 8). The D-Ala² (20 μg)-induced behavioral change was blocked by treatment with 1 mg/kg of naloxone.

DISCUSSION

The present results show that γ-endorphin, leu- and met-enkephalins similarly increased the 1/1 size of movement (linear locomotion), unlike β-endorphin which reduced most of the movement sizes (depression of almost all activities). In addition, almost all the movement spectra induced by morphine and the endorphins except for DTγE were antagonized by the injection of naloxone (1 mg/kg). The inability of naloxone to antagonize the DTγE-induced increase in 1/2, 1/4 and 1/8 sizes of movement (rearing behavior) within 15–30 min after intracerebral injection was in good agreement with the results reported by De Wied *et al.* [6]. It is possible then that the opiate receptors play an important role in the individual behavioral patterns induced by morphine and endorphins. On the contrary, it is noted that naloxone plus morphine actually produced a significant decrease in the 1/1 size of movement during the first 15 min period as compared with the saline-treated group. Although the earlier findings support the hypothesis that the effects of naloxone are due to the blockade of opiate receptors, there are an increasing number of reports which indicate that naloxone may have pharmacological actions unrelated to opiate receptor blockade [21]. The significant decrease in the 1/1 size of movement induced by morphine plus naloxone may be due to the non-specific effects of naloxone.

It is well known that D-Ala² which is resistant to the enkephalin-degrading enzymes *in vivo* exerts a stronger an-

algesic action than met-enkephalin [17]. However, the present results indicate that the behavioral effect of D-Ala^2 is qualitatively different from that of met-enkephalin as exploited by the Animex II. The results that D-Ala^2 (10 and 20 μg), like β-endorphin, decreases most of the movement sizes within 15 min after intracerebral injection support the evidence reported by Segal *et al.* [22]. The behavioral evidence regarding met-enkephalin and D-Ala^2 is in good agreement in that the effects of these peptides on the metabolism of dopamine and norepinephrine in the rat brain are each different [1,4]. However, Segal *et al.* [22] have demonstrated that D-Ala^2 produces a biphasic effect with increases in motility 2–4 hr after intracerebral administration. In this experiment, behavior was observed for 30 min after intracerebral administration, since the direct pharmacological effects of drugs seem to be revealed rather rapidly when drugs are administered intracerebrally. Therefore, a biphasic effect of D-Ala^2 with increases in activity 2–4 hr after intracerebral administration reported by Segal *et al.* [22] might be one of the indirect actions of D-Ala^2. Moreover, we showed a relatively limited dose-range of compounds, because the doses presented could exert the most specific behavioral patterns and biochemical aspects [14,15] among the previously tested doses.

According to the statistically significant differences in the movement sizes of mice using the multi-dimensional behavioral analyser (Animex II), the psychopharmacological action of morphine, endorphins and DTγE can be readily classified into: (1) enkephalin group (leu- and met-enkephalins) (2) β-endorphin group (β-endorphin), (3) γ-endorphin group (γ-endorphin), (4) morphine group (morphine), (5) DTγE group (DTγE) and (6) D-Ala^2 group (D-Ala^2). This classification includes consideration of the onset and duration of action, whether each of the movements divided into nine sizes is increased or decreased and whether naloxone reverses the effects induced by the tested compounds. The previous data indicate that α-endorphin should be classified into the γ-endorphin group, since α-endorphin causes a marked increase in the 1/1 size of movement (mainly linear locomotion) within 15 min after administration [13]. It is of interest that each of these peptides shows a specific effect on the movement patterns of animals.

It has been reported that α-endorphin (20 μg) decreases the content of homovanillic acid (HVA) in the striatum and that γ-endorphin (10 μg) decreases the contents of dopamine (DA), 3,4-dihydroxyphenylacetic acid and HVA, while β-endorphin (1 and 2 μg) has no effects on the DA metabolism in the mouse brain [15]. Additionally, the changes in the DA metabolism induced by α- and γ-endorphins are readily reversed by treatment with naloxone (1 mg/kg) [15]. The evidence that the biochemical features of α- and γ-endorphins are similar to each other is compatible with the results that they produce similar behavioral patterns.

It is likely that the different behavioral effects induced by the opioid peptides is associated with the stimulation of different opioid receptors. It is known that multiple opiate receptors such as μ-, κ-, σ-, δ- and ϵ-receptors possess a relatively specific ligand: morphine, ketocyclazocine, a benzomorphan derivative (SKF-10047) and nalorphine, enkephalins, and β-endorphin, respectively, and that each of them differs in various brain and peripheral tissues [5,27]. In addition the interaction between these receptors and the corresponding ligands elicits specific pharmacological profiles [12]. Unfortunately, the behavioral profile of the receptors is not well known. In particular, the psychopharmacological function of δ- and ϵ-receptors still remains unclear [26]. From the present results, it appears that the qualitative differences in the behavior induced by morphine and endorphins, except for DTγ-E which is not associated with opiate receptors, reflect the degree of affinity for each of the specific opiate receptors in the brain. Additionally, the increase in 1/1 size of movement (linear locomotion) induced by leu- and met-enkephalins as well as α- and γ-endorphins may be elicited through the mediation of the δ-receptor, while β-endorphin-induced behavioral patterns (significant decrease in spontaneous locomotor activities) by the ϵ-receptor. Wood [26] has described that D-Ala^2 acts on both δ- and μ-receptors in the brain. The finding that the D-Ala^2-induced behavioral patterns are somewhat different from the other tested ligands suggests that the behavioral patterns induced by D-Ala^2 are both δ- and μ-receptors-mediated.

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