Pharmacology Biochemistry & Behavior, Vol. 20, pp. 815–818, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

BRIEF COMMUNICATION

Naloxone Reverses the Inhibitory Effects of Dynorphin A on Motor Activity in the Mouse

MAKOTO UKAI, SHIGEYUKI YAMADA AND TSUTOMU KAMEYAMA[1]

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan

Received 15 August 1983

UKAI, M., S. YAMADA AND T. KAMEYAMA. *Naloxone reverses the inhibitory effects of dynorphin A on motor activity in the mouse.* PHARMACOL BIOCHEM BEHAV **20**(5) 815–818, 1984.—Dynorphin-(1–17) (dynorphin A) significantly reduced the linear locomotion, rearing and grooming behaviors in mice using a newly devised multi-dimensional behavioral analyser. The behaviors inhibited by dynorphin A were dose-dependently antagonized by prior treatment with naloxone. The results suggest that dynorphin A-induced behavioral depression is mediated via opioid receptors in the mouse brain.

Dynorphin A Naloxone Behaviors Mouse

IT has been reported that dynorphin A, a newly identified opioid peptide in the porcine pituitary [1] and in the duodenum [10], is the most potent opioid in inhibiting electrically stimulated contractions of peripheral tissues such as the guinea pig ileum and mouse vas deferens [1]. Ukai and Kameyama [11] have previously demonstrated that dynorphin-(1–13)[D13A], one of the dynorphin A fragments, markedly increases linear locomotion and grooming behaviors in mice and those effects are antagonized by pretreatment with naloxone. More recently, Walker *et al.* [12] have found that dynorphin-(1–17), the full sequence of dynorphin A, consistently inhibits hippocampal unit activity in rats, and these effects are not antagonized by pretreatment with high doses of naloxone. Although Herman [3] has compared the effects (antinociception, motor) of spinally administered dynorphin A with those of D13A in rats, there is little information as to the behavioral effects of dynorphin A in mice.

It has previously been shown that α-, β-, γ- and [des-tyrosine[1]]-γ-endorphins, leucine-, methionine-enkephalins as well as [d-alanine[2], methionine[5]]-enkephalinamide have different behavioral patterns, as revealed by a multi-dimensional behavioral analyser (Animex II) which classifies animals' movements into nine degrees according to amplitude measures [6,7]. We have also found that these behavioral changes elicited by endorphins are correlated with alterations of dopamine metabolism in the mouse brain [8].

In the present experiment, Animex II was used to analyse the behavioral effects of dynorphin A administered to mice. It was also determined if the behavioral patterns elicited by dynorphin A are mediated by opioid receptors in the mouse brain.

METHOD

Animals

Male ddY mice (Shizuoka Experimental Animal Agricultural Cooperative Association, Japan) weighing between 18 and 25 g were used in the experiments. The mice were given free access to food and water, and housed in individual cages in a temperature-controlled room with a 12-hr light-dark cycle (lights on 0800 to 2000 hr).

Drugs and Treatments

The following drugs were used: dynorphin A (Protein Research Foundation, Japan) and naloxone hydrochloride (Sankyo Co. Ltd. Japan). Dynorphin A was dissolved in physiological saline in polypropylene containers and given intracerebrally. The unilateral injection site was 2 mm from either side of the midline on a line drawn through the anterior roots of the ears [2]. The injection was made with a 3 mm long needle attached to a 25 μl Hamilton microsyringe. The needle was inserted perpendicularly through the skull and into

[1]Requests for reprints should be addressed to T. Kameyama.

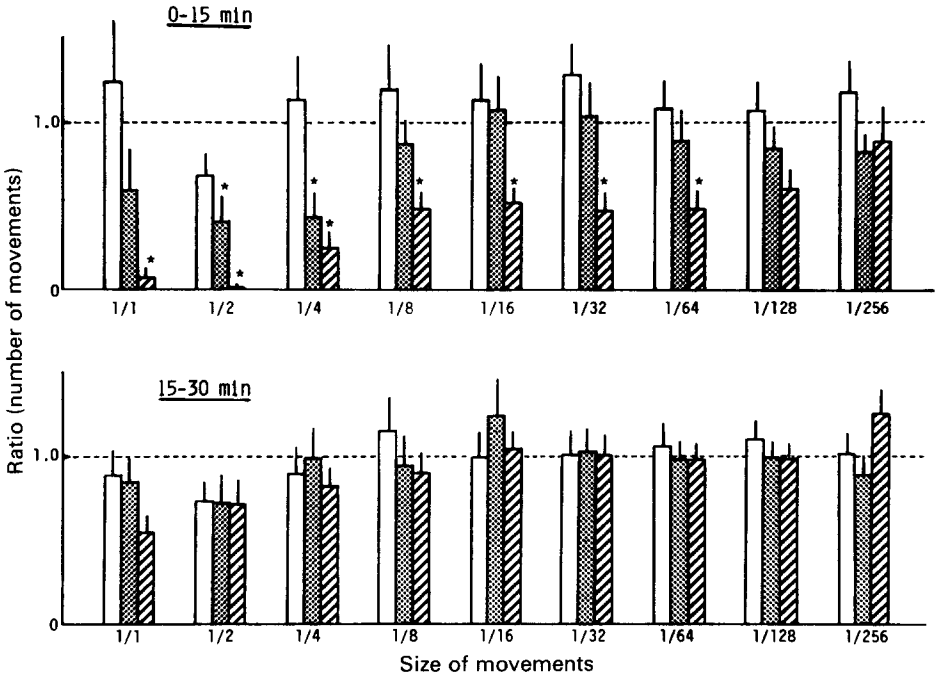

FIG. 1. Spontaneous movements in mice after dynorphin A. Values depict the mean±S.E. for 10 mice. Within 15 min after injection, ANOVA revealed a significant relation in the following sizes of movement: $F(3,27)=3.81$, $p<0.05$ in 1/1 size, $F(3,27)=7.62$, $p<0.01$ in 1/2 size, $F(3,27)=5.63$, $p<0.01$ in 1/4 size, $F(3,27)=3.06$, $p<0.05$ in 1/8 size, $F(3,27)=3.89$, $p<0.05$ in 1/16 size, $F(3,27)=3.39$, $p<0.05$ in 1/32 size and $F(3,27)=3.14$, $p<0.05$ in 1/64 size. *Denotes significant difference from saline control, $p<0.05$. -------: Saline, open column: dynorphin A 1 μg/mouse, IC, dotted column: 3 μg/mouse, IC, striped column: 10 μg/mouse, IC.

the brain of the unanaesthetized mouse in a volume of 10 μl per mouse over a period of 15 sec as previously described [6,7]. The site was checked by injecting a 1:10 dilution of Indian ink in isotonic saline (0.9% NaCl, pH 7.5). Histological examinations revealed particles of the ink in the lateral and 3rd ventricles but not in the others. As previously described [7], neither insertion of the needle nor injection of 10 μl of isotonic saline solution had a significant influence on behavior.

Procedure

Behavioral measurements were made for 30 min immediately after the peptide administration. The Animex II equipped with an electric microcomputer, was used for the measurements and permitted comparison of activities among the animals with different weight. The sensor consisted of three pairs of electrodes and formed a capacitor bridge. Once a mouse 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 a digital form, the DC-voltage movements 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 a drug were registered on the counters as follows, linear locomotion on 1/1 and 1/2, rearing and circling on 1/4 and 1/8, grooming on 1/16, 1/32 and 1/64 and convulsion on 1/128 [6]. The sensitivity (%) of the device depended on the body weight of each mouse as follows, 18 g=29%, 19 g=28%, 20–21 g=27%, 22–23 g=26% and 24–25 g=25%.

The ordinate in the figures was labelled "Ratio (number of movements)." This ratio was derived using the formula: Ratio (number of movements)=(value of drug-treated animal)/(mean value of controls).

Statistical Analysis

The results were analysed by a one-factor analysis of variance (ANOVA). This was followed by Newman-Keuls tests. A p value of less than 0.05 was taken as the level of statistical significance.

RESULTS

Spontaneous Movements in Mice after Dynorphin A

Within 15 min after injection, a 3 μg dose of dynorphin A significantly decreased the 1/2 and 1/4 sizes of movement (linear locomotion, rearing and circling) (Fig. 1). In addition, a 10 μg dose of dynorphin A significantly inhibited most of

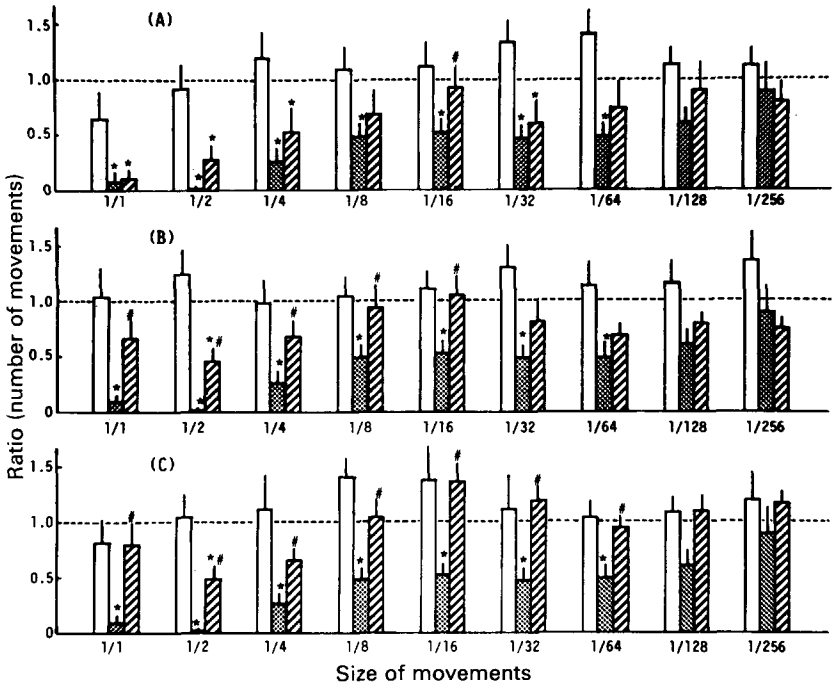

FIG. 2. Spontaneous movements in mice within 15 min after dynorphin A injection and the influence of naloxone. Values depict the mean±S.E. for 10 mice. Animals were pretreated with 0.5, 1 and 2 mg/kg of naloxone at −10 min. (A) ANOVA revealed a significant relation in the following sizes of movement: $F(3,27)=7.89$, $p<0.01$ in 1/1 size, $F(3,27)=6.03$, $p<0.01$ in 1/2 size, $F(3,27)=6.37$, $p<0.01$ in 1/4 size, $F(3,27)=3.05$, $p<0.05$ in 1/8 size, $F(3,27)=3.52$, $p<0.05$ in 1/16 size, $F(3,27)=6.55$, $p<0.01$ in 1/32 size and $F(3,27)=5.34$, $p<0.01$ in 1/64 size. -------: Saline, open column: naloxone 0.5 mg/kg, SC, dotted column: dynorphin A 10 μg/mouse, IC, striped column: naloxone 0.5 mg/kg, SC + dynorphin A 10 μg/mouse, IC. (B) ANOVA showed a significant relation in the following sizes of movement: $F(3,27)=5.62$, $p<0.01$ in 1/1 size, $F(3,27)=12.37$, $p<0.01$ in 1/2 size, $F(3,27)=5.18$, $p<0.01$ in 1/4 size, $F(3,27)=4.01$, $p<0.01$ in 1/8 size, $F(3,27)=4.61$, $p<0.01$ in 1/16 size, $F(3,27)=5.34$, $p<0.01$ in 1/32 size and $F(3,27)=4.96$, $p<0.01$ in 1/64 size. -------: Saline, open column: naloxone 1 mg/kg, SC, dotted column: dynorphin A 10 μg/mouse, IC, striped column: naloxone 1 mg/kg, SC + dynorphin A 10 μg/mouse, IC. (C) ANOVA revealed a significant effect in the following sizes of movement: $F(3,27)=5.33$, $p<0.01$ in 1/1 size, $F(3,27)=8.36$, $p<0.01$ in 1/2 size, $F(3,27)=4.97$, $p<0.01$ in 1/4 size, $F(3,27)=7.13$, $p<0.01$ in 1/8 size, $F(3,27)=5.11$, $p<0.01$ in 1/16 size, $F(3,27)=3.4$, $p<0.05$ in 1/32 size and $F(3,27)=5.05$, $p<0.01$ in 1/64 size. -------: Saline, open column: naloxone 2 mg/kg, SC, dotted column: dynorphin A 10 μg/mouse, IC, striped column: naloxone 2 mg/kg, SC + dynorphin A 10 μg/mouse, IC. *Denotes significant difference from saline control, $p<0.05$. #Denotes significant difference from dynorphin A (10 μg), $p<0.05$.

the movement sizes other than the 1/128 and 1/256 sizes of movement. Fifteen to 30 min, however, 1, 3 and 10 μg doses of dynorphin A had no significant effects on the movement sizes in mice.

Spontaneous Movements in Mice within 15 min After Dynorphin A Injection and the Influence of Naloxone

A 0.5 mg/kg dose of naloxone reversed only the decrease in the 1/16 size of movement induced by a 10 μg dose of dynorphin A (Fig. 2A). A dose of 1.0 mg/kg of naloxone antagonized the dynorphin A (10 μg)-induced decrease in the 1/1, 1/2, 1/4, 1/8 and 1/16 sizes of movement but not the decrease in the 1/32 and 1/64 sizes (Fig. 2B). In addition, a 2 mg/kg dose of naloxone completely reversed the dynorphin A-induced decrease in the 1/1, 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64 sizes of movement (Fig. 2C).

DISCUSSION

It has been suggested that dynorphin A has two active sites in the amino acid sequence which consists of (1) an opioid active site, and (2) a non-opioid active site [12]. Walker *et al.* [12] have reported that dynorphin A consistently inhibits hippocampal unit activity, and that a high dose of naloxone fails to reverse this effect. This effect may be modulated by the non-opioid active site. It is also conceivable that the effects of D13A on monoamine neurotransmission are only partly mediated by endogenous opioid binding sites in the brain [9]. Herman *et al.* [5] have demonstrated

that a relatively high dose (100 nmol, 160 μg) of D13A elicits catalepsy (platform immobility, righting loss) and that naloxone is less effective in antagonizing the cataleptic effects of D13A than of D-ala^2-dynorphin-(1–11) or β_c-endorphin. Thus, they suggest that catalepsy may be mediated by more than one opioid receptor in the brain. D13A catalepsy is reportedly mediated by an opioid receptor other than the μ opiate receptor in the brain [4]. In the present experiment, dynorphin A (10 μg) significantly inhibited the 1/1, 1/2 (linear locomotion), 1/4, 1/8, 1/16 (rearing and circling), 1/32, 1/64 (grooming) sizes of movement in mice using a multi-dimensional behavioral analyser (Animex II). In addition, the behavioral depressions induced by dynorphin A (10 μg) were readily antagonized by low doses of naloxone (0.5, 1 and 2 mg/kg). The results suggest that the behavioral depressions induced by dynorphin A are mediated by opioid receptors in the mouse brain, unlike the electrophysiological effects reported by Walker *et al.* [12].

On the other hand, we have previously demonstrated that lower doses of D13A significantly increase linear locomotion within 15–30 min after injection [11]. The reason for the different behavioral patterns between D13A and dynorphin A is not known.

The dynorphin A-induced behavioral changes that we have observed involve an opioid-sensitive neural substrate, since these effects are blocked by naloxone. Similar results in rats have been obtained by Zwiers *et al.* [13] with D13A.

ACKNOWLEDGEMENTS

This research was supported by a Grant No. 57771539 from the Ministry of Education. Naloxone was kindly provided by Sankyo Co., Ltd., Japan. We thank Mr. K. Fukaya for his technical assistance in preparing the figures.

REFERENCES

1. Goldstein, A., S. Tachibana, L. I. Lowney, M. Hunkapiller and L. Hood. Dynorphin-(1–13), an extraordinarily potent opioid peptide. *Proc Natl Acad Sci USA* **76:** 6666–6670, 1979.
2. Haley, T. J. and W. G. McCormick. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br J Pharmacol* **12:** 12–15, 1957.
3. Herman, B. H. Intrathecal dynorphin induces antinociception and paralysis. *Soc Neurosci Abstr* **8:** 1982.
4. Herman, B. H. and A. Goldstein. Cataleptic effects of dynorphin-(1–13) in rats made tolerant to a mu opiate receptor agonist. *Neuropeptides* **2:** 13–22, 1981.
5. Herman, B. H., F. Leslie and A. Goldstein. Behavioral effects and in vivo degradation of intraventricularly administered dynorphin-(1–13) and D-ala^2-dynorphin-(1–11) in rats. *Life Sci* **27:** 883–892, 1980.
6. Kameyama, T. and M. Ukai. Multi-dimensional analyses of behavior in mice treated with α-endorphin. *Neuropharmacology* **20:** 247–250, 1981.
7. 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:** 671–677, 1983.
8. Kameyama, T., M. Ukai, S. Noma and M. Hiramatsu. Differential effects of α-, β- and γ-endorphins on dopamine metabolism in the mouse brain. *Brain Res* **244:** 305–309, 1982.
9. Kovacs, G. L. and G. Telegdy. Effects of intraventricular dynorphin treatment on brain monoamines. *Neuropeptides* **1:** 371–375, 1981.
10. Tachibana, S., K. Araki, S. Ohya and S. Yoshida. Isolation and structure of dynorphin, an opioid peptide from porcine duodenum. *Nature* **295:** 339–340, 1982.
11. Ukai, M. and T. Kameyama. The antagonistic effects of naloxone on dynorphin-(1–13)-induced hypermotility in mice using a multi-dimensional behavioral analysis. *Neuropharmacology*, in press, 1984.
12. Walker, J. M., H. C. Moises, D. H. Coy, G. Baldrighi and H. Akil. Nonopiate effects of dynorphin and des-tyr-dynorphin. *Science* **218:** 1136–1138, 1982.
13. Zwiers, H., V. J. Aloyo and W. H. Gispen. Behavioral and neurochemical effects of the new opioid peptide dynorphin-(1–13): comparison with other neuropeptides. *Life Sci* **28:** 2545–2551, 1981.