Pharmacology Biochemistry & Behavior, Vol. 35, pp. 231–235. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Inhibitory and Stimulatory Effects of Morphine on Locomotor Activity in Mice: Biochemical and Behavioral Studies

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Received 11 May 1989

SAITO, H. *Inhibitory and stimulatory effects of morphine on locomotor activity in mice: Biochemical and behavioral studies.* PHARMACOL BIOCHEM BEHAV **35**(1) 231–235, 1990.—A possible interaction between the opposite effect (inhibition and stimulation) of morphine on locomotor activity in mice and monoaminergic systems in the striatum was studied. Ten minutes after systemic administration, morphine at 1.25 mg/kg decreased locomotor activity and the levels of 3-methoxytyramine (3-MT), whereas at 20 mg/kg locomotor activity and 3-MT levels increased. At the same time, no change in the other monoamine metabolite (DOPAC, HVA, MHPG, and 5-HIAA) levels was observed. Sixty minutes after administration, morphine at 1.25 mg/kg did not induce any change in locomotor activity or in all the monoamine metabolite levels measured. On the other hand, morphine at 20 mg/kg maintained an initial increase in locomotor activity and increased not only 3-MT levels, but also other metabolite (DOPAC, HVA, MHPG, and 5-HIAA) levels. These results suggest that, at low dosages, the inhibitory effect of morphine on locomotor activity in mice may be related to a decrease of the presynaptic dopamine release in the striatum and that the stimulatory effect of morphine, at high dosages, may be related to an increase of the presynaptic dopamine release in the striatum.

Inhibition Stimulation Locomotor activity Morphine Mice Striatum Monoamine-related substrates

MANY investigators have shown that morphine causes a dose-related opposite effect (inhibition and stimulation) or a time-related biphasic effect (initial inhibition followed by stimulation) on locomotor activity in rats (3, 6, 8) and hamsters (21). Nevertheless, on locomotor activity in mice, only a few reports have succeeded in detection of an opposite (4, 11, 12) or a biphasic (11,24) effect of morphine on locomotor activity, since morphine generally induces stimulated locomotor activity termed "running- fit," over a wide dose range in this species (20,22). As regards the stimulatory effect of morphine on locomotor activity in mice, several neurochemical investigations have indicated the participation of the central monoaminergic nervous systems in the striatum (18, 19, 29). But the neurochemical mechanisms by which the inhibitory effect of morphine is mediated have not yet been clarified.

In the present study, a possible interaction between the dose-related opposite effect of morphine on locomotor activity in mice and the central dopaminergic (DA), noradrenergic (NE) and serotonergic (5-HT) systems in the striatum was investigated.

METHOD

Animals

Male mice of ddy strain, weighing 25–28 g, were used in the experiments. The mice were housed in our animal room, maintained under a 12-hr light-dark cycle at 23 ± 2°C, and were given food and tap water ad lib. Mice were used only once and experiments were always carried out between 9:00 and 13:00.

Locomotor Activity

The horizontal spontaneous locomotor activity in mice was measured using our infrared ray photo-coupler counter as described previously (12). Briefly, the apparatus consisted of a plastic box (16 cm wide × 29 cm deep × 30 cm high), and locomotor activity was counted by an infrared photo-coupler mounted at a height of 1.8 cm. Immediately after injection of a 0.9% physiological saline solution (saline) or morphine (0.32–20 mg/kg, SC), the mice were placed in the plastic box and their cumulative counts for locomotor activity were measured every 10 min for 60 min.

Biochemistry

The levels of monoamine-related substrates in the striatum were measured by our method (15) using high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Briefly, mice were killed 10 and 60 min after saline or morphine administration by immersing into dry ice-ethanol solution to minimize the postmortem increase of 3-methoxytyramine (3-MT) in the striatum. The striatum was isolated from the nearly frozen brain on a cooled glass plate according to a modification of the method of Glowinski and Iversen (10), weighed and stored at −80°C until extraction. The frozen tissues were homogenized

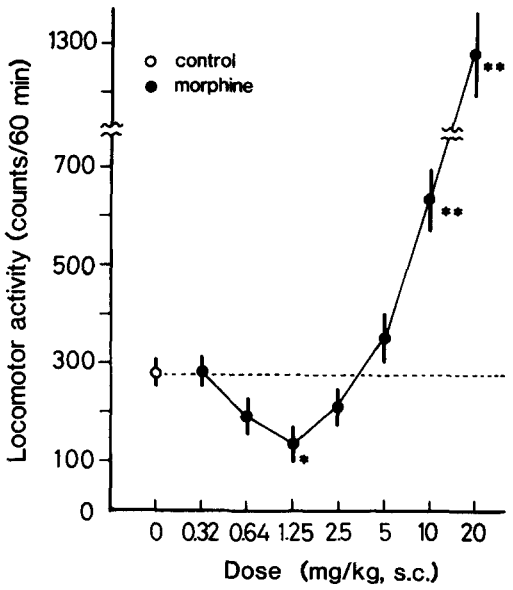

FIG. 1. Dose-effects of morphine on locomotor activity in mice. Points and vertical bars represent mean ± S.E. (n = 8). Asterisks indicate the significance of differences from controls (*$p<0.05$; **$p<0.01$).

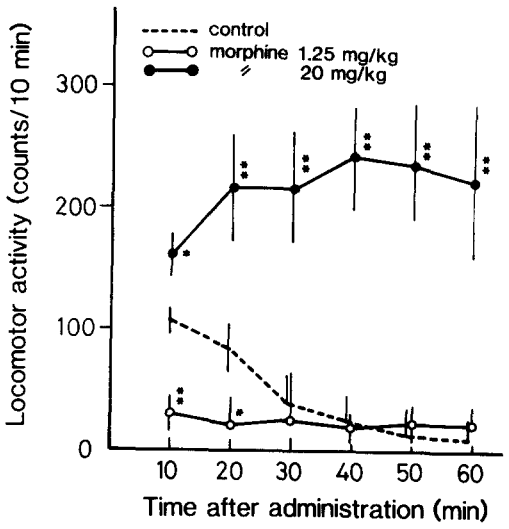

FIG. 2. Time-effects of morphine on locomotor activity in mice. Points and vertical bars represent mean ± S.E. (n = 8). Asterisks represent the significance of differences from controls (*$p<0.05$; **$p<0.01$).

with an ultrasonic cell disruptor (Model 200, Branson, CT) in 200 μl of 0.1 M perchloric acid containing 0.1 mM EDTA. Homogenates were centrifuged at 12,000 × g for 10 min at 4°C and the supernatants were decanted and filtered through a 0.45 μm filter. Thirty μl of filtrates were injected onto the HPLC system. The HPLC system consisted of a delivery pump (L-5000, Yanagimoto, Japan), an analytical column (EICOMPAK, 250 × 4.6 mm, Eicom, Japan) and a guard column (Eicom). The electrochemical detector (VMD-501, Yanagimoto) with a graphite electrode (WE-3G, Eicom) was used at a voltage setting of +0.83 V vs. an Ag/AgCl reference electrode. The mobile phase was 0.02 M sodium acetate/0.0125 M citric acid buffer, pH 3.92, containing 16% methanol, 0.033% heptanesulfonic acid and 0.1 mM EDTA. The flow rate was set to 2.1 ml/min with a column temperature of 23°C.

Drugs

Morphine hydrochloride (Takeda Yakuhin, Japan) was dissolved in saline. The injection volume was 0.05 ml/10 g body weight.

Statistics

The behavioral data were statistically analyzed by one-way analysis of variance (for dose-effects of morphine) or two-way analysis of variance (for time-effects of morphine) followed by Dunnett test. The biochemical data were analyzed by one-way analysis of variance followed by Scheffé test.

RESULTS

Effect of Morphine on Locomotor Activity

Figure 1 shows the mean of locomotor activity for 60 min after the beginning of the test in saline and morphine-treated mice. Morphine clearly induced a dose-related opposite effect on locomotor activity in mice. That is, when compared with saline controls, morphine at 1.25 mg/kg significantly decreased locomotor activity and at 10 and 20 mg/kg significantly increased locomotor activity. Figure 2 shows the effect of morphine at 1.25 and 20 mg/kg on locomotor activity recorded every 10 min after the beginning of the test for 60 min. Morphine at 1.25 mg/kg significantly decreased locomotor activity only for the first and the second period of the 10-min recordings, when compared with the saline controls. Morphine at 20 mg/kg produced a significant increase in locomotor activity at the first 10-min period and this increase was maintained for the duration of the test (60 min).

Effect of Morphine on the Levels of 10 Monoamine-Related Substrates

On the basis of the results shown in Fig. 2, the effect of morphine at 1.25 and 20 mg/kg on the levels of ten DA, NE and 5-HT-related substrates in the striatum was examined 10 and 60 min after administration. Ten minutes after administration, morphine at 1.25 mg/kg significantly decreased only the 3-MT levels, whereas morphine at 20 mg/kg significantly increased only the 3-MT levels. No change in the levels of the other monoamine-related substrates, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA), was observed. These results are shown in Table 1. Sixty minutes after administration, morphine at 1.25 mg/kg induced no changes in all monoamine-related substrate levels studied, whereas morphine at 20 mg/kg significantly increased not only 3-MT levels, but also DOPAC, HVA, MHPG, and 5-HIAA levels. These results are shown in Table 2.

DISCUSSION

The present results demonstrate that acute morphine caused a dose-dependent opposite effect on both locomotor activity and the levels of 3-MT: ten minutes after administration, morphine at 1.25 mg/kg decreased locomotor activity and only 3-MT levels among 10 monoamine-related substrates measured in the striatum, whereas morphine at 20 mg/kg increased locomotor activity and only 3-MT levels in the striatum. The early studies (9, 14, 23) have reported that during the morphine-induced hypermotility period, the levels

TABLE 1

EFFECT OF MORPHINE ON THE LEVELS OF MONOAMINE-RELATED SUBSTRATES IN THE MOUSE STRIATUM

	10 Min After Administration			
Substrates	Saline	Mor 1.25[a]	Saline	Mor 20[a]
Tyrosine	12066 ± 968[b]	11134 ± 818	11468 ± 1050	11431 ± 221
DA	13048 ± 1068	13069 ± 937	12617 ± 1555	12125 ± 1018
DOPAC	1023 ± 120	963 ± 48	1082 ± 195	1075 ± 108
HVA	1477 ± 303	1600 ± 109	1449 ± 201	1457 ± 221
3-MT	113 ± 12	90 ± 9*	108 ± 10	146 ± 9*
NE	46 ± 8	50 ± 14	44 ± 8	43 ± 7
MHPG	45 ± 4	45 ± 4	43 ± 7	43 ± 4
Tryptophan	3576 ± 416	3331 ± 323	2919 ± 283	2660 ± 192
5-HT	704 ± 108	753 ± 71	802 ± 159	684 ± 42
5-HIAA	634 ± 114	681 ± 80	632 ± 34	732 ± 110

[a]mg/kg. [b]Values (ng/g wet tissue weight) represent means ± S.D., n=8.
*$p<0.01$ (compared to saline controls, Scheffé test). Mor: morphine.

of 3-MT and dopamine release in the striatum of C57BL/6J mice increased. Furthermore, in DBA/2J mice, morphine induced neither stimulation of locomotor activity nor changes in 3-MT levels in the striatum (14, 18, 19). These findings, including the present results, suggest that effects of morphine on locomotor activity in mice are closely related to changes in 3-MT levels in the striatum.

Many investigators have used 3-MT as an indirect index of released DA from the nerve terminal (1, 17, 26). Although some doubt was cast on the significance of 3-MT (25), recent studies have shown that the decreased 3-MT levels reflect a decrease of presynaptic DA release in the striatum (25, 27, 28). Thus, the present results suggest that the initial inhibition of locomotor activity which was produced by a low dosage of morphine may be related to a decrease of the presynaptic DA release in the striatum.

Initial stimulation of locomotor activity produced by 20 mg/kg of morphine was accompanied by only the increase of 3-MT levels in the striatum of mice. Several authors (13,28) have pointed out that high dosages of morphine caused hypermotility by increasing the presynaptic DA release in the striatum. More recently, some authors (7) have reported that in the brain dialysis study, morphine-induced stimulation of the behavior including locomotion was associated to stimulation of DA release in the striatum and nucleus accumbens of the rat. Thus, the increase of 3-MT levels observed in the present study appears to reflect an increase of DA release in the striatum of mice. Presumably, initial stimulation of locomotor activity produced by high dosages of morphine may be related to an increase of presynaptic DA release in the striatum of mice.

The stimulating effect of morphine on locomotor activity was noted in the first 10-min period and maintained for 60 min after administration. Sixty minutes after administration, morphine at 20 mg/kg increased significantly not only 3-MT levels, but also DOPAC, HVA, MHPG and 5-HIAA levels in the striatum, when

TABLE 2

EFFECT OF MORPHINE ON THE LEVELS OF MONOAMINE-RELATED SUBSTRATES IN THE MOUSE STRIATUM

	60 Min After Administration			
Substrates	Saline	Mor 1.25[a]	Saline	Mor 20[a]
Tyrosine	10837 ± 2214[b]	9727 ± 1416	11630 ± 1364	11266 ± 678
DA	12380 ± 1031	12786 ± 1162	11582 ± 1102	11701 ± 595
DOPAC	894 ± 129	965 ± 161	971 ± 113	1233 ± 103†
HVA	1271 ± 188	1341 ± 190	1378 ± 78	1686 ± 157†
3-MT	119 ± 22	123 ± 17	93 ± 10	118 ± 24*
NE	57 ± 10	56 ± 13	51 ± 6	52 ± 10
MHPG	52 ± 5	43 ± 7	51 ± 15	78 ± 12*
Tryptophan	2507 ± 504	2323 ± 423	3121 ± 441	3732 ± 395
5-HT	650 ± 75	575 ± 87	742 ± 85	693 ± 160
5-HIAA	578 ± 64	657 ± 78	675 ± 56	839 ± 67†

[a]mg/kg. [b]Values (ng/g wet tissue weight) represent means ± S.D., n=8.
*$p<0.05$ and †$p<0.01$ (compared to saline controls, Scheffé test). Mor: morphine.

compared with those of the control mice. These results show that morphine at 20 mg/kg simultaneously increased the DA, NE and 5-HT turnover in the striatum. Several investigators have suggested that not only the DA system, but also the central NE or 5-HT systems are related to the neurochemical mechanisms of central effects of morphine at least in part (2,16). The present data at 60 min after administration agree with these neurochemical findings (2,16). However, it is not clear whether the striatal NE and 5-HT systems are also involved in the neurochemical mechanisms related to the stimulating effect of morphine on locomotor activity in mice, because initial stimulation of locomotor activity produced by morphine at 20 mg/kg did not accompany changes in all the metabolite (DOPAC, HVA, MHPG, and 5-HIAA) levels except for 3-MT.

The present results show that systemic administration of morphine induced a dose-related opposite effect on locomotor activity in mice. When compared to the saline-treated mice, the inhibitory effect of morphine was the most marked in the first 10-min period after administration of 1.25 mg/kg, and this inhibition was not followed by stimulation during 60 min after administration. These characteristics of the inhibitory effect of morphine are in agreement with the early study showing effects of morphine administered intracerebroventriculary in mice (4), but are somewhat inconsistent with other report in mice (24). Like the present results, the former report (4) has shown only the inhibitory effect of morphine during 30 min after administration. On the other hand, the latter report (24) has indicated only the inhibitory effect of morphine during first 60 min after subcutaneous administration, but this inhibition was followed by stimulation during subsequent 60 min. The latter (24) is of interest because the locomotor pattern after morphine administration was a time-related biphasic response, similar to that after morphine on the locomotor activity in rats (3, 6, 8) and on the wheel-running activity in hamsters (21). It has been shown that the effect of morphine on locomotor activity in mice depends upon the observation period (11) and differs among the various strains used (5, 14, 18). For instance, morphine induces a stimulation of locomotor activity in the C57BL/6J mice, but not in the DBA/2J mice. The disagreement between our results and others (24) is not explained by the present results, but it may be due to the differences in the experimental conditions, such as the observation period, morphine dosages, and/or strain differences used.

In conclusion, the present data indicate that morphine produces dose-related opposite effects on locomotor activity in mice, and suggest that the inhibition of locomotor activity produced by low dosages of morphine may be related to a decrease of presynaptic DA release in the striatum, whereas the stimulation of locomotor activity produced by high dosages of morphine may be related to an increase of presynaptic DA release in the striatum.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Itoh and Dr. Murai for their helpful advice. This work was supported by a grant from Keiryoukai Research Foundation No. 25.

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