Pharmacology Biochemistry & Behavior, Vol. 27, pp. 193–197. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

BRIEF COMMUNICATION

Effects of Methamphetamine and Morphine on the Vertical and Horizontal Motor Activities in Mice

TADANOBU ITOH, SHIGEO MURAI, HIROSHI YOSHIDA, YOSHIKATSU MASUDA, HIROKO SAITO AND CHING HSUN CHEN

Department of Pharmacology, School of Dentistry, Iwate Medical University 3-27, 1 Chome, Chuodori, Morioka, 020, Japan

Received 8 May 1986

ITOH, T., S. MURAI, H. YOSHIDA, Y. MASUDA, H. SAITO AND C. H. CHEN. *Effects of methamphetamine and morphine on the vertical and horizontal motor activities in mice.* PHARMACOL BIOCHEM BEHAV 27(1) 193–197, 1987.—The effects of methamphetamine and morphine on the vertical (VMA) and the horizontal motor activities (HMA) in male ddY mice (six weeks of age) was investigated between 9:00 and 13:00, using an apparatus which can differentiate spontaneous motor activity into VMA and HMA, measuring their activities simultaneously. VMA and HMA were evaluated by counting the number of times that an infrared ray was blocked by the mouse in the activity cage. Nine infrared photo-couplers were used to measure the VMA and one to measure the HMA. All measurements were taken at 10 min intervals during the 180 min period after subcutaneous injection of methamphetamine (0.1, 1 and 10 mg/kg) or morphine (2, 10 and 50 mg/kg). A small dose of methamphetamine (0.1 mg/kg) did not exert influence on the counts of the VMA and the HMA, whereas a large dose enhanced both activities, especially at 10 mg/kg, where each activity showed qualitatively different biphasic patterns. On the other hand, three doses of morphine significantly inhibited the VMA for 20 min after administration, while morphine at 2 mg/kg depressed the HMA for 10 min after administration and at 10 mg/kg or more markedly enhanced it during the 180 min observation period. These results show that different doses of methamphetamine and morphine exert different effects on the VMA and the HMA in mice.

Methamphetamine Morphine Vertical locomotion Horizontal locomotion Mouse

IT is well known that spontaneous motor activity in animals reflects various functions of the central nervous system. Thus, as one of the tests for evaluating drug effects on the central nervous system, measurement of the response to spontaneous motor activity in small animals has often been performed by using the photo-electric cell method [5, 18, 28, 31] and the opto-varimex method [15,19].

The photo-electric cell method measures relatively purposeful ambulatory activity, such as walking and running movements, while the opto-varimex method can differentiate locomotor activity in small animals into different kinds of movements and measure those activities.

Recently, we [13, 14, 21] have developed an apparatus using the infrared ray, which can measure locomotor activity in mice and which can (a) divide spontaneous motor activity into two different locomotor components, vertical (VMA) and horizontal motor activity (HMA), (b) measure their activities simultaneously, and (c) at a much lower cost.

There are many reports about the effects of methamphetamine [3, 5, 10, 11, 25, 28, 32] and morphine [2, 7, 8, 10, 11, 23, 27] on the locomotor activity in mice, but only a few reports about the effects of those drugs on locomotor activities dividing simultaneously spontaneous motor activity into two different behavior components. In the present paper, the authors' method is introduced and the effects of methamphetamine and morphine on the VMA and the HMA in mice are demonstrated.

METHOD

Animals

Seventy male ddY strain mice, aged 6 weeks (Shizuoka Experimental Animal Agricultural Cooperative Association, Japan), weighing between 24 and 28 g, were used in this experiment. The mice were housed in a community plastic cage, 32 cm wide × 13 cm high × 22 cm deep, and were given a solid diet (MF: Oriental Yeast Co., Tokyo) and tap water ad lib. The room was kept at a temperature of 23–26°C and at a humidity of 55–65% with lights on from 6:00 to 18:00.

Seven days before the beginning of the experiment, their tails had been cut about 10 mm from the root under ether anesthesia because the motion of their tails exerts adversely

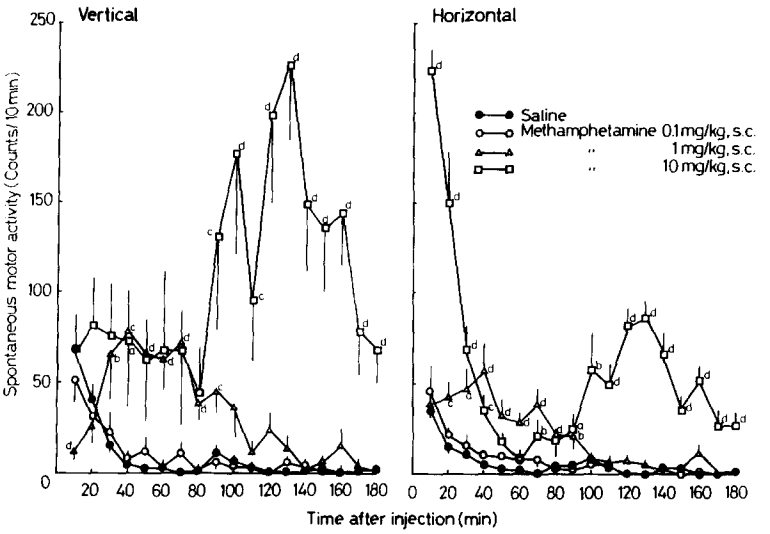

FIG. 1. The time-courses of the vertical and horizontal motor activities after administration of saline or methamphetamine (0.1, 1.0 and 10 mg/kg SC). Vertical: vertical motor activity, Horizontal: horizontal motor activity. Mean values ±S.E. Significances a, b, c and d: $p<0.05$, $p<0.025$, $p<0.01$ and $p<0.005$ for motor activity count of methamphetamine-treated mice vs. saline-treated mice at the same time, respectively (multiple comparison analysis of Kruskal-Wallis and Student's *t*-test).

affects the VMA and HMA count when measuring their locomotor activities. Mice were used only once and always between 9:00 and 13:00 in every experiment.

Apparatus

The apparatus used in the experiment can differentiate spontaneous motor activity of mice into two different behavior components, VMA and HMA, and measure their activities simultaneously. The method for measuring the VMA and the HMA is to count the number of times that an infrared ray is blocked by the mouse in the activity cage. The apparatus consists of a detector and a counter.

Detector. An activity cage which is made of black opaque plastic (5 mm in thickness) consists of the saucer (160 mm wide × 20 mm high × 290 mm deep), the frame (130 mm wide × 300 mm high × 260 mm deep) and the lid (130 mm wide × 260 mm deep) with 8 breathing holes.

Detection for VMA is performed with nine infrared ray photo-couplers placed on the side of the box at a height of 65 mm from the base at 30 mm intervals. Detection for HMA is done with one infrared ray photo-coupler set up on the same side at a height of 18 mm from the base.

An infrared ray photo-coupler consists of two elements, an illuminator and a light beam sensor. The driving voltage and electric current were determined from the results of preparatory experiments done at 200 mV and 20 mA, respectively. A photo-transistor and an IC volt-comparator were introduced into the circuit to amplify the brightness of the beam. Sensitivity to the photo-detecting was regulated by correlating the several output voltages using a sensitivity amplifier.

Counter. This instrument registers the number of times which VMA and HMA is detected. One counter has the ability to process individually all the information relative to the locomotor activities, which is sent from five detectors. In addition, a C-Mosic is incorporated into the circuit of the counter, and the electric current and voltage which was consumed for registering the information was fixed at 25 mA per figure, with a voltage between +4 and +7.

Procedures

Prior to administration of the drugs, the mice were allowed to be in the activity cage for 10 min. Then the mice were removed and injected with methamphetamine hydrochloride (0.1, 1 and 10 mg/kg; Dainihon Seiyaku Co., Japan) or morphine hydrochloride (2, 10 and 50 mg/kg; Takeda Yakuhin Co., Japan).

Immediately after the administration of drugs, the mice were again placed in the activity cage and their cumulative locomotor activities for VMA and HMA were measured every 10 min in the same room for 180 min.

The drugs were diluted in a 0.9% w/v NaCl solution (saline; Otsuka Seiyaku, Japan) and injected subcutaneously into the dorsal at 0.1 ml/10 g. Control injection was of saline only.

Statistical Analysis

Data was collected for every successive 10 min segment and was statistically analyzed by multiple comparison analysis of Kruskal-Wallis and Student's *t*-test.

RESULTS

Methamphetamine-Induced VMA and HMA

Figure 1 shows the time-course changes in the mean VMA and HMA count in the mice after administration of methamphetamine and saline.

In the treatment with methamphetamine at 0.1 mg/kg, the

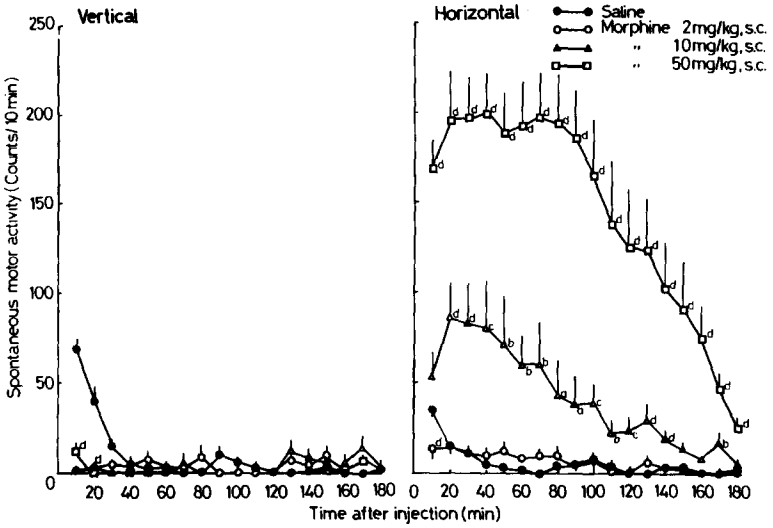

FIG. 2. The time-courses of the vertical and horizontal motor activities after administration of saline or morphine (2, 10 and 50 mg/kg SC). Vertical: vertical motor activity, Horizontal: horizontal motor activity. Mean values ±S.E. Significances a, b, c and d: $p<0.05$, $p<0.025$, $p<0.01$ and $p<0.005$ for motor activity count of morphine-treated mice vs. saline-treated mice at the same time, respectively (multiple comparison analysis of Kruskal-Wallis and Student's t-test).

time-course in the VMA count showed similar change to that of the control group.

In the treatment with methamphetamine at 1.0 mg/kg, the VMA count during the first 10 min period after administration significantly decreased over that of the control group. Thereafter, the count increased gradually, and between 30 min and 70 min after administration became higher than that of the control group. Eighty min after administration, however, the VMA count gradually approached that of the control group.

In the treatment with methamphetamine at 10 mg/kg, the counts between 10 min and 70 min after administration showed the same value as the locomotor activity count observed between 30 min and 70 min after treatment with methamphetamine at 1.0 mg/kg. Thereafter, the VMA count showed a gradual but remarkable increase and peaked 130 min after administration. After 140 min, there was a trend toward recovery in the VMA count, but even after 180 min the count showed a value significantly higher than that of the control group.

Subsequently, after the treatment with methamphetamine at 0.1 mg/kg the time-course in the HMA count showed the same pattern as that of the control group.

In the treatment with methamphetamine at 1.0 mg/kg, the HMA count during the first 10 min period after administration showed the same value as that of the control group, and then the count lapsed, maintaining a value higher than that of the control group.

The HMA count with methamphetamine at 10 mg/kg showed a remarkable increase during the first 10 min period after administration, and then there was a recovery in the count. After 60 min, no differences in the HMA count between the treated and control groups were noted. After 70 min, however, the HMA count showed an increase again and peaked between 120 min and 130 min after administration. Furthermore, after 140 min there was a trend toward recovery in the HMA count, but even after 180 min the count showed a value significantly higher than that of the control group.

Morphine-Induced VMA and HMA

Figure 2 shows the time-course changes in the mean VMA and HMA count in mice after administration of morphine and saline.

In the case of VMA, treatments with three doses of morphine induced significant decreases in the counts of locomotor activities for 20 min after administration. After 30 min, the time-courses in the VMA count showed the same pattern as that of the control group.

On the other hand, although in treatment with morphine at 2 mg/kg the HMA count showed significant decrease during the first 10 min period after administration, after 20 min, the time course in the HMA count showed the same pattern as that of the control group, while with 10 and 50 mg/kg of morphine, the HMA count increased significantly more than that of the control group, depending on the doses of morphine.

DISCUSSION

Many investigators have reported the effects of methamphetamine and morphine on spontaneous motor activity in mice. However, there are only a few reports which differentiate the spontaneous motor activity into two different behavior components, VMA and HMA, and simultaneously analyze their activities.

It is known that methamphetamine induces an increase in locomotor activity in mice [5, 25, 32]. The present study, however, found that the counts of both VMA and HMA in ddY strain mice induced by the treatment with methamphetamine at 0.1 mg/kg showed the same time-course changes as those of the control group. This finding differs

from the results of past studies where decreased locomotor activity was induced by a low dose of apomorphine, which seems to act on dopamine receptors also existing at the presynaptic sites in dopaminergic neurons [8, 9, 26]. Over against this, treatment with methamphetamine at 1.0 mg/kg induced an increase in the counts of both VMA and HMA and markedly prolonged the duration of the effects. These stimulant effects of methamphetamine on locomotor activity are now generally assumed to be produced by a mechanism that induces the stimulation of catecholamine release and the inhibition of catecholamine reuptake at the presynaptic sites in dopaminergic neurons [6, 12, 26, 30].

Treatment with a large dose of methamphetamine (10 mg/kg) induced a marked increase in the counts of both VMA and HMA, and the counts of those activities showed biphasic changes during the 180 min observation period. In the treatment with a large dose of methamphetamine, the changes in the counts of both VMA and HMA showed qualitatively different patterns from each other in the early stage and similar patterns in the late stage. These biphasic changes in the VMA and the HMA are thought to be mediated by the dopaminergic system. In particular, changes in the VMA and HMA counts observed in the late stage may be related to the development of stereotyped behavior such as sniffing, licking, head shaking, gyrating and moving backward [3].

Subsequently, treatment of any dose of morphine used in the behavioral test experiment significantly decreased the VMA count for 20 min after administration in ddY strain mice. Because there is no report concerning the inhibitory effect of morphine on VMA mice, the present paper may be the first.

On the other hand, the pattern in the HMA count in ddY strain mice after treatment with a low dose of morphine (2 mg/kg) showed the same change as that of control group and a large dose of morphine (10 and 50 mg/kg) induced a dose-dependent increase in the HMA count. This increase in the HMA count agrees with the results of morphine-induced hypermotility in mice reported by Filibeck *et al.* [7], Hirabayashi *et al.* [10], Loh and Ross [20] and Oliverio *et al.* [23]. However, although Babbini and Davis [2], Hecht and Shiorring [8] and Szekely *et al.* [27] have reported that morphine induces an initially depressed effect followed by a delayed excitatory effect on locomotor activity in mice, in the present study these phenomenon with morphine were not found in HMA.

It has been reported that during the morphine-induced hypermotility period in mice, morphine decreases the dopamine-turnover at the striatum in ddY strain mouse brain [17], increases the release of dopamine and the amount of 3-methoxytyramine in C57BL/6J strain mouse brain [20] (in our unpublished observations, we also recognized an increased amount of 3-methoxytyramine at the striatum in ddY strain mouse brain), and does not change the amount of enkephalin and the number of opiate-receptors in the mouse brain [4].

Conversely, it has been reported that in DBA/2J strain mice, morphine does not produce a stimulation of locomotor activity and decreases the amount of 3-methoxytyramine at the striatum [20,26].

As described above, it is known that morphine-induced hypermotility in mice is mediated by the brain catecholamine system [20], that the effect of morphine on the brain depends on the region, and that the mode of morphine action on the locomotor activity varies with the strain and the species [1].

It is also suggested that morphine-induced analgesia and hypermotility in mice are produced by different mechanisms [20]. Therefore, it is considered that the mechanism of an increase in morphine-induced HMA may differ from that of morphine-induced analgesia, and that the effects of morphine on VMA and HMA may also be developed by different mechanisms.

Furthermore, because the opiate-receptors are also known to exist at the presynaptic sites in dopaminergic neurons [29], it is suggested that at first a low dose of morphine may act on opiate-receptors at the site, and induce a decrease in the release of dopamine and an increase of dopamine-turnover [22]. Consequently, it is assumed that the development of a decreased locomotor activity induced by a low dose of morphine may have been due to the regulation by the mechanism of positive feedback on the biosynthesis of dopamine in the dopaminergic system.

Hitherto, although it is known that the catecholamine system is involved in an increase of locomotor activity induced in mice by morphine [20], it has recently been suggested that the muscarinic [24], H2-histaminergic [24], and serotonergic [16] system is also involved in the development of hypermotility induced by morphine in animals.

REFERENCES

1. Alper, R. H., K. T. Dermarest and K. E. Moore. Morphine differentially alters synthesis and turnover of dopamine in central neuronal systems. *J Neural Transm* **48:** 157–165, 1980.
2. Babbini, M. and W. H. Davis. Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. *Br J Pharmacol* **46:** 213–224, 1972.
3. Balsara, J. J., T. R. Bapat, V. P. Gada and A. G. Chandorkar. Small doses of apomorphine induce catalepsy and antagonize methamphetamine stereotypy in rats. *Psychopharmacology (Berlin)* **78:** 192–194, 1982.
4. Brunello, N., A. Volterra, A. M. Di Giulio, V. Cuomo and G. Racagni. Modulation of opioid system in C57 mice after repeated treatment with morphine and naloxone: Biochemical and behavioral correlates. *Life Sci* **34:** 1669–1678, 1984.
5. Dews, P. R. The measurement of the influence of drugs on voluntary activity in mice. *Br J Pharmacol* **8:** 46–48, 1953.
6. Ernst, A. M. Mode of action of apomorphine and dexamphetamine gnawing compulsion in rats. *Psychopharmacologia* **10:** 316–326, 1967.
7. Filibeck, U., C. Castellano and A. Oliverio. Development of morphine induced changes of activity in the mouse. *Brain Res* **254:** 602–605, 1981.
8. Hecht, A. and E. Shiorring. Behavioral effects of low and high acute doses of morphine in solitary mice. *Psychopharmacology (Berlin)* **64:** 73–79, 1979.
9. Herman, J. P., L. Stinus and M. Le Moal. Repeated stress increases locomotor response to amphetamine. *Psychopharmacology (Berlin)* **84:** 431–435, 1984.
10. Hirabayashi, M., F. Iwai, M. Iizuka, T. Mesaki, M. R. Alam and S. Tadokoro. Individual differences in the accelerating effect of methamphetamine, d-amphetamine and morphine on ambulatory activity in mice. *Folia Pharmacol Jpn* **75:** 683–693, 1979.
11. Hirabayashi, M., M. Iizuka and S. Tadokoro. Simple and easy method for measurement of ambulatory activity in mice. *Folia Pharmacol Jpn* **74:** 629–639, 1978.

12. Hughes, R. V. and A. M. Greig. Effects of caffein, methamphetamine and methylphenidate on reaction to novelty and activity in rats. *Neuropharmacology* **15:** 673–676, 1976.
13. Itoh, T., S. Murai, Y. Masuda and H. Saito. Effects of several drugs on vertical and horizontal movement activities in mice. *Jpn J Pharmacol* Suppl **39:** 238p, 1985.
14. Itoh, T., S. Murai, H. Yoshida, Y. Masuda and H. Saito. A simply designed apparatus and its use for measuring vertical and horizontal motor activities in methamphetamine-treated mice. *Jpn J Psychopharmacol* **5:** 19–23, 1985.
15. Iwamoto, E. T. The opto-varimex and behavior processor model BP-80-3C. *J Electrophysiol Tech* **10:** 141–145, 1983.
16. Johnston, C. A. and K. E. Moore. The effect of morphine on 5-hydroxytryptamine synthesis and metabolism in the striatum, and several discrete hypothalamic regions of the rat brain. *J Neural Transm* **57:** 65–73, 1983.
17. Kameyama, T., M. Ukai, S. Soma and M. Hiramatsu. Differential effect of α-, β- and γ-endorphin on dopamine metabolism in the mouse brain. *Brain Res* **244:** 305–309, 1982.
18. Kinnard, W. J., Jr. and C. J. Carr. A preliminary procedure for the evaluation of central nervous system depressants. *J Pharmacol Exp Ther* **121:** 354–361, 1957.
19. Lemly, A. D. and R. J. F. Smith. A behavioral assay for assessing effects of pollutants on fish chemoreception. *Ecotoxicol Environ Safety* **11:** 210–218, 1986.
20. Loh, H. H. and D. H. Ross. Opiate action, catecholamines and locomotor activity in mice. *Adv Biochem Psychopharmacol* **20:** 369–370, 1979.
21. Murai, S., Y. Nakamoto, O. Itsukaichi, J. Nakamura and T. Itoh. Biphasic effects of morphine on locomotion in mice. *Jpn J Pharmacol* Suppl **39:** 35p, 1985.
22. Murakami, H. and T. Segawa. Effect of apomorphine on morphine induced decrease in locomotor activity and increase in dopamine turnover in rats. *Jpn J Pharmacol* **30:** 565–567, 1980.
23. Oliverio, A., C. Castellano, F. Pavone and J. Vetulani. Caffeine interferes with morphine-induced hyperactivity but not analgesia. *Pol J Pharmacol Pharm* **35:** 445–449, 1983.
24. Pant, K. K., S. Gurtu, C. Nath, J. N. Sinha and K. P. Bharagava. Evidence for the involvement of central muscarinic cholinergic and H2-histaminergic receptors in morphine induced hyperactivity in the mouse. *Indian J Med Res* **78:** 587–592, 1983.
25. Peachey, J. E., B. Rogers, J. E. Brien, A. Maclean and D. Rogers. Measurement of acute and chronic behavioral effect of methamphetamine in the mouse. *Psychopharmacologia* **48:** 271–275, 1976.
26. Seemen, P. Brain dopamine receptors. *Pharmacol Rev* **32:** 229–313, 1981.
27. Szekely, J. I., E. Miglecz and A. Z. Ronai. Biphasic effects of a potent enkephalin analogue (D-MET 2, Pro 5)-enkephalinamide and morphine on locomotor activity in mice. *Psychopharmacology (Berlin)* **71:** 299–301, 1980.
28. Ueki, S. and H. Sugano. A quantitative method for continuous recording of spontaneous activity in small animals. *Jpn J Pharmacol* **17:** 82–89, 1967.
29. Van Loon, C. R. and C. Kim. β-endorphin-induced increase in striatal dopamine turnover. *Life Sci* **23:** 961–970, 1978.
30. Van Rossum, J. M. Mode of action of psychomotor stimulant drugs. *Int Rev Neurobiol* **12:** 307–383, 1970.
31. Winter, C. A. and L. Flataker. The effect of cortisone, desoxycorticosterone, and adrenocorticotrophic hormone upon the responses of animals to analgesic drugs. *J Pharmacol Exp Ther* **103:** 93–105, 1951.
32. Yamanaka, Y., T. Yamamoto and T. Egashira. Methamphetamine-induced behavioral effects and releases of brain catecholamines and brain concentrations of methamphetamine in mice. *Jpn J Pharmacol* **33:** 33–40, 1983.