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Effects of Methionine-Enkephalin and Morphine on Spontaneous Locomotor Activity: Antagonism by Naloxone

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BHARGAVA, H. N. *Effects of methionine-enkephalin and morphine on spontaneous locomotor activity: Antagonism by naloxone*. PHARMAC. BIOCHEM. BEHAV. 9(2) 167–171, 1978.—Methionine-enkephalin (MEK), a postulated endogenous ligand for the opiate receptors, when administered intracerebroventricularly (ICV) in a dose of 1.75 μmoles/kg) of morphine sulfate had no effect on motor activity. Higher doses (7.0 μmoles/kg) of both MEK and morphine produced profound depression in motor activity; the decrease was significant from 6 to 30 min after their administration. Subcutaneous administration of naloxone (1 mg/kg) did not alter the motor activity. However, administration of naloxone (1 mg/kg) 2 min prior to MEK (7.0 μmoles/kg) administration completely blocked the effect of the latter for 15 min while antagonizing the effect of 7.0 μmoles/kg of morphine for 10 min. At 30 min after 7.0 μmoles/kg of either MEK or morphine administration, the motor activity in these groups was identical with that of the naloxone treated group. Methionine-enkephalin in doses of 0.2 and 0.4 μmole/kg had no effect on motor activity for 1 hr observation period. A significant increase in motor activity was recorded 45 and 60 min after 0.2 μmole/kg of morphine sulfate, whereas 0.4 μmole/kg dose of morphine showed significant increase in motor activity only at 60 min after its administration. Furthermore, 0.2 μmole/kg of morphine produced a greater increase in motor activity compared with 0.4 μmole/kg dose at 45 and 60 min after administration. It is concluded that morphine and MEK produce differential effects on mouse spontaneous motor activity with respect to time and dose and that naloxone can inhibit the effects of MEK more effectively than that of morphine on motor activity.

Methionine-enkephalin Morphine Naloxone Motor activity Time course Antagonism

ENKEPHALINS isolated from the brain have been identified as a mixture of two pentapeptides, Tyr-Gly-Gly-Phe-Met (methionine-enkephalin, MEK) and Try-Gly-Gly-Phe-Leu (leucine-enkephalin, LEK) [15,23] and have been proposed to be endogenous ligands for the opiate receptors in the brain [14, 18, 19]. These enkephalins exhibit pharmacological profiles analogous to morphine in vitro [15,23]. Similarities in pharmacological effects have also been demonstrated in vivo. For example, analgesic activity of enkephalins has been observed in mice [7] and rats [2]. Thus, it is possible that the receptors for enkephalins and morphine may be similar or identical.

The action of opiate narcotics on locomotor activity in rodents has been of considerable interest. It is well known that morphine and other opioids produce opposite effect on locomotor activity in mice and rats. In mice, morphine induces an increase in running activity [8] whereas in rats, the locomotor activity is decreased [17]. However, findings contrary to above have been reported. Thus, Pert and Sivit [20] found that morphine-injected into the nucleus accumbens increased spontaneous motor activity in rats. Morphine (1, 3, and 10 μg) and MEK (100, 200 and 400 μg) increased locomotor activity in mice in a dose related manner during the first hour after administration [10,21]. Furthermore, it was shown that β-endorphin and etorphine, the potent narcotic agonists failed to increase motor activity in mice [26] indicating some differences between the enkephalins and other opiates.

In many studies motor activity has been recorded at various times after morphine administration. Similarly, the activity has been recorded for times ranging from 5 min to 60 min [9, 25, 26]. These variables can affect the outcome of behavioral and biochemical investigations.

More recently in our laboratory, it was shown that MEK and LEK behave like opiates in inhibiting the naloxone-induced morphine abstinence syndrome in morphine-dependent mice [3,4]. In the present communication, the effects of different doses of MEK and morphine sulfate administered intracerebroventricularly (ICV) on the motor activity of mice are reported. The effect of drugs on time course of changes on the motor activity has been examined. Finally, the effect of pretreatment with opiate antagonist, naloxone, on the effects of morphine and MEK induced changes on the motor activity has been determined.

METHOD

Male Swiss Webster mice, weighing 20–25 g (Scientific Small Animals, Arlington Heights, IL.) were used in all experiments. Food and water were allowed ad lib and the animals were housed under the following conditions: temperature (23 ± 1°C), humidity (65 ± 2%) and light (L. 0600–1800 hr).

Locomotor activity was measured by means of 3 sets of circular activity cages. Each cage was 35 cm in dia. and 20 cm in height, and was equipped with 6 light sources and 6 photocells placed just above the floor levels. The lights were placed orthogonally to each other so that the light beams crossed in the center of the cages. The measure of activity was the number of times the light beam was broken within a specified period of time. The activity was recorded automatically on a counter. Methionine-enkephalin (Calbiochem, San Diego, CA.; Lot 700291) and morphine sulfate were dissolved in saline and were injected ICV [13] in a volume such that each mouse received 0.5 μl/g body weight. Brain sections taken after the injection of India ink revealed that the injection sites were the third ventricles. The injection method has been shown to be reproducible as evidenced by behavioral and biochemical changes reported by us [5,11] and by others [24]. Immediately after the injection of the drug or the vehicle, the animals were put in the activity cages. The activity of animals was recorded at each dose (1.75 and 7.0 μmoles/kg) at various time intervals for a period of 30 min. The experiments were repeated four times using three animals per determination. For assessing the effect of naloxone on methionine-enkephalin or morphine induced changes in locomotor activity, naloxone (1 mg/kg) was injected subcutaneously 2 min prior to narcotic agonist and the motor activity recorded as described above. The dose of naloxone was selected because it was high enough to precipitate abstinence in morphine dependent mice [3,4]. In studying the effect of naloxone on motor activity changes induced by morphine and enkephalin, only the higher dose (7.0 μmoles/kg) of the narcotic agonists was used since they produced profound and longer lasting effects. The effects of relatively low doses (0.2 and 0.4 μmole/kg) of MEK and morphine on motor activity were also determined. Motor activity was measured at the same time of the day to minimize the diurnal variations. The results obtained from drug treated animals in each group are expressed as means ± S.E.M.

Statistics

The data analysed by two way analysis of variance upon the untransformed scores using a split-plot design for repeated measures [16]. For significant F ratios, Scheffe's [22] S method was used to make all possible comparisons among means.

RESULTS

Depending upon the dose employed and time at which observations were made, intracerebroventricular administration of morphine and MEK produced depression and stimulation in mouse locomotor activity. The effects of 1.75 μmoles/kg of morphine and MEK on the cumulative motor activity for a 30 min period are presented in Table 1. Analysis of variance data and Scheffe's test revealed that MEK significantly ($p<0.05$) affected the motor activity only at 30 min after its administration compared with the saline controls, at which time the activity was depressed; however at other time intervals no change in activity was noticed. Morphine, however, at the same dose (1.75 μmoles/kg) failed to show any effect at any of the time intervals. A dose of 7.0 μmoles/kg of morphine and MEK produced profound drop in the locomotor activity. As shown in Table 2, the motor activity of MEK and morphine treated mice were depressed significantly ($p<0.05$) compared with saline at all time intervals beginning with 6 min after their administration. The activities in both MEK and morphine treated mice were approximately 15 percent of controls, whereas, at 30 min after their administration they were 49 and 41 percent, respectively. Although the recovery in the morphine treated mice appeared to be slower than in MEK treated mice, the two groups did not differ significantly at any time.

Administration of naloxone (1 mg/kg) did not affect the motor activity in mice for 15 min; however, a statistically significant ($p<0.05$) decrease was noted at 30 min observation period. As shown in Table 3, pretreatment with naloxone antagonized the effects of 7.0 μmoles/kg doses of morphine and MEK on motor activity. The effect of MEK was antagonized completely for 15 min, whereas, morphine effect was abolished for 10 min. The naloxone-morphine treated mice had significantly lower motor activity at 15 min after its administration compared with naloxone-saline or the saline-saline group. At 30 min after the administration there was no difference between the naloxone-saline vs.

TABLE 1

EFFECT OF ENKEPHALIN AND MORPHINE ON SPONTANEOUS LOCOMOTOR ACTIVITY

Treatment	Cumulative motor activity Counts ± SEM (N=4) Time after injection (min)						
	2	4	6	8	10	15	30
Saline	12.0 ± 1.1	40.7 ± 8.3	58.3 ± 5.5	91.8 ± 2.9	136.5 ± 4.4	256.9 ± 22.6	733.6 ± 106.7
MEK (1.75 μmol/kg)	1.2 ± 0.4	3.8 ± 1.2	44.6 ± 9.2	84.3 ± 9.4	122.4 ± 7.9	192.0 ± 10.7	510.0 ± 40.1*
Morphine Sulfate (1.75 μmol/kg)	1.9 ± 0.5	3.0 ± 0.8	16.0 ± 3.1	44.9 ± 2.5	83.3 ± 4.3	246.9 ± 16.0	791.7 ± 101.4

MEK=methionine-enkephalin; *$p<0.05$ vs. saline control

TABLE 2

EFFECT OF METHIONINE-ENKEPHALIN AND MORPHINE ON SPONTANEOUS LOCOMOTOR ACTIVITY

Treatment	Cumulative motor activity (Counts + SEM; N=4) Time after injection (min)						
	2	4	6	8	10	15	30
Saline	51.8 ± 4.1	117.0 ± 12.5	168.8 ± 16.5	228.4 ± 22.0	287.2 ± 32.0	451.9 ± 34.0	920.0 ± 97.3
MEK (7 μmol/kg)	5.4 ± 1.3	12.3 ± 4.3	26.7 ± 10.3*	50.7 ± 17.9*	91.8 ± 16.5*	201.7 ± 20.0*	452.0 ± 24.3*
Morphine Sulfate (7 μmol/kg)	3.7 ± 0.6	12.1 ± 2.4	26.0 ± 5.7*	42.8 ± 13.9*	59.0 ± 19.5*	92.2 ± 25.0*	383.2 ± 34.1*

*$p<0.05$ vs. saline control; MEK=methionine-enkephalin

TABLE 3

EFFECT OF NALOXONE PRETREATMENT ON SPONTANEOUS MOTOR ACTIVITY CHANGES INDUCED BY METHIONINE-ENKEPHALIN AND MORPHINE

Treatment	Cumulative motor activity (Counts ± SEM; N=4) Time after injection (min)						
	2	4	6	8	10	15	30
Sal-Sal	8.9 ± 1.3	41.0 ± 6.6	64.9 ± 6.1	88.8 ± 12.4	119.9 ± 20.2	183.6 ± 25.9	442.4 ± 54.0
Nal-Sal	16.2 ± 1.9	44.3 ± 10.0	66.7 ± 11.8	105.0 ± 8.5	132.1 ± 9.2	166.9 ± 15.2	242.4 ± 25.6†
Nal-MEK	15.1 ± 4.3	24.4 ± 4.1	45.5 ± 4.0	80.1 ± 10.7	115.4 ± 11.1	169.8 ± 11.8	281.8 ± 29.2†
Nal-M.S.	12.6 ± 3.4	25.1 ± 3.7	39.3 ± 3.5	50.4 ± 4.6	61.7 ± 2.7	90.7 ± 8.6*	200.3 ± 28.3†

Sal=saline; Nal=naloxone; MEK=methionine-enkephalin; M.S.=morphine sulfate
*$p<0.05$ vs. Sal-Sal, Nal-Sal and Nal-M.S. groups; †0.05 vs. Sal-Sal group

TABLE 4

EFFECT OF METHIONINE-ENKEPHALIN ON SPONTANEOUS MOTOR ACTIVITY

Treatment	Cumulative motor activity Counts ± SEM (N=4) Time after injection (min)			
	15	30	45	60
Saline	491.7 ± 87.0	718.1 ± 112.0	735.9 ± 115.0	745.3 ± 119.0
Enkephalin				
0.2 μmol/kg	672.3 ± 119.0	910.8 ± 146.5	936.6 ± 140.5	982.7 ± 144.5
0.4 μmol/kg	704.5 ± 94.0	900.2 ± 106.0	1054.7 ± 139.0	1071.6 ± 145.0

naloxone-MEK and naloxone-saline vs. naloxone-morphine groups. However, at this time the naloxone-saline group showed lower activity compared with saline treated groups. Furthermore, the motor activity at 30 min in naloxone-MEK treated group was significantly greater than in naloxone-morphine group.

As shown in Table 4, MEK in doses of 0.2 and 0.4 μmole/kg, had no effect at any time period tested during 1 hr observation period. In contrast, morphine (0.2 μmole/kg) increased the motor activity in mice significantly at 45 and 60 min following its administration relative to the controls. There were no differences, however, at 15 and 30 min periods in the activities of morphine and saline controls. Interestingly, the higher dose 0.4 μmole/kg of morphine significantly stimulated the motor activity which could be observed only at 60 min after its administration and this dose produced significantly ($p<0.05$) less of an increase in the motor activity compared with the lower dose (0.2 μmole/kg) or morphine.

DISCUSSION

The present studies indicate that intracerebral administration of MEK and morphine sulfate produce both stimulatory and inhibitory effect on locomotor activity in mice depending upon the dose employed and the time at which the observations are recorded following the drug administration. In the present studies the motor activities were recorded at short time intervals to more clearly observe the changes produced by drugs, particularly the peptide MEK which has been shown to have a short duration of action.

Methionine-enkephalin (0.2–0.4 μmole/kg) did not pro-

TABLE 5

EFFECT OF MORPHINE SULFATE ON SPONTANEOUS LOCOMOTOR ACTIVITY

Treatment	Cumulative motor activity Counts ± SEM (N=4) Time after injection (min) 15	30	45	60
Saline	245.0 ± 16.5	571.0 ± 39.3	887.2 ± 226.5	935.1 ± 240.6*
Morphine Sulfate				
0.2 μmol/kg	426.9 ± 26.9	1110.8 ± 260.0	1969.0 ± 72.6*	2553.0 ± 167.6*
0.4 μmol/kg	169.5 ± 42.0	679.9 ± 212.9	1246.3 ± 175.0†	1673.5 ± 244.0*†

*$p<0.05$ vs. saline group; †$p<0.05$ vs. M.S. 0.2 μmol/kg

duce any significant effect on motor activity, whereas, morphine (0.2–0.4 μmole/kg) stimulated the activity. The latter was not evident for 45 or 60 min after the administration. Doses of MEK in the same range have been shown to stimulate activity [26] when it was measured for one hour after the administration. Another synthetic peptide, Try-D-Ala-Gly-Phe-D-Leu in doses greater than 0.04 μg and morphine at greater than 0.3 μg significantly increased the motor activity [1]. The discrepancy between the present data and others [10,17] may perhaps be explained on the basis of strain differences used. The effect of genetic make-up on morphine induced changes in the motor activity is well documented [9]. However, in the present studies only one strain of mice was used and yet dose and time related changes in motor activity were observed following administration of the opiate agonists. Thus in contrast to 0.2–0.4 μmole/kg doses or « (2.4–4.8 μg/20 g mouse) which did not affect motor activity doses of 1.75 and 7.0 μmole/kg (21–84 μg/mouse) of MEK significantly depressed the motor activity.

MEK (1.75 μmole/kg) showed decreased activity only at 30 min after injection, whereas, 7.0 μmoles/kg dose, showed significant inhibition in activity at 6 min after administration and similar effect was clearly evident at each time period for up to 30 min. On the other hand, morphine at 1.75 μmoles/kg was without any effect but 7.0 μmole/kg dose produced profound depression similar to that produced by an equivalent dose of MEK. These studies indicate that some differences between morphine and endogenous opiate may exist. Wei *et al.* [26] observed increased motor activity in mice following morphine and enkephalins; however, in their studies β-endorphin and etorphine, the potent narcotic agonists had no effect on motor activity.

It is also shown that administration of naloxone prior to morphine or MEK antagonized the effect of the latter on motor activity. The antagonism was of short duration and was much more effective against MEK than against morphine. Naloxone is perhaps a less potent inhibitor of morphine binding than enkephalin binding to brain opiate receptors. Naloxone has been shown to be a potent inhibitor of ^{3}H-enkephalin binding to receptors in rat brain [27].

Recent studies have shown that MEK and morphine produce similar depressant actions on brain stem neurons in the rat and cat [6,12]. Both morphine and enkephalins have been shown to inhibit some signs of morphine abstinence in morphine-dependent mice [3,4]. Since MEK and morphine (both at 1.75 μmole/kg) had no effect on motor activity at 15 min after their administration, yet were active in inhibiting the morphine abstinence, it appears that a negative correlation exists between the two effects. A similar negative correlation has been shown between the motor activity and analgesia in mice [9].

The mode of action of morphine in producing their effect on locomotor activity has not been established with certainty, although it is clear that the effect is at least partly mediated by cerebral monoamines. Carroll and Sharp [8] concluded that dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine are all involved in the normal activation response of mice to morphine with dopaminergic mechanisms being of primary importance. Kuchinsky and Hornykiewicz [17] showed that in mice, morphine produces locomotor hyperactivity by releasing DA from the presynaptic terminals in the striatum, thus increasing the dopaminergic activity in this structure and NE may have an important auxiliary function. In the above study, mice were placed for 15 min into the cages and the running activity recorded from 5 to 10 min at times which coincided with the peak of the drug effect. In the present studies since the drugs were injected directly in the brain, the activities were recorded right after putting the animals in the cages.

In summary, in the present investigation it is shown that differential dose and time related effects of locomotor activity are observed in mice following ICV injection of morphine and MEK. Whether these differential changes in motor activity are related to differential changes in the function of cerebral monoamines are currently under investigation. Finally, it appears that a relationship between motor activity changes induced by opiate narcotics and inhibition of morphine abstinence in morphine-dependent mice does not exist.

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