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Strain Dependent Effects of the Enkephalin Analogue FK 33-824 on Locomotor Activity in Mice

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CASTELLANO, C. *Strain dependent effects of the enkephalin analogue FK 33-824 on locomotor activity in mice.* PHARMAC. BIOCHEM. BEHAV. 15(5) 729–734, 1981.—Administration of the enkephalin analogue FK 33-824 was followed in the DBA/2 (DBA) strain by dose related depressant effects which, after the injection of 40 mg/kg, were still present 6 hrs after treatment; on the contrary, in the C57BL/6 (C57) strain, activity levels were enhanced by treatment, except for the dose of 40 mg/kg, which induced a short lasting behavioral stimulation followed by activity depression and catatonia and later on again by behavioral stimulation. All the effects of FK were naloxone reversible. Cross tolerance between FK and morphine was moreover observed both as concerns the excitatory effects (C57 strain) and the depressant effects (DBA strain) evident following their acute administration. The results are discussed in terms of differences in type, number and/or distribution of the receptors responsible, in the two strains, for the behavioral responses to opiate administration.

FK 33-824 Naloxone Morphine Locomotor activity Inbred mice

THE effects of the natural opioid peptides (enkephalins), and of some of their synthetic analogues, on locomotor activity in rodents have been investigated in a number of researches, with sometimes contrasting results. Following their administration, for example, activity decrements and catatonia [1,14] as well as motor excitement [19], have been demonstrated in rats, and behavioral activation [12], no effect [9] or depression of activity [25] have been observed in mice. A relevant role in these effects seems to be played, according to some investigations, by dose level and time interval following injection [1, 3, 10, 25].

The compound FK 33-824 (D-Ala^2, MePhe^4, Met(O)^5-ol-enkephalin) has been recently obtained from met-enkephalin [23]. This peptide, which has high affinity to the opiate receptor, exerts central effects after systemic administration [20]. Compared with morphine, it has shown to be twice as potent subcutaneously in inducing analgesia in rats and mice [20]. Its administration is followed in rats and rabbits by an initial behavioral excitation, followed by catatonia, which is more marked at high doses [1]. Dependence and cross tolerance with morphine for the analgesic effects, in mice, and akinesia, in rats and rabbits, have been observed when high doses of this peptide are injected [11]. Moreover, FK induces in the rat a naloxone reversible catatonia, following SC administration (32 mg/kg). Differences in the site of action between FK and morphine have been finally demonstrated by some investigations [1,23].

In recent years, inbred strains of mice have proved to be a very useful tool in psychopharmacological investigations. As concerns locomotor activity, in particular, two strains: C57BL/6 (C57) and DBA/2 (DBA), which show regional brain differences in the content of dopamine (DA) and acetylcholine (ACH) [18] have been shown to react differently to opiate administration. Intraperitoneal injections of morphine or heroin are in fact followed by behavioral excitation (running fit) [17], related to activation of DA neurons in the striatum [21], in the former, and by motility depression in the latter strain [24].

In the present research the effects of intraperitoneal administrations of FK on locomotor activity were studied in C57 and DBA mice. The naloxone reversibility of the effects observed, and the occurrence of cross tolerance between FK and morphine were also investigated. The results were finally compared with those obtained following opiate administration in the same strains of mice in previous investigations [4, 17, 24].

METHOD

Subjects

The subjects were naive male mice belonging to the strains C57BL/6 and DBA/2 (River Lab., Como, Italy), weighing 23–25 g at the beginning of the experiments. All mice were maintained upon their arrival in the laboratory (2 weeks before the experiments) in groups of 8 in clear plastic pens with food and water available ad lib. In all experiments the animals were tested once.

Locomotor Activity

Locomotor activity was measured as previously de-

TABLE 1

MEAN NUMBER OF CROSSINGS (±SEM) IN THE TOGGLE BOX OF DIFFERENT GROUPS OF 8 DBA AND 8 C57 MICE FOLLOWING TREATMENT WITH SALINE OR FK

Group	mg/kg	C57		Group	mg/kg	DBA	
		1–10	11–60			1–10	11–60
Saline		28.6 ± 1.2	92.1 ± 9.1	Saline		29.8 ± 1.7	72.5 ± 4.2
FK	1.0	27.3 ± 1.4	88.3 ± 8.3	FK	0.25	31.3 ± 1.0	66.6 ± 3.2
"	2.5	25.2 ± 1.0	182.5 ± 14.8	"	0.05	28.5 ± 1.1	37.3 ± 1.3
"	5.0	27.2 ± 0.9	280.3 ± 25.7	"	5.0	19.5 ± 1.4	13.2 ± 2.3
"	10.0	40.1 ± 1.1	560.5 ± 23.5	"	10.0	9.1 ± 0.7	5.8 ± 1.0
"	20.0	60.2 ± 3.2	262.6 ± 15.1	"	20.0	7.6 ± 0.7	1.3 ± 0.4
"	40.0	73.2 ± 3.9	5.8 ± 0.8	"	40.0	2.3 ± 0.5	0.1 ± 0.1

The animals were tested immediately after the injection for a single 60 min long session. For each group the mean number of crossings of the first period of 10 min (1–10) and of the following 50 min (11–60) is reported. ANOVA (1 factor, 7 levels) showed, for both strains, significant differences between groups, $F(6,49)=110.5$ (1–10) and 179.9 (11–60) (DBA), 76.5 (1–10) and 129.2 (11–60) (C57); $p<0.001$ (see also Tables 2 and 3).

scribed [17]. The mice were tested in Plexiglas toggle-floor boxes (24.5×9.0 cm). The number of crossings from one side to the other of the box was automatically recorded by means of a microswitch connected to the tilting floor of the box, and constituted the score of the mouse. Circuitry was arranged so that whenever the mouse crossed the cage, a cumulative counter was advanced. A light located 1.5 m above the top of the boxes was the source of illumination (0.25 ft-c., at the cage floor level).

In a first series of experiments different groups of 8 mice for each strain were injected with different doses of FK 33-824 and tested in the toggle box immediately after treatment for a single 60 min long session. Their performances were compared with those of groups of 8 saline injected mice. The doses of FK injected were 1, 2.5, 5.0, 10.0, 20.0, and 40 mg/kg for the C57 mice, and 0.25, 0.5, 5.0, 10.0, 20.0, and 40.0 mg/kg for the DBA mice. Observations were carried out (for a maximum of 5 hrs) at the different dose levels in other groups of C57 and DBA mice (4 per group) to examine the occurrence of stereotypies or of other behavioral changes following FK treatment. The C57 and the DBA mice injected with 40 mg/kg of FK were also tested for eventual loss of the righting reflex.

In a second series of experiments, for each strain, different groups of 8 mice were injected with FK (40 mg/kg and tested in the toggle box for single 60 min long sessions, at different time intervals (1, 2, 3, 4 and 5 hrs) following administration. The performance of each group of mice was compared with that of a different group of saline injected controls.

In a third series of experiments different groups of 8 DBA and 8 C57 mice were injected with saline, naloxone (0.5 mg/kg), FK, or naloxone + FK combinations and tested in the toggle box for a single 30 min session. Control groups were injected with saline 90 and 30 min before testing. Naloxone groups were injected with naloxone 90 min and with saline 30 min before testing. FK groups were injected with saline 90 min and with FK 30 min before testing. Finally, naloxone + FK groups were injected with naloxone 90 min and with FK 30 min before testing. The dose of naloxone and the time interval before testing were chosen on the basis of previous findings [8] and of preliminary experiments. The C57 mice were injected with 10 or 40 mg/kg of FK, the DBA mice with 40 mg/kg of FK.

In a fourth series of experiments the occurrence of cross tolerance between FK and morphine was investigated. For this purpose, According to a previously described schedule [17], for each strain different groups of 8 animals were pretreated with saline, FK (10 mg/kg) or morphine (20 mg/kg) twice a day (09.00 and 19.00 for two consecutive days. On the third day (09.00) the animals were injected with saline, FK or morphine, and were tested for motor activity in the toggle box, immediately after the injection, for a 60 min long session (see also Table 4). The dose of morphine was chosen on the basis of previous investigations [17] and of preliminary experiments.

FK 33-824 (Sandoz, Basel), Naloxone (HCl) (Endo, Garden City, NY) and morphine (HCl) (Erba, Milan) were dissolved in 0.9% NaCl and injected at the volume of 4 ml/kg. 0.9% NaCl (4 ml/kg) was used for control injections. All injections were given intraperitoneally.

The results were statistically evaluated by ANOVA [6]. Additional post hoc analyses, utilizing the error variance of the general analysis, were carried out, when necessary, in order to obtain individual between treatment comparisons [15].

RESULTS

FK Alone

1 hr recordings. As compared with the saline groups, the administration of FK to mice tested for a single 60 min session was followed by dose related activity depressant effects in the DBA mice, and by dose related activity stimulating effects in the C57 mice (Table 1). In particular, already for the first ten min after treatment, activity was significantly depressed, for the DBA strain, in the groups injected with 5, 10, 20, or 40 mg/kg of FK, while it was enhanced, in the C57 strain, in the groups injected with 10 or 20 mg/kg of this peptide. Moreover, in the C57 mice injected with 40 mg/kg of FK, behavioral stimulation was initially evident (first ten min after injection), the activity was depressed but still present during the following 20 min, and lack of movements was recorded during the last 30 min of the testing (see also Tables 2, and 3).

TABLE 2

C57 STRAIN

Comparisons	mg/kg	1–10 F(1,49)	p	11–60 F(1,49)	p
Saline–FK	1.0	0.1	>0.05	0.02	>0.05
" – "	2.5	1.1	"	15.6	<0.001
" – "	5.0	0.1	"	68.1	"
" – "	10.0	13.9	<0.001	421.6	"
" – "	20.0	105.1	"	55.8	"
" – "	40.0	209.3	"	14.2	"
FK 5.0 FK	10.0	17.4	"	150.18	"
" " "	20.0	114.4	"	0.6	>0.05
" " "	40.0	222.4	"	144.8	<0.001
FK 10.0 FK	20.0	42.5	"	170.5	"
" " "	40.0	115.3	"	591.2	"
FK 20.0 FK	40.0	17.7	"	126.6	"

Statistical analysis of some relevant data reported in Table 1 (Individual between-treatment comparisons).

TABLE 3

DBA STRAIN

Comparisons	mg/kg	1–10 F(1,49)	p	11–60 F(1,49)	p
Saline–FK	0.25	0.8	>0.05	3.2	>0.05
" – "	0.5	0.7	"	115.7	<0.001
" – "	5.0	41.4	<0.001	329.3	"
" – "	10.0	165.6	"	416.5	"
" – "	20.0	190.4	"	474.6	"
" – "	40.0	290.9	"	491.4	"
FK 0.5–FK	5.0	31.1	"	54.6	"
" – "	10.0	144.4	"	63.1	"
" – "	20.0	167.6	"	121.6	"
" – "	40.0	262.6	"	130.1	"
FK 5.0–FK	10.0	41.4	"	5.1	<0.05
" – "	20.0	54.2	"	13.2	<0.001
" – "	40.0	112.8	"	16.1	"
FK 10.0–FK	20.0	0.8	>0.05	1.9	>0.05
" – "	40.0	17.5	<0.001	3.1	"
FK 20.0–"	40.0	10.6	<0.01	0.1	"

Statistical analysis of some relevant data reported in Table 1 (individual between-treatment comparisons).

The observations, which were carried out for a maximum of 5 hrs, showed, in the DBA mice, following all the doses of FK tested, (except 0.25 mg/kg) activity depression of dose dependent duration, with long lasting periods of immobility at the highest doses. In the C57 strain, FK (from 2.5 until 20 mg/kg) always induced behavioral stimulation (running fit) of dose dependent duration. In this strain, a certain degree of muscular rigidity was observed (from the 10th to the 60th min about after injection) in the mice injected with 20 mg/kg of FK. The C57 mice injected with 40 mg/kg showed an initial behavioral stimulation (first 10 min) followed by activity depression, and after (from the 30th to the 90th min about after injection) by catatonia (never evident at lower doses), characterized by a marked muscular rigidity and (from the 30th to the 60th min about after injection) by complete loss of the righting reflex. Later on behavioral stilmulation was

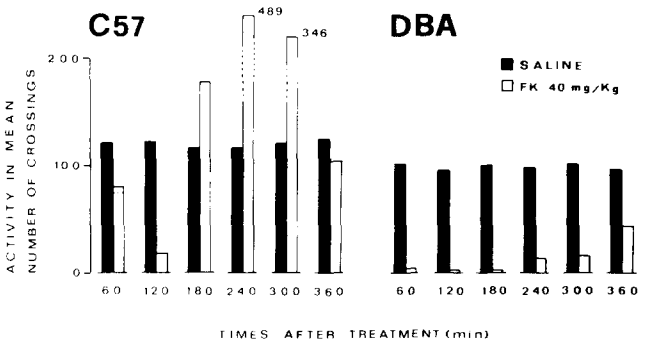

FIG. 1. Mean number of crossings in the toggle box of different groups of 8 saline and 8 FK (40 mg/kg) injected C57 (left) and DBA (right) mice. All animals were tested for a single 60 min long session at increasing time intervals following treatment. The dotted line inside the column corresponding to the C57 group tested immediately after treatment indicates the mean number of crossings carried out during the first 10 min of the session, corresponding to the initial period of behavioral stimulation (see Table 1). All other crossings were carried out in this group during the following 20 min. All crossings of the C57 group tested 60 min after injection were recorded during the last part (from the 30th to the 60th min about after treatment) of the session.ANOVA (2 factors, 2×5) showed for both strains, significant effects for treatments, $F(1,70)= 2509.8$ (DBA), 177.0 (C57), $p<0.001$, for times, $F(4,70) =12.5$ (DBA), 110.4 (C57), $p<0.001$, and a significant treatments × times interaction, $F(4,70) = 12.2$ (DBA), 115.9 (C57), $p<0.001$. Individual between-treatment comparisons showed for both strains significant differences between the performances of the FK injected mice and those of their controls: $p<0.001$ for all groups, except for the C57 mice tested 3 hrs after treatment ($p<0.01$), and 5 hrs after treatment ($p<0.05$).

TABLE 4

MEAN NUMBER OF CROSSINGS (±SEM) IN THE TOGGLE BOX OF DIFFERENT GROUPS OF 8 DBA AND 8 C57 MICE INJECTED WITH SALINE, NALOXONE, FK OR FK+NALOXONE COMBINATIONS

Strains	Pretreatment	mg/kg	Treatment	mg/kg	Crossings
C57	Saline		Saline		83.5 ± 2.3
	Naloxone	0.5	"		80.6 ± 2.5
	Saline		FK	10.0	408.8 ± 6.8
	"		"	40.	0.1 ± 0.1
	Naloxone	0.5	"	10.0	103.2 ± 16.0*
	"		"	40.0	70.2 ± 11.3*
DBA	Saline		Saline		74.6 ± 1.9
	Naloxone	0.5	"		71.1 ± 2.6
	Saline		FK	40.0	0.1 ± 0.1
	Naloxone	0.5	"	40.0	61.5 ± 2.7*

The animals were tested for a single 30 min long session (see text).

ANOVA (2 factors, 2×2 (DBA), 2×3 (C57)) showed for both strains significant effects for both FK and naloxone treatments (DBA: $F(1,28)=182.1$ and 384.9, $p<0.001$; C57: $F(1,42)=127.7$, $p<0.001$ and $F(2,42)=365.2$, $p<0.001$.

*Significantly different ($p<0.001$) from the corresponding FK group (individual between-treatment comparisons).

again evident. It was decided to study in particular this effect in additional experiments (see Method), in which also the DBA mice were tested.

6 hrs recordings (FK 40 mg/kg). As compared with the saline groups, FK induced, in the DBA strain, activity depressant effects, still present 6 hrs after treatment (Fig. 1). In the C57 mice activity depressant effects were observed in the mice tested 1 hr after the injection. In this group, lack of movements (corresponding to catatonia, as shown by the observations carried out in this strain), was evident during

TABLE 5

TOLERANCE: MEAN NUMBER OF CROSSINGS (±SEM) IN THE TOGGLE BOX OF DIFFERENT GROUPS OF 8 DBA AND 8 C57 MICE

	Pretreatment	mg/kg	Treatment	mg/kg	C57	DBA
(a)	Saline		Saline		125.0 ± 2.9	106.2 ± 2.4
(b)	"		Morphine	20	626.1 ± 27.8	13.0 ± 1.2
(c)	"		FK	10	615.1 ± 24.2	16.7 ± 1.1
(d)	Morphine	20	Saline		133.0 ± 2.8	103.5 ± 3.3
(e)	"	"	Morphine	20	166.3 ± 3.8	90.8 ± 1.6
(f)	"	"	FK	10	150.5 ± 6.0	104.3 ± 1.6
(g)	FK	10	Saline		123.8 ± 3.0	102.3 ± 2.2
(h)	"	"	Morphine	20	152.0 ± 3.0	102.2 ± 2.6
(i)	"	"	FK	10	155.5 ± 4.0	91.6 ± 2.5

The animals were tested for a single 60 min long session on the third day, following two days of pretreatment (two injections per day), immediately after the fifth injection (Treatment) (for further explanations see text).

ANOVA (2 factors, 3×3) showed in both strains significant effects of pretreatments, $F(2,63)=588.5$ (DBA), 599.0 (C57), $p<0.001$, of treatments, $F(2,63)=238.5$ (DBA), 211.3 (C57), $p<0.001$, and a significant pretreatments × treatments interaction, $F(4,63)=173.1$ (DBA), 153.2 (C57), $p<0.001$.

For both strains: e vs b, i vs c, f vs c, h vs b, b vs a, c vs a: $p<0.001$; f vs h, b vs c, d vs a, g vs a: $p>0.05$ (individual between-treatment comparisons).

the first 30 min of the session; the last part of the session was characterized by a gradual reappearance of the crossings. The activity levels were, in the C57 mice, significantly higher, as compared with controls, 3, 4 and 5 hrs after treatment, and the activity levels of the FK and the saline injected mice were not significantly different 6 hrs after the administration of FK.

FK After Naloxone

The administration of naloxone antagonized the depressant effects exerted by FK (40 mg/kg) in the DBA mice; in teh C57 strain naloxone antagonized both the locomotor stimulation induced by 10 mg/kg, and the catatonia induced by 40 mg/kg, of FK (see also Table 4).

Cross Tolerance Between FK and Morphine

Tolerance developed to both the depressant effects exerted in the DBA mice by FK (40 mg/kg) or morphine (20 mg/kg), and to the stimulating effects evident in the C57 mice following FK (10 mg/kg) or morphine (20 mg/kg) administrations. Cross tolerance between FK and morphine was moreover evident (see also Table 5).

DISCUSSION

The results of the present research, in which the pharmacogenetic approach based on DBA/2 (DBA) and C57BL/6 (C57) was used, clearly show the existence of genetic influences on the reactivity of these strains to the administration of the enkephalin analogue FK 33-824 (FK). In fact, FK depressed activity in the DBA strain, and enhanced it in the C57 strain. Moreover, biphasic effects were evident, only in the C57 mice, when 40 mg/kg of the peptide were injected. In both strains all the effects observed were naloxone reversible, and cross tolerance between FK and morphine, suggesting the existence of similar mechanisms of action, was evidentiated.

Previous researches [17,24] have shown that morphine, or heroin, administrations are followed by dose related motor stimulation in the C57 mice, and by decrement of locomotor activity in the DBA mice. Other experiments [4] have shown that, differently from what has been observed in the present research following FK administration, heroin never induces, in the C57 strain, even at high doses (150 mg/kg) muscular rigidity or loss of righting reflex; moreover, at these dose levels, only motor depressant effects , with long lasting immobility periods, are evident in the DBA mice, as after FK administration in the present research. Aloisi *et al.* [1] have shown recently that FK induces in the rat a short period of excitation, followed by sedation, catatonia and rigidity. The existence of biphasic effects on locomotor activity following morphine [2,7] or enkephalins [1,25] administrations, and of differences in righting responses, muscular rigidity, etc. [10,27] between opiates and enkephalins have led to the hypothesis [2, 10, 25] that multiple types of receptors mediate the effect of opiates in the central nervous system. The existence of more than one class of opiate receptors has been in fact demonstrated in recent years [5, 13, 16, 27]. In particular, the morphine μ receptors (for which FK has high affinity [5]) preferentially bind opiates, while the endogenous opioid peptides interact at least with the μ receptors and the δ receptors, with which they interact perferentially when these receptors are present. Differences in the ratio of morphine binding sites to enkephalin binding sites depending on brain areas have been moreover shown in the rat [5]. As concerns the DBA and C57 mice it has been hypothesized [22] that differences in the ratios of different types of receptors might be responsible for the strain differences observed in the analgesic and motor responses to morphine administration. Thus, it could be possible that differences between

DBA and C57 mice in type, number and/or distribution of the receptors involved might account for the strain differences observed, in the present research, following the administration of FK. In the C57 strain, in particular, at least two types of receptors may be present: one type, responsible for the excitatory responses to FK, which is activated earlier, and when the concentrations of the peptide in the CNS are lower, the other, responsible for catatonia following FK administration, which is later activated and at higher concentrations of FK. Further researches will however be necessary to clarify these points.

In conclusion, the results of the present research clearly underline the role played by genetic make-up in modulating the effects of the enkephalin analogue FK 33-824 on locomotor activity in mice. Moreover, they support the hypothesis of the existence of multiple receptor populations responsible for the motor responses of rodents to opiate administration.

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