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Opioid-Induced Linear Running in Obese (*ob/ob*) and Lean Mice

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CALCAGNETTI, D. J., J. J. FLYNN AND D. L. MARGULES. *Opioid-induced linear running in obese* (ob/ob) *and lean mice*. PHARMACOL BIOCHEM BEHAV **26**(4) 743–747, 1987.—Earlier research has shown that opioids stimulate behavioral activation in mice whereas opioid antagonists attenuate this activation. We conducted an experiment to determine the dose-response curve of FK33824, a potent Met-enkephalin analogue. FK33824 produced an unusual form of behavioral activation we called "linear running" in which the mice ran continuously in one direction and were nearly oblivious to environmental stimuli. This may be the kind of running that occurs naturally during migration. Wheel running activity of genetically obese (*ob/ob*) and lean (C57BL/6J ?/+) mice was measured following the intracerebroventricular infusion of 0.1, 1.0, 10.0 and 100.0 ng of FK33824. The lowest dose did not alter baseline running, whereas the 1.0 and 10.0 ng doses significantly increased running in both genotypes. We found a genotype difference with the highest dose tested, the lean mice ran at baseline levles and displayed ataxia whereas the obese mice continued to show increased running without ataxia. We hypothesize that genetic differences in the enkephalin mechanisms of C57 lean and obese mice are responsible for linear running.

FK33824 Enkephalin Obese mice Linear running Intracerebroventricular Migration
Naloxone Naltrexone Naltrexone methobromide MR-2266

IN mammals acutely administered opioid ligands (e.g., morphine, methadone, etonitazene and heroin) induce behavioral depression in some species (rat, dog, monkey, human), and excitatory behavior in others such as the mouse [6, 11, 35]. Following morphine administration mice display increased exploration, rearing, hopping, grooming and nonlinear "running fits" [7, 12, 34, 43]. Naloxone readily reverses opiate-induced behavioral activation in mice [6] but fails (at doses of 1.0, 3.0, and 10.0 mg/kg) to effect baseline running [7,47]. These findings suggest an opioid component mediating behavioral activation in the mouse.

Intracerebroventricular (ICV) injections of the relatively stable enkephalin (ENK) analogues D-Ala-2-Met-enkephalin-amide and D-Ala-2-Leu-enkephalin-amide (25 and 50 μg/mouse), into the mouse, significantly increased behavioral activation as measured by activity platforms [27]. This activation was rather brief (20 min) and was readily reversed by naloxone (4 and 8 mg/kg). Similar increases in locomotion were found in mice given ICV MET-enkephalin (100 μg), Leu-enkephalin (200 μg), gamma-endorphin (5 and 10 μg) [23], alpha-endorphin (20 μg) [22], and dynorphin (0.3 and 1 μg) [25, 46]. Met- and Leu-enkephalin have been shown to produce 2–15 min increases in analgesia and locomotion probably because they are readily degraded [8,23]. These studies did not provide information on the question of linear running necessary for navigation toward a goal. Linear running was first reported by Ukai and Kameyama [47] however, these authors did not relate it to migration.

Of several strains of mice tested, the C57BL/6J mouse strain displays the greatest locomotor activation [7,34] and lowest analgesia after peripheral administration of morphine [14, 35, 36, 38]. In the C57 mouse, morphine releases striatal dopamine (DA) [37,38] and leads to an activation of striatal DA receptors [44]. It has been suggested that this genetic difference among mouse strains is due to the larger proportion of delta to mu opioid receptors on DA striatal fibers of the C57BL/6J strain [3]. The genetically obese mutant mouse (*ob/ob*) which differs from its lean counterpart by a single gene is available on the C57BL/6J backround. This mutant has multiple hormonal and behavioral abnormalities [15,31] including obesity, overeating, hyperinsulinemia, hyperglycemia, reproductive problems, impaired thermoregulation, thyroid insufficiency, growth retardation [4], shortened life span and *greatly reduced locomotor activity* [29,49]. In a pilot study a 1 μg ICV injection of FK33824 in mice produced "linear running" (best characterized as a sustained, unidirectional and compulsive wheel running). Two of eight pilot obese mice died within two hours (possibly from exhaustion), none of the nine ataxic lean mice died.

Based on these findings, we hypothesized that both obese and lean mice of the C57BL/6J strain would display linear running given a long-acting ENK analogue ICV. The syn-

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thetic Met-enkephalin analogue, FK33824, was chosen for two reasons: (1) it is highly resistant to degradation by peptidases [19, 39, 40], and (2) it has high affinity for the mu and delta opioid binding sites [48]. In the present work we measured wheel running of obese and lean mice from the C57BL/6J strain to determine the dose-response curve of FK33824-induced linear running in both lean and obese mice after ICV injection.

METHOD

Subjects

The subjects were male obese mice *(ob/ob)* and lean *(+/?)* littermates of the C57BL/6J strain raised in our colony. All subjects were six months old at the time of surgery. The mice were individually housed in stainless steel shoe box cages and provided ad lib food (Purina mouse chow No. 5015) and distilled water. They were housed and tested in an isolated room maintained at 23°C with a 12:12 hr light:dark cycle with dark onset at 1130 hr.

Surgery

Mice were anesthetized with Nembutal (80 mg/kg). A 26 gauge stainless steel outer cannula (Plastic Products Co., Roanoke, VA) was stereotaxically implanted into the right lateral ventricle (coordinates used were 3 mm posterior to bregma, 1 mm lateral to midline, and 2 mm ventral to the surface of the cortex) with the skull leveled between lambda and bregma.

Drugs and Injection

FK33824 [Tyr-D-Ala-Gly-MePhe-Met(O) ol, a gift from Dr. D. Roemer of Sandoz Ltd., Basel, Switzerland] was dissolved in 0.1 M acetic acid and aliquotted into siliconized glass bottles. The peptide was lyophilized, stoppered under vacuum, and then stored at 4°C. FK33824 was reconstituted on the day of use with filter sterilized (Millex-GV 0.22 μm, Millipore Corp., Bedford, MA) artificial cerebrospinal fluid (ACSF) containing 78.0 mM NaCl, 2.5 mM KCl, 50 mM $NaHCO_3$, 1.3 mM $CaCO_3$, and 1.3 mM $NgCl_2$ [45].

The ICV injections were performed by backloading the drug up a 33 gauge internal cannula (Plastic Products) into PE-20 tubing (Intramedic No. 7406). An injection volume of 1 μl was delivered by a 2 μl syringe (Hamilton No. 7002) mounted in a repeating pushbutton device (Hamilton No. PB 00-1) at a rate of 1 μl/40 sec. Four doses of FK33824 (100.0, 10.0, 1.0, 0.1 ng/mouse) were administered in a descending dose order. ACSF served as vehicle as well as control injection.

Apparatus

The running of each mouse was measured with a rat activity wheel (Lafayette Instrument Co., No. 86041) modified for mice by substituting a common pet store running wheel (diameter=16 cm) for the galvanized steel wheel. The wheel was connected to the counter such that one wheel revolution yielded one count. All wheels were sealed on both sides to prevent the mice from escaping.

Procedure

The mice were given 2 weeks to recover from surgery.

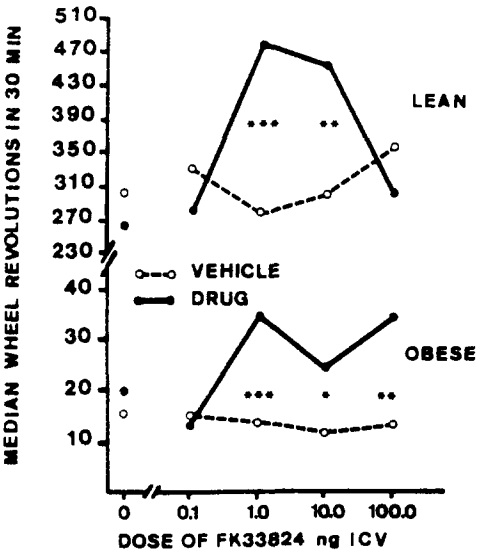

FIG. 1. Median wheel revolutions per 30 min for obese (n=9) and lean (n=9) mice are shown with one of four doses of FK33824 (0.1, 1.0, 10.0 and 100.0 ng/mouse) ICV. The asterisks indicate significance using Wilcoxon's test comparison (*=$p<0.05$, **=$p<0.02$ and ***=$p<0.01$).

For the next seven days the mice were adapted to the running wheels. Adaptation consisted of removing and replacing the dummy cannula followed by a one hour wheel running session (beginning at 1530 hr) on two consecutive days, once a week. Daily testing began on the following week with obese and lean body weights averaging 62.0 g (SD=4.1) and 29.4 g (SD=2.5) respectively. Pilot data (with other subjects not included in this study) suggested wheel running could be sampled best by the following schedule: ICV injection of vehicle followed in 15 min by a 30 min running session (the control session); 15 min in the home cage followed by an ICV vehicle or drug injection and an additional 15 min of rest after which followed a second 30 min running session (the test session).

The testing schedule began with 2 days of ICV vehicle injections (1 μl ACSF) for both the vehicle and test running sessions. For the next four days, vehicle was injected 15 min prior to the control session and FK33824 was injected 15 min prior to the test session.

Statistical Analysis

Since we made no assumption regarding the form of the population distribution and due to initial heterogeneity of variance across cells, statistical analysis was performed by non-parametric tests. A within-subjects repeated measures design was used to evaluate drug effects. The wheel running counts were anlayzed with Friedman's non-parametric 2-way ANOVA by ranks [28] followed by Wilcoxon's Matched-Pair signed-ranks test to determine significant differences between control and test sessions as well as day to day comparisons [42]. Wheel running was quantified by the number of wheel revolutions (counts per 30 min) for control and test sessions.

RESULTS

On every occasion all lean mice ran more than obese mice

TABLE 1

DEPICTS THE MINIMUM (MIN) AND MAXIMUM (MAX) WHEEL RUNNING SCORES OF OBESE AND LEAN MICE (RANGE PER VEHICLE AND FK33824 TREATMENT)

Dose	0.0	0.1	1.0	10	100
			Obese Mice		
FK33824					
Min	4	5	12	7	8
Max	68	77	164	267	195
Vehicle					
Min	2	5	9	4	2
Max	71	70	47	41	61
			Lean Mice		
FK33824					
Min	107	5	58	103	93
Max	572	643	595	770	582
Vehicle					
Min	78	7	93	79	156
Max	563	635	429	603	500

(it follows that all comparisons were made within each genotype). FK33824 produced significant dose-related increases in running for both obese and lean mice [Friedman's analysis yielded $\chi r^2(4)=20.11$, $p<0.002$] and [$\chi r^2(4)=14.8$, $p<0.02$ respectively].

Figure 1 summarizes the median wheel revolutions (counts per 30 min) for obese and lean mice in both the control and test sessions. Vehicle alone and the 0.1 ng dose of FK33824 did not produce reliable differences from baseline running for either genotype. The three highest doses of FK33824 produced significant increases in obese mice running relative to baseline (Wilcoxon tests yielded $p<0.01$, $p<0.05$ and $p<0.01$ respectively). Significantly increased wheel running was also found in the lean mice with the 1.0 and 10.0 ng doses (Wilcoxon tests yeilded $p<0.01$ and $p<0.02$ respectively). However, the 100.0 ng dose did not produce reliably increased wheel running in the lean mice; at this dose the lean mice became ataxic. Table 1 depicts the minimum and maximum range of wheel running scores for lean and obese mice during vehicle and FK33824 1/2 hr running sessions.

At the conclusion of the experiments, the mice were injected ICV with 2 μl of ink and immediately sacrificed by cervical dislocation. The brains were removed and coronal sections were made along the cannula tract. Visual inspection confirmed that all cannulae placements allowed access to the right lateral ventricle as shown by ink staining.

DISCUSSION

We measured linear running in contrast to published measurements of nondirected locomotor activation [25–27, 43, 49] or graded movement in mice [22–24, 46]. Nanogram quantities of ICV FK33824 produced significant increases in coordinated wheel running in both genotypes. This effect of FK33824 is in contrast to the locomotor activation by morphine and related compounds which do not produce solely linear running, but rather produce grooming, hopping and intermittent nondirected running fits. The mice given FK33824 also displayed stereotypic straub tails, recumbent ears, an elevated rear posture and muscle rigidity.

The lowest dose did not significantly increase running. However, the 1.0 and 10.0 ng doses significantly increased running in both genotypes. The running of obese mice was more dramatically increased as a percentage of baseline. At the highest dose tested, the obese mice continued to display increased running relative to baseline, but the lean mice ran only at baseline levels and displayed ataxia. This finding indicates that genotype is an important factor. Additional evidence further suggests genotype differences since peripheral injection of FK33824 (3.0, 6.0 and 12.0 mg/kg SC) produced significant linear running in obese but not lean mice for up to 2, 5 and 7 hours respectively [9].

Pilot data using mice (n=4 lean and n=4 obese) run according to the procedure suggests that opioid-induced running is centrally mediated. We observed that subcutaneous (SC) injections of the centrally active opioid antagonists, naloxone (2.0 mg/kg), naltrexone (1.25 mg/kg) and the kappa specific opiate receptor antagonist, MR-2266 (2.0 mg/kg), effectively stopped FK33824-induced running within a two min period after ICV administration of FK33824 (3.0 ng). However, running resumed after a period of time (20 min–1 hr) in which it was reasonable to assume that the antagonist wore off within the 2 hr period in which FK33824 is known to remain effective. These mice were also given the quaternary form of naltrexone (naltrexone methobromide, QNTX), which dose not cross the blood-brain barrier at moderate doses [5]. QNTX (6 mg/kg SC) failed to block FK33824- (6 mg/kg SC and 3.0 ng ICV) induced running nor did it block the behavioral activation produced by morphine (3 mg/kg SC, manuscript in preparation). Collectively these data support the hypothesis that FK33824-induced linear running is centrally mediated.

We do not propose that the behavioral activation produced by opioid ligands is specific to opioids just because their effects are reversed by naloxone. More pharmacological evidence is necessary to establish specificity since a variety of substances produce a general behavioral activation in the mouse including scopolamine [1,41], phencyclidine [13], ethanol [30], and ACTH [18]. Exposure to ionizing radiation and nitrous oxide also stimulate locomotion in mice [20,33]. Several direct and indirect dopamine (DA) agonists (L-dopa, apomorphine, pergolide, bromoergocryptine and d-amphetamine) also produce dose-dependent increases in locomotion in mice [2, 16, 21, 32, 49]. The behavioral activation of these substances/techniques also is reversed by naloxone [10, 20, 33]. Naloxone reversibility suggests that a common underlying opioid mechanism exists in the regulation of hyperlocomotion and this remains to be demonstrated and documented.

In summary, FK33824 produces a specific form of activation we call linear running that is different from the "running fit" produced by morphine. FK33824 binds selectively to mu and delta type opioid receptors [48]. The delta receptor is pharmacologically defined as the receptor with high affinity for ENK but low affinity for morphine [17]. One hypothesis why FK33824-induced linear running is different than the morphine running fit is that FK33824 may act primarily at opioid receptors of the delta type on DA cells in the mouse striatum that are critical for initiating and maintaining perseverative linear locomotion. Further study using more selective mu and delta agonists and antagonists is required to

elucidate the mechanism producing linear running. It has not escaped our notice that this opioid-induced compulsive locomotion in mice may serve as a laboratory model to study migration-like behavior.

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