Stimulation of Locomotor Activity of Genetically Obese Mice by Amphetamine

Previous studies[1] have shown that in several types of genetically obese mice, obesity was caused by factors other than inactivity. Before they became corpulent, none of these mice genetically destined to become obese was less active than normal mice. In the case of 'obese mice' (C57BL/6J-*ob/ob*) and 'diabetic mice' (C57BL/KsJ-*db/db*), inactivity was secondary to obesity, i.e., the mice were less active after they became corpulent. In the case of 'viable yellow mice' (VY/Wf-A^{vy}/a), 'lethal yellow mice' (YS/ChWf-A^{y}/a) and New Zealand Obese mice (NZO/BlWfL), even obesity did not reduce their locomotor activity. This report presents data on the stimulation of locomotor activity by amphetamine in *ob/ob* and *db/db* mice.

Mice and methods. Only male mice were used in this study. C57BL/6J-*ob/ob* mice and littermates, and C57BL/KsJ-*db/db* and normal mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. The littermates of *ob/ob* mice were of mixed genotypes, either homozygous for the wild type allele (ob^{+}/ob^{+}) or heterozygous (ob/ob^{+}). They are thus designated ?/+ in this report. The normal mice of the C57BL/KsJ strain were not littermates of *db/db* mice. All mice were fed Purina Laboratory Chow and water ad libitum except during the activity test. Lights in the mouse room were on from 06.00 h to 18.00 h.

Activity cages manufactured by Woodard Research Corp. were used to test the locomotor activity. They have been described previously[1]. Basal locomotor activity of a

[1] T. T. Yen and J. M. Acton, Proc. Soc. exp. Biol. Med. *140*, 647 (1972).

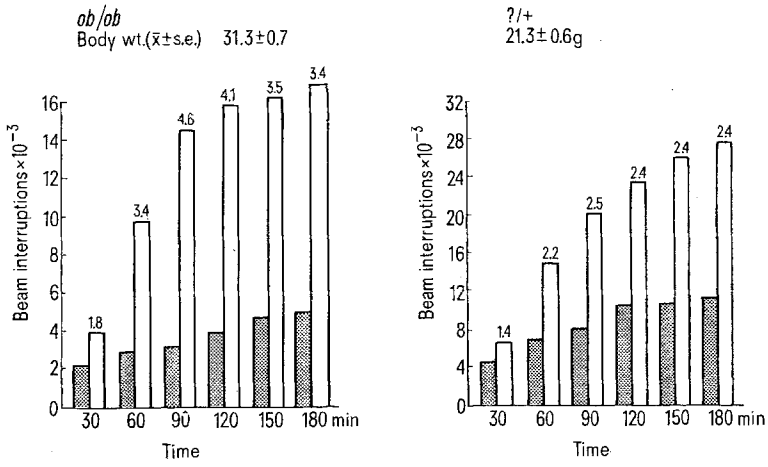

Fig. 1. The basal (shaded bar) and D-amphetamine-stimulated (5 mg/kg, i.p. blank bar) locomotor activity of C57BL/6J-*ob/ob* and normal (?/+) mice. Each bar represents the cumulative count of beam interruptions over the time period indicated on the abscissa by a group of 6 mice being tested simultaneously in the same activity cage. The basal and amphetamine-stimulated activity was obtained from the same set of mice on 2 different days but at the same time of the day. The number inside the blank bar is the ratio of amphetamine-stimulated activity to basal activity of the same testing period.

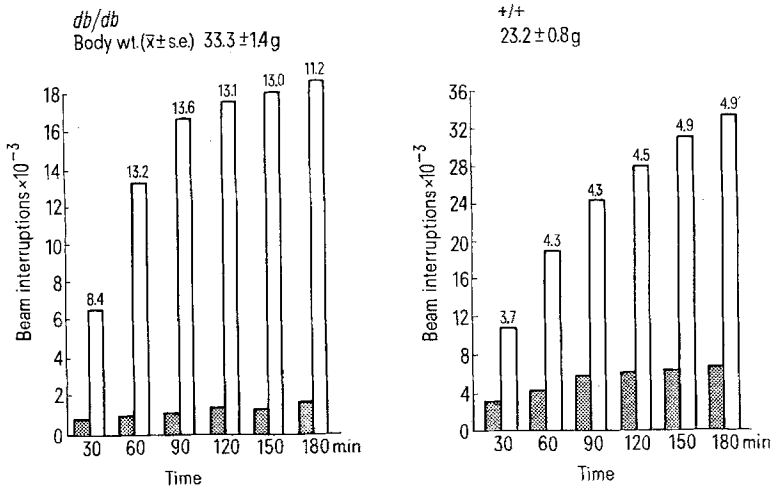

Fig. 2. The basal (shaped bar) and D-amphetamine-stimulated (5 mg/kg, i.p., blank bar) locomotor activity of C57BL/Ks-*db/db* and normal (+/+) mice. Each bar represents the cumulative count of beam interruptions over the time period indicated on the abscissa by a group of 6 mice being tested simultaneously in the same activity cage. The basal and amphetamine-stimulated activity was obtained from the same set of mice on 2 different days but at the same time of the day. The number inside the blank bar is the ratio of amphetamine-stimulated activity to basal activity of the same testing period.

group of 6 mice was measured simultaneously in the same activity cage for a period of 3 h after they were given saline, i.p. During the same time on the next day, the same mice were given D-amphetamine, 5 mg/kg, i.p. before their activity was measured again. The numbers of beam interruptions registered on the impulse counters were recorded every 30 min. No other activity was allowed in the room during the testing period. Amphetamine was dissolved in saline just prior to injection.

Results and discussion. The stimulation of locomotor activity of *ob/ob* and normal mice by amphetamine is shown in Figure 1; that of *db/db* and normal mice is presented in Figure 2. The basal activity of both *ob/ob* and *db/db* mice was much lower than that of the corresponding normal mice. It should be pointed out that this difference is probably not only due to the difference in body weight but also due to the difference in the interactions among mice. In our previous study[1], only 1 mouse was tested at a time in order to avoid interactions among mice. In the present study the effect of amphetamine over a period of 3 h was monitored, making it impractical to test each mouse separately.

The response of both *ob/ob* and *db/db* mice to amphetamine is better than that of normal mice of the same strain when the data are expressed on the basis of percentage of the basal level. This indicates that the center that regulates motor activity in these mice is not defective. This observation is compatible with our previous conclusion that in these mice, there is no genetic predisposition for inactivity and that inactivity in these mice is secondary to obesity[1].

Zusammenfassung. Die motorische Aktivität von genetisch obesen (*ob/ob*) und diabetischen (*db/db*) Mäusen wurde durch D-Amphetamin stärker gesteigert als bei normalen Mäusen, wobei das Regulationszentrum für die motorische Aktivität intakt zu sein scheint.

T. T. YEN and JUNE M. ACTON

Biochemical and Physiological Research Division, Eli Lilly and Company, 307 East McCarty Street, Indianapolis (Indiana 46206, USA), 21 May 1973.

Mutagenecity in the Mal Regions of *Escherichia coli*

N-methyl-N′-nitro-N-nitrosoguanidine (MNG) is a powerful mutagen for several kinds of bacteria[1–4]. ADELBERG et al.[2] have reported that treatment of cells in *Tris*-Maleate (TM) buffer instead of a broth reduced the lethal effects of MNG without reducing mutagenecity. OLMEDO and HANAWALT[4] found that bacteria are equally sensitive to MNG mutagenesis whether grown in minimal or complex medium. The present investigations were undertaken to find the relative efficiency of MNG as a mutagen for *E. coli*, when used in growth media and various buffers. Forward mutations from the ability to ferment maltose (Mal^+) to inability (Mal^-) were examined.

Materials and methods. Strain B 251 of *E. coli*, a Mal^+ derivative of strain B (gift of Dr. A. ARBER)[5], was used throughout. The media used have been previously described[6,7]. The bacteria were grown overnight in different media (J.N. or DM or DM-maltose), diluted 1:25 in 5 ml of fresh medium, and then incubated until log phase ($\sim 5 \times 10^8$ viable cells/ml) was reached. Only cells in log phase of growth were used. Cells were centrifuged, washed, and resuspended in the appropriate medium for the treatment with MNG. MNG was used at a concentration of 50 µg/ml. Mal mutants were visualized by spreading the treated bacterial suspension on Penassay-tetrazolium medium (TTC), supplemented with maltose[6], and confirmed, after reisolation, by their inability to grow on minimal (DM)[6] maltose plates, though they were

Survival and mutagenesis[a]

Treatment in (MNG-50µg/ml)	Time of treatment (min)	Survival fractions	Maltose negative mutants per 1000 survivors
DM-glucose	Before MNG addition	1.1×10^9	1.7
	Immediately after	2.8×10^{-1}	8.8
	15 after	4.4×10^{-5}	55.3
	30 after	2.6×10^{-7}	50.3
J. N. Broth	Before MNG addition	3.6×10^9	0.76
	Immediately after	1.3×10^{-1}	—
	15 after	2.4×10^{-3}	29.1
	30 after	1.3×10^{-4}	42.8
	60 after	3.0×10^{-5}	46.8
Phosphate-buffer (DM)	Before MNG addition	5.8×10^8	—
	Immediately after	6.3×10^{-1}	9.3
	15 after	3.3×10^{-4}	20.3
	30 after	7.5×10^{-5}	30.1
	60 after	3.7×10^{-5}	89.8
Tris-buffer	Before MNG addition	2.1×10^8	—
	Immediately after	3.5×10^{-1}	—
	15 after	1.1×10^{-1}	9.0
	30 after	2.0×10^{-2}	20.9
	60 after	1.3×10^{-2}	21.3
Citrate-buffer	Before MNG addition	4.2×10^8	—
	Immediately after	4.3×10^{-1}	4.3
	15 after	1.3×10^{-2}	27.7
	30 after	3.6×10^{-3}	32.8
	60 after	3.3×10^{-4}	60.0

[a] Mean of 3 replications.

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