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Amphetamine Anorexia and Hypothalamic Catecholamines in Genetically Obese Mice (*obob*)

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KUPRYS, R. AND G. A. OLTMANS. *Amphetamine anorexia and hypothalamic catecholamines in genetically obese mice* (obob). PHARMAC. BIOCHEM. BEHAV. 17(2) 271–282, 1982.—Genetically obese mice (*obob*) and their lean littermates were acclimated to a restricted food-access schedule of six hours and then treated with various doses of amphetamine (0, 3, 5 or 10 mg/kg). Saline-treated *obob* mice maintained on this schedule retained the primary characteristics of *obob* mice fed ad lib, i.e., hyperphagia, hyperglycemia, elevated hypothalamic norepinephrine (NE) levels. Both lean and obese mice treated with amphetamine showed a dose-dependent decrease in food intake and hypothalamic NE levels. In *obob* mice amphetamine treatment reduced food intake and hypothalamic NE levels to values which were not significantly different from those of similarly treated lean mice. When the drug dose was administered on a body weight basis, however, brain amphetamine levels were twice as high in *obob* as in lean mice. When the amphetamine dose was adjusted to produce approximately equivalent brain levels of amphetamine in *obob* and lean mice, the *obob* mice ate significantly more than lean mice. The results indicate that amphetamine is an effective anorectic agent capable of reducing food intake, body weight, and hypothalamic NE levels in *obob* mice.

Amphetamine Anorexia Hypothalamus Norepinephrine Dopamine *obob* mice
Genetic obesity Hyperphagia Obesity Food intake

RECENT studies have indicated the presence of central nervous system abnormalities in several rodent models of genetically transmitted obesity. These models include the mouse mutations *obob* and *dbdb*, and the rat mutation *fafa*. Among the abnormalities are significant changes in norepinephrine (NE) levels in specific hypothalamic nuclei of the *fafa* rat [11,27], and increased levels of hypothalamic NE in the *dbdb* mouse [31]. A number of abnormal neurochemical and anatomical conditions have been reported in the *obob* mutant. These include increased hypothalamic NE levels [15, 31, 36], increased pituitary dopamine (DA) levels [30], decreased cortical cholecystokinin [39], decreased luteinizing-hormone-releasing hormone activity [34], decreased neuronal size [4], and altered dendritic orientation [5].

Although the precise relationship of the central nervous system abnormalities to the hyperphagia and obesity has not been established in any of these mutations, it is interesting to note that in all cases there are alterations in hypothalamic catecholamines. Since other work [19, 24, 25, 32] has indicated that hypothalamic catecholamine systems may play an important role in the regulation of ingestive behavior, it is possible that genetically-determined abnormalities in these systems lead to alterations in feeding behavior. This suggests that pharmacological manipulation of the catecholamine systems might modify feeding behavior in the mutants.

Initial studies of the effects of catecholamine-modifying drugs on food intake in the *obob* mutant have provided inconclusive results. Feldman and Blalock [14] found that administration of a monoamine oxidase inhibitor to either *obob* or lean mice did not modify the pattern of weight gain in either group. In contrast, Batt *et al.* [3] found that administration of the tyrosine hydroxylase inhibitor, α-methyltyrosine, produced significant increases in food intake in the *obob* mice. Since other work indirectly indicates that *obob* mice may have decreased postsynaptic activity of NE [35,37], it is possible that the α-methyltyrosine treatment potentiated this effect by decreasing the amount of noradrenergic neurotransmitter available for release. If this is the case, then agents which increase the activity of the noradrenergic systems might decrease feeding in the *obob* mutant.

To study this possibility the catecholamine-releasing agent, amphetamine [2,9], was administered to *obob* mice and their lean littermate controls. The effects of the amphetamine treatment on food intake and brain catecholamines were then compared between the two groups. In addi-

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tion, plasma glucose levels were measured to determine if the hyperglycemic features of the obesity syndrome in the *obob* mouse [6] would be altered by the drug treatment.

METHOD

Subjects were female C57BL/6J-*ob* mice (*ob/ob*) and their lean littermates (*OB/?*) obtained from the Jackson Laboratories, Bar Harbor, ME. Prior to the beginning of the experiments the mice had ad lib access to both water and food (Purina Lab Chow Pellets) and were maintained on a reverse day-night cycle (lights off 9:00 a.m. to 9:00 p.m.).

In order to study the effects of amphetamine treatment on food intake the animals were adapted to a 6-hour feeding schedule and to the injection procedures using the following format: Animals were food-deprived for 18 hours, and one-half hour prior to being allowed access to food they received an IP injection of isotonic saline (0.01 ml/g body weight). At the time of injection (3 hours after the beginning of the dark phase of the light-dark cycle) the mice were removed from their home cages (group housing, 3–5 mice per cage) and placed in individual testing cages. They were given a preweighed amount of food (Purina Lab Chow Pellets) 30 minutes later. Food intake was determined three and six hours later by weighing the remaining pellets, taking special care to collect spillage. After 6 hours of food access the mice were returned to the group-housing conditions. This procedure was followed until food intake had stabilized for at least seven consecutive days. When this criterion was reached half of the animals received an IP injection of d-amphetamine sulfate (calculated as the base, dissolved in saline; Sigma Chemical Co.) in place of the saline injection. Drug injections were administered for a period of 6 to 14 days, with half of each group (*obob* and lean) receiving amphetamine injections and half receiving saline injections. The dose of amphetamine was 3, 5, or 10 mg/kg, depending upon the conditions of the specific experiment.

On the day following the end of the period of behavioral testing the animals received their usual drug dose and were sacrificed by decapitation either 1.5 or 5.0 hours after drug administration. The telencephalon and hypothalamus were removed [31], frozen in liquid nitrogen, and stored at −80°C until analyzed for NE and DA content. Hypothalamic content of NE and DA was determined by the radioenzymatic method of Coyle and Henry [10] and telencephalic content of NE and DA was determined by the fluorimetric method of Jacobowitz and Richardson [20]. Neurochemical values are reported as μg of amine per gram fresh weight of brain. In some experiments trunk blood was collected for determination of plasma glucose levels (glucose oxidase-peroxidase method; Sigma Technical Bulletin No. 510).

Four experiments were conducted. These experiments examined the effects of drug dose and the number of days of testing on food intake, and the effect of the time of sacrifice after final drug treatment on the neurochemical variables. In Experiment 1 the mice were treated with 0, 3, or 10 mg/kg of amphetamine on each day and food intake was measured for 6 days. On the seventh day the mice were sacrificed 5 hours after drug treatment. In Experiment 2 the mice were treated each day with either 0 or 3 mg/kg of amphetamine and food intake was measured for 11 days. On day 12 the mice were sacrificed 1.5 hours after drug treatment. In Experiment 3 lean mice were treated each day with 0 or 10 mg/kg and *obob* mice were treated with 0, 5, or 10 mg/kg of amphetamine. Food intake was measured for six days and on the seventh day the mice were sacrificed 1.5 hours following drug treatment. In Experiment 4 the mice were treated each day with either 0 or 10 mg/kg of amphetamine and food intake was measured for 14 days. This experiment was designed to determine if behavioral tolerance to the anorectic effect of the high dose of amphetamine would develop; biochemical data were not collected for this experiment.

Since the obese animals weighed substantially more than the lean mice, they received larger absolute amounts of amphetamine. Under these conditions if there were a differential tissue distribution of amphetamine in the two groups this might result in different brain concentrations of amphetamine in lean and obese mice. To examine this possibility brain levels of amphetamine were determined by injecting the mice (IP) with either a 3 or 10 mg/kg dose of amphetamine containing a 150 μCi/kg dose of (^{3}H)-d-amphetamine sulfate (New England Nuclear; specific activity=16.5 Ci/mmol). The mice were sacrificed 1.5, 4, or 6.5 hours after treatment and the regional brain levels of (^{3}H)-amphetamine were measured by the method of Glowinski *et al.* [17]. In brief, hypothalamic and telencephalic tissue was removed, homogenized in 0.4 N perchloric acid, and centrifuged (6,000 g). Two ml aliquots of the supernatant were added to 0.5 ml of 3 N sodium hydroxide and the ^{3}H-amphetamine was then extracted into 6 ml of a toluene/isoamyl alcohol solution (50:1) shaking for 10 minutes. This mixture was centrifuged (60 g) and a 4 ml aliquot of the organic phase was transferred to scintillation vials containing 5 ml of Beckman Ready Solv GP and counted in a Beckman scintillation counter. Amphetamine levels were normally determined following acute amphetamine treatment although in one case the levels were determined in a group of mice adapted to the restricted food access schedule and given chronic amphetamine treatment (11 days, 10 mg/kg).

To determine if the food-restriction schedule would modify the neurochemical parameters, a group of lean animals were maintained on the schedule for 26 days and then compared to a group of mice fed ad lib.

Food intake data were analyzed using an analysis of variance with repeated measures factor for days. For the catecholamine assays only one measure was being compared and the data were analyzed using either a simple analysis of variance or a *t*-test. Differences in sample sizes between behavioral and biochemical data are due to loss of the sample in the biochemical assay. Table values are presented as the Mean±Standard Deviation.

RESULTS

Acclimation Period

No significant differences in either hypothalamic or telencephalic NE or DA levels were found between the mice maintained on the restricted food schedule and those fed ad lib (Table 1). These data indicate that the food-restriction schedule itself did not produce significant changes in the neurochemical variables under consideration.

The amounts eaten during the 6 hours of food access on the last day of the acclimation period for each of the four experiments are presented in Table 2. These results show that at the end of this period the obese mice were consuming significantly more food than lean mice (*obob*=3.4 g, lean=2.4 g, all experiments). As expected, the total food intake of the lean and obese mice for the 6-hour period of food access was less than that found for a 24-hour period of

TABLE 1

BRAIN CATECHOLAMINE LEVELS IN LEAN MICE MAINTAINED ON EITHER THE RESTRICTED OR AD LIB FOOD ACCESS SCHEDULE

Brain section	Amine	Amine Concentration* (μg/g) Ad lib Access	Restricted Access
Hypothalamus	NE	2.00 ± 0.24 (n=9)	2.00 ± 0.09 (9)
	DA	0.33 ± 0.09 (9)	0.34 ± 0.09 (9)
Telencephalon	NE	0.40 ± 0.03 (9)	0.40 ± 0.05 (9)
	DA	1.45 ± 0.30 (8)	1.31 ± 0.08 (9)

*Mean ± S.D.

TABLE 2

TOTAL SIX-HOUR FOOD INTAKE OF LEAN AND *obob* MICE ON THE LAST DAY OF THE ACCLIMATION PERIOD

Experiment	Food Intake* (g) lean	*obob*
1	2.5 ± 0.3 (n=15)	3.5 ± 0.4† (15)
2	2.4 ± 0.3 (15)	3.5 ± 0.6† (15)
3	2.5 ± 0.3 (15)	3.2 ± 0.5† (15)
4	2.4 ± 0.3 (15)	3.4 ± 0.3† (15)

*Mean ± S.D.
†Differs from lean values, $p<0.01$.

ad lib food access (lean~4.0; *obob*~5.3 g, see [36]). However, the percent excess in food intake of the obese mice (+39%) compared to the lean mice was similar to that found for 24-hour feeding periods (about +30%; see [21, 28, 33]). This hyperphagia continued throughout the treatment period for the saline-treated *obob* mice in all 4 experiments.

Effects of Amphetamine Treatment on Food Intake

Analysis of the data indicated that the primary anorectic effect of amphetamine treatment was during the initial 3 hours of food access, while the subsequent 3 hours constituted a recovery period (Figs. 1–4). This is consistent with the expected time-course for amphetamine in mice [13]. Consequently, the behavioral and neurochemical data are presented in terms of these two phases.

Hours 0–3

In Fig. 1A the effects of the saline, 3 and 10 mg/kg treatments over hours 0–3 can be compared for a 6-day treatment period (Experiment 1). During the first three hours of food access saline-treated *obob* ate significantly more than saline-treated lean mice, $F(1,24)=7.53$, $p<0.05$. The low dose of amphetamine (3 mg/kg) produced a significant reduction in food intake in both lean (−27%, $F(1,24)=13.3$, $p<0.001$) and *obob* (−30%, $F(1,24)=29$, $p<0.001$) mice compared to their respective saline-injected controls. Increasing the dose to 10 mg/kg produced a larger reduction in food intake in both *obob* (−78%, $F(1,24)=175$, $p<0.001$) and lean (−74%, $F(1,24)=105$, $p<0.001$) animals. In addition, a direct comparison of the effects of amphetamine treatment between the lean and obese mice indicated that drug treatment produced comparable food intake in these two groups at both the 3 mg/kg, $F(1,24)=0.96$, $p>0.20$, and 10 mg/kg, $F(1,24)=0.05$, $p>0.20$, doses, thus eliminating the difference found between the lean and *obob* mice receiving saline injections.

The effects of longer periods of treatment with the 3 and 10 mg/kg doses can be seen in Figs. 2A and 3A, respectively (Experiments 2 and 4). Analysis of the data for Experiment 2 indicated that during the 11 days of amphetamine treatment the 3 mg/kg dose produced significant reductions in food intake in both lean and *obob* mice compared to their saline-treated controls (lean mice, $F(1,26)=11.8$, $p<0.01$; *obob* mice, $F(1,26)=18.5$, $p<0.01$). This reduction resulted in comparable levels of food intake in both groups of treated mice, $F(1,26)=0.62$, $p>0.10$. In Experiment 4 the 10 mg/kg dose also produced a significant reduction in the food intake of lean and *obob* mice when compared to the food intake of saline controls during the 14-day period (lean mice, $F(1,26)=73.6$, $p<0.01$; *obob* mice, $F(1,26)=165$, $p<0.01$). Direct comparison of the daily food intake in the drug-treated groups indicated that the *obob* and lean animals did not differ in the amount eaten during days 1–9, $F(1,26)=2.42$, $p>0.10$, but that on days 10–14 the lean animals ate significantly more than the obese animals, $F(1,12)=6.2$, $p<0.05$. A trend analysis of the data obtained from the amphetamine-treated lean mice indicated that there was a progressive increase in food intake by these animals during the treatment period, $F(1,12)=30$, $p<0.001$. This effect was not found in the *obob* mice. This may represent the development of behavioral tolerance to the anorectic effects of amphetamine in the lean mice.

In Experiment 3 (0, 5, 10 mg/kg doses) the effects of the 10 mg/kg dose essentially replicated the findings of Experiments 1 and 4 (Fig. 4). Other results of Experiment 3 will be presented in more detail following presentation of the drug-distribution data.

Hours 3–6

During the second three-hour period of food access (hours 3–6), amphetamine treatment no longer had an anorectic effect. The results of Experiment 1 (6 days of drug treatment) are shown in Fig. 1B. These results indicate that amphetamine-treated lean mice ate 86% (3 mg/kg group) and 109% (10 mg/kg group) of the amount eaten by their saline-

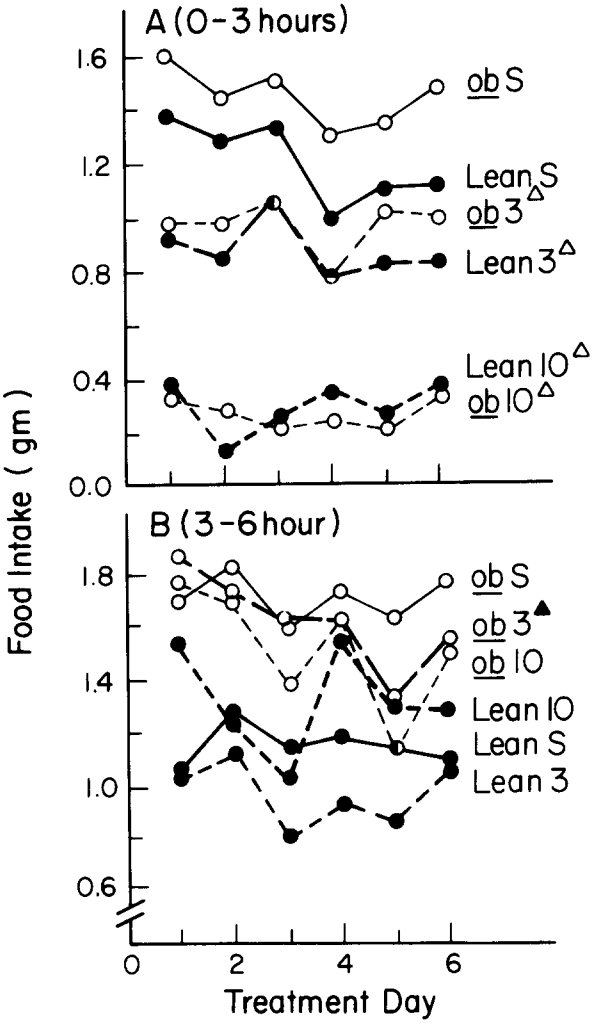

FIG. 1. Experiment 1. The daily average food intakes of lean (●) and *obob* (○) mice treated with saline (−), 3 mg/kg (− −) or 10 mg/kg (---) of amphetamine for a six-day period are presented for the 0–3 hour and 3–6 hour periods. Panel A; hours 0–3. Saline-treated *obob* mice ate more than saline-treated lean mice. Amphetamine treatment significantly reduced food-intake in both lean and *obob* mice. Lean and *obob* mice treated with the same dose of amphetamine were not significantly different in food-intake. Panel B; hours 3–6. The *obob* mice treated with saline or 3 mg/kg of amphetamine ate more than similarly treated lean mice. The *obob* and lean mice treated with 10 mg/kg of amphetamine were not significantly different in food intake during this period. △ Differs significantly from saline treated group with same genotype. ▲ Differs significantly from similarly treated lean group.

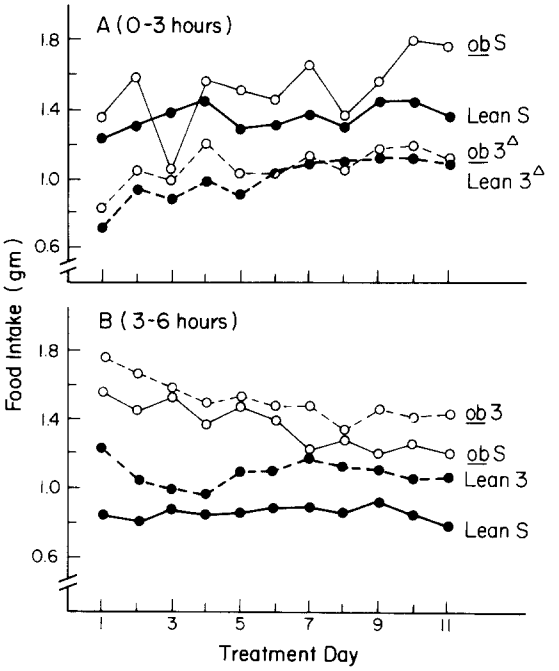

FIG. 2. Experiment 2. The daily average food-intakes of lean (●) and *obob* (○) mice treated with saline (−) or 3 mg/kg (− −) of amphetamine for 11 days are presented for the 0–3 hour and 3–6 hour periods. Panel A; hours 0–3. Amphetamine-treated lean and *obob* mice ate significantly less than saline-treated controls, but did not differ from each other. Panel B; hours 3–6. Amphetamine-treated *obob* mice ate significantly more than amphetamine-treated lean mice. Amphetamine-treated mice did not differ from their respective saline-treated controls. △ Differs significantly from saline treated group with same genotype.

treated controls, while *obob* mice ate 95% (3 mg/kg group) and 89% (10 mg/kg group) of the amount eaten by their saline-treated controls. None of these values differed significantly from control values. Increasing the length of treatment (14 days) with the high amphetamine dose (10 mg/kg, Experiment 4, Figure 3B) produced a significant increase in the food intake of drug-treated lean mice during the last 10 days of treatment when compared to the food consumption of saline-treated lean mice during the same period. In contrast, the food consumption of amphetamine-treated *obob* mice did not differ significantly from that of saline-treated *obob* controls during this period. Thus, the amphetamine-treated lean mice showed a rebound hyperphagia over the last 10 days of treatment. Direct comparison of the amphetamine-treated groups in this experiment indicates that the lean and obese mice did not differ significantly in food intake during the 3–6 hour period, $F(1,26)=2.69$, $p>0.10$.

Food intake during the 3–6 hour period was not significantly increased in amphetamine-treated lean and *obob* mice compared to saline-treated controls (lean mice, $F(1,26)=3.9$, $p<0.05$; *obob* mice, $F(1,26)=1.4$, $p>0.05$) after extended treatment (11 days) with the 3 mg/kg dose of amphetamine (Fig. 2B). Direct comparison between *obob* and lean mice indicated that the obese mice ate more than the lean mice during the 3–6 hour period, $F(1,26)=12.9$, $p<0.001$.

In summary, amphetamine treatment produced an initial anorectic phase (hours 0–3) followed by a recovery phase (hours 3–6). During the anorectic phase the reduction in food intake was dose-dependent for both lean and obese mice. Furthermore, although saline-treated obese mice ate more than saline-treated lean mice during the 0–3 hour period, both the low (3 mg/kg) and high (10 mg/kg) doses of amphetamine decreased food intake in the obese mice to amounts which were not significantly different from those of comparably treated lean mice. During the recovery phase

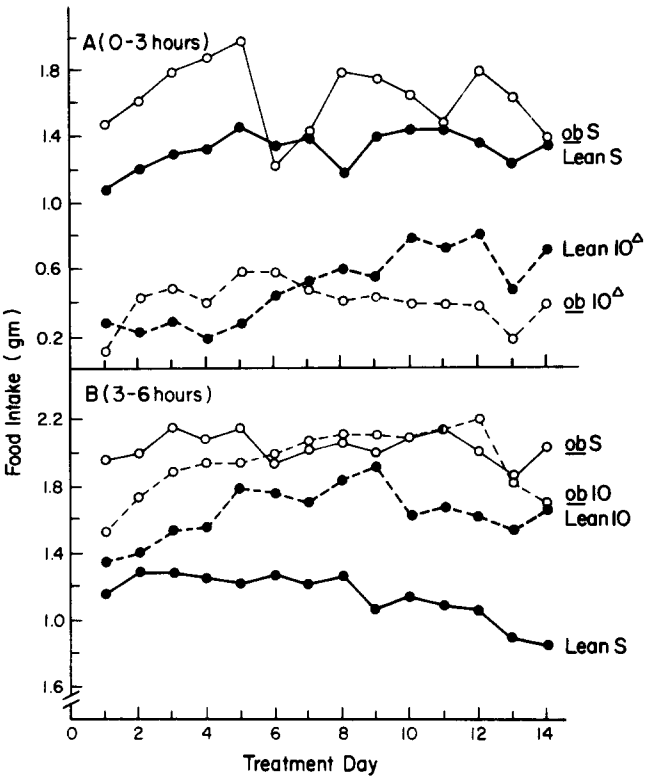

FIG. 3. Experiment 4. The daily average food-intakes of lean (●) and *obob* (○) mice treated with saline (–) or 10 mg/kg of amphetamine (---) for 14 days are presented for the 0–3 hour and 3–6 hour periods. Panel A; hours 0–3. Saline-treated *obob* mice ate more than saline-treated lean mice. Amphetamine-treated mice ate significantly less than their respective control groups. For days 1–9 amphetamine-treated lean and *obob* mice were not different, but on days 10–14 lean mice ate significantly more than *obob* mice. Panel B; hours 3–6. Saline-treated *obob* mice and amphetamine-treated lean and *obob* mice all ate more than saline-treated lean mice, but were not significantly different from each other. Δ Differs significantly from saline treated group with same genotype.

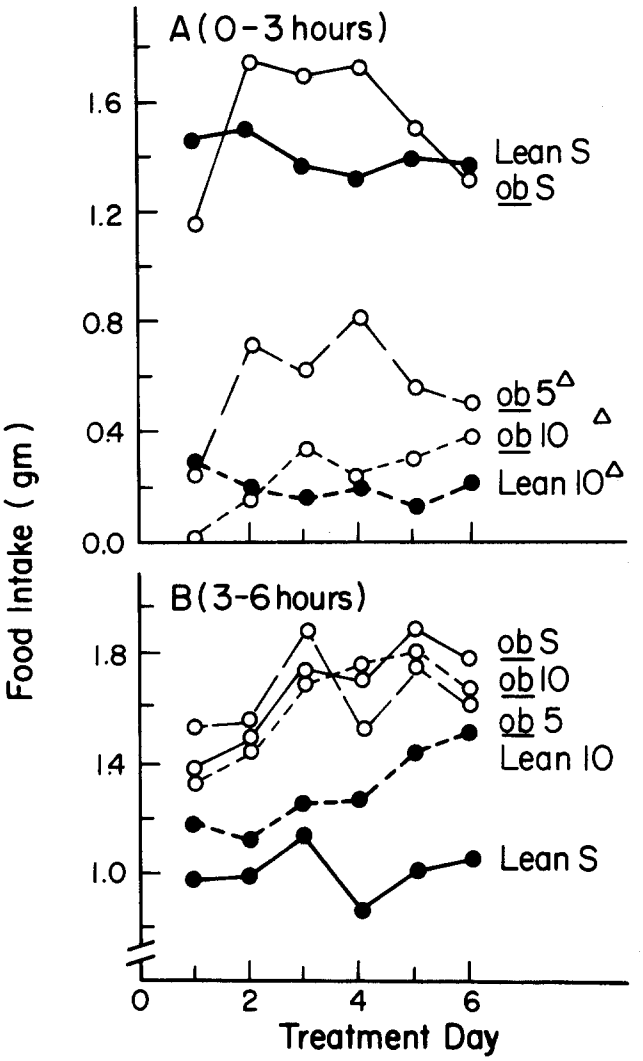

FIG. 4. Experiment 3. The daily average food-intakes of lean (●) and *obob* (○) mice treated with saline (–) 5 mg/kg (– –) or 10 mg/kg (---) of amphetamine are presented for the 0–3 hour and 3–6 hour periods. Panel A; hours 0–3. Amphetamine-treated mice ate significantly less than their respective controls. The *obob* mice treated with 5 mg/kg of amphetamine ate more than either lean or *obob* mice treated with 10 mg/kg of amphetamine, while the latter two groups were not significantly different. Panel B; hours 3–6. Saline-treated lean mice ate less than all other groups. Δ Differs significantly from saline treated group with same genotype.

(hours 3–6) both lean and *obob* mice treated with the low dose of amphetamine (3 mg/kg) ate amounts which were not significantly different from their respective controls over an 11-day period. With the high dose of amphetamine (10 mg/kg) the lean mice showed a significant hyperphagia during the last 10 days (out of 14) of treatment. This hyperphagia increased food-intake values in the amphetamine-treated lean mice to levels which were comparable to those of obese mice (both treated and control) during this same period.

Effects of Amphetamine Treatment on Brain Catecholamine Levels

To obtain neurochemical measures corresponding to the phases of the distinct behavioral effects of amphetamine, the animals were sacrificed either 1.5 (anorectic period) or 5.0 (recovery period) hours after amphetamine injection on the day following the completion of the behavioral testing.

In confirmation of the findings of other studies [15, 31, 37], hypothalamic NE levels in saline-treated *obob* mice were significantly higher (+22% for all experiments) than those in saline-treated lean mice (Tables 3 and 5). Hypothalamic and telencephalic DA levels did not differ significantly between these two groups (Tables 3–5).

Following 11 days of treatment with the low dose of amphetamine (3 mg/kg; Experiment 2) there was a significant reduction in hypothalamic NE levels in both lean (89% of saline-treated lean control) and *obob* (84% of saline-treated *obob* control) mice (Table 3) 1.5 hours after drug treatment. A direct comparison between the two groups of drug-treated animals indicates that at this time there was no significant

TABLE 3

HYPOTHALAMIC CATECHOLAMINE LEVELS OF LEAN AND *obob* MICE AFTER AMPHETAMINE TREATMENT (1.5 HOURS POST-INJECTION)

Experiment	Drug	Days of Treatment	Amine	Amine Concentration* (μg/g) lean	*obob*
2	Saline	11	NE	1.70 ± 0.06 (n=7)	1.96 ± 0.24† (7)
			DA	0.58 ± 0.25 (7)	0.57 ± 0.11 (7)
2	Amphetamine (3 mg/kg)	11	NE	1.51 ± 0.15‡ (8)	1.64 ± 0.14‡ (8)
			DA	0.43 ± 0.04 (8)	0.43 ± 0.08‡ (8)
3	Saline	6	NE	2.46 ± 0.31 (8)	3.10 ± 0.58† (5)
			DA	0.47 ± 0.14 (8)	0.55 ± 0.09 (5)
3	Amphetamine (5 mg/kg)	6	NE	—	2.23 ± 0.38‡ (4)
			DA	—	0.45 ± 0.06 (5)
3	Amphetamine (10 mg/kg)	6	NE	1.94 ± 0.23‡ (6)	2.04 ± 0.26‡ (4)
			DA	0.36 ± 0.06 (6)	0.44 ± 0.15 (4)

*Mean ± S.D.
†Differs from respective lean value in same experiment, $p<0.05$.
‡Differs from respective saline value in same experiment, $p<0.05$.

difference in hypothalamic NE levels between the lean and *obob* mice. The low-dose amphetamine treatment did not alter hypothalamic DA levels in lean mice, $t(13)=1.7$, $p>0.05$, but did significantly reduce hypothalamic DA levels in *obob* mice, $t(13)=2.8$, $p<0.05$. The low-dose amphetamine treatment also changed telencephalic catecholamine levels (Table 4), producing significant increases in telencephalic DA (both lean and *obob* mice) and a significant decrease in telencephalic NE levels (*obob* mice only).

At 1.5 hours after drug treatment the high dose of amphetamine (10 mg/kg; Experiment 3) produced even larger decreases in hypothalamic NE levels than the low dose of amphetamine. Hypothalamic NE levels in lean and *obob* mice were 79% and 66%, respectively, of their saline-treated controls (Table 3). As in the case of the low-dose amphetamine treatment, lean and *obob* mice did not differ significantly from each other in hypothalamic NE levels following treatment with 10 mg/kg of amphetamine. The high dose of amphetamine did not produce any significant changes in hypothalamic DA in either obese or lean animals (Table 3), but did produce significant decreases in telencephalic NE in both groups (Table 4).

These results indicate that the amphetamine treatment produced multiple effects upon brain catecholamines in both lean and obese mice 1.5 hours after drug treatment. However, comparison of the behavioral (Figs. 3A and 4A) and neurochemical data (Tables 3 and 4) indicates that the only change in catecholamine levels which consistently corresponded to the anorectic effect of the drug treatment was the decrease in the hypothalamic NE content. In this respect, the period of equivalent food intake by the lean and obese mice corresponded to the period during which hypothalamic NE levels were not significantly different between the two groups. This applied to both the 3 and 10 mg/kg amphetamine treatments.

The neurochemical results obtained from animals sacrificed at a time corresponding to the period of behavioral recovery are presented in Table 5. Five hours after the administration of the low dose of amphetamine (3 mg/kg) there were no significant differences in hypothalamic NE levels between the amphetamine-treated groups and their respective controls (lean=98% of saline-treated control; *obob*=106% of saline-treated control). Direct comparison of the two drug-treated groups indicated that at this time the hypothalamic NE levels were significantly higher in *obob* than in lean mice, $t(8)=4.6$, $p<0.01$. These results correspond to the time period in which food intake in amphetamine-treated *obob* mice was significantly higher than in amphetamine-treated lean mice, but when neither of these groups differed from their respective saline-treated controls (Fig. 1B). At this time hypothalamic DA levels were significantly increased, and telencephalic NE and DA levels significantly decreased in amphetamine-treated *obob* mice when compared to saline-treated *obob* controls. There were no significant changes in these latter measures in lean mice.

These results indicate that following chronic treatment (6 days) with the low dose of amphetamine the hypothalamic NE levels had returned to control levels in both lean and *obob* mice within 5 hours of treatment. This in turn suggests that the significant decreases in hypothalamic NE levels found 1.5 hours after chronic treatment with the 3.0 mg/kg dose represent a decrease in content produced on the day of treatment.

Five hours after chronic treatment with the high dose of amphetamine (10 mg/kg) there was still a significant decrease in hypothalamic NE levels in *obob* mice (81% of saline-treated *obob* control), but not in lean (89% of saline-treated lean control). When compared to the hypothalamic NE levels 1.5 hours following chronic treatment with 10 mg/kg of amphetamine (*obob*=66% of saline-treated *obob* control; lean=79% of saline-treated control), these results indicate partial or complete recovery of hypothalamic NE levels within 5.0 hours of the drug treatment. Direct comparison of amphetamine-treated lean and obese mice indicated that the

TABLE 4

TELENCEPHALIC CATECHOLAMINE LEVELS OF LEAN AND *obob* MICE AFTER AMPHETAMINE TREATMENT (1.5 HOURS POST-INJECTION)

Experiment	Drug	Days of Treatment	Amine	Amine Concentration* (μg/g) lean	*obob*
2	Saline	11	NE	0.35 ± 0.02 (n=7)	0.41 ± 0.03† (7)
			DA	1.68 ± 0.15 (7)	1.72 ± 0.12 (7)
2	AMPH (3 mg/kg)	11	NE	0.35 ± 0.03 (8)	0.32 ± 0.03‡ (8)
			DA	2.00 ± 0.16‡ (8)	1.95 ± 0.22‡ (8)
3	Saline	6	NE	0.46 ± 0.06 (8)	0.58 ± 0.13† (5)
3	AMPH (5 mg/kg)	6	NE	—	0.37 ± 0.02‡§ (5)
3	AMPH (10 mg/kg)	6	NE	0.29 ± 0.07‡ (6)	0.35 ± 0.09‡ (5)

*Mean ± S.D.
†Differs from respective lean value in same experiment, $p<0.05$.
‡Differs from respective saline value in same experiment, $p<0.05$.
§Differs from 10 mg/kg amphetamine-lean value in same experiment, $p<0.05$.

hypothalamic NE levels did not differ significantly between these two groups (Table 5). This corresponds to the period in which the food intakes of these two groups (Figs. 1B and 3B) were not significantly different. Hypothalamic and telencephalic DA levels in amphetamine-treated mice did not differ significantly from those in their respective saline-treated controls, while telencephalic NE levels were significantly decreased in both *obob* and lean mice at this time (Table 5).

Correlation of Food Intake and Neurochemical Results

When behavioral effects were considered in conjunction with the neurochemical results, the best correspondence found was between the amount of food eaten and hypothalamic NE levels. Because of the inter-experiment variability in brain amine assays, in order to compare the neurochemical and behavioral results across experiments the neuro-

TABLE 5

HYPOTHALAMIC AND TELENCEPHALIC CATECHOLAMINE LEVELS OF LEAN AND *obob* MICE AFTER 6 DAYS OF AMPHETAMINE TREATMENT (EXPERIMENT 1, 5.0 HOURS POST-INJECTION)

Brain Section	Drug Treatment	Amine	Amine Concentration* (μg/g) lean	*obob*
Hypothalamus	Saline	NE	2.01 ± 0.18 (n=6)	2.28 ± 0.24† (6)
		DA	0.43 ± 0.06 (6)	0.50 ± 0.08 (6)
Hypothalamus	AMPH (3 mg/kg)	NE	1.96 ± 0.17 (5)	2.43 ± 0.15† (5)
		DA	0.52 ± 0.13 (4)	0.68 ± 0.04†‡ (5)
Hypothalamus	AMPH (10 mg/kg)	NE	1.79 ± 0.28 (5)	1.84 ± 0.20‡ (5)
		DA	0.55 ± 0.15 (4)	0.46 ± 0.05 (5)
Telencephalon	Saline	NE	0.38 ± 0.06 (6)	0.52 ± 0.03† (6)
		DA	1.43 ± 0.19 (6)	1.61 ± 0.14 (6)
Telencephalon	AMPH (3 mg/kg)	NE	0.37 ± 0.05 (5)	0.43 ± 0.03‡ (5)
		DA	1.37 ± 0.15 (5)	1.39 ± 0.10‡ (5)
Telencephalon	AMPH (10 mg/kg)	NE	0.31 ± 0.03‡ (5)	0.30 ± 0.06‡ (5)
		DA	1.45 ± 0.18 (5)	1.61 ± 0.22 (5)

*Mean ± S.D.
†Differs from respective lean value, $p<0.05$.
‡Differs from respective saline value, $p<0.05$.

TABLE 6

BRAIN AMPHETAMINE LEVELS OF LEAN AND *obob* MICE FOLLOWING TREATMENT WITH EITHER 3 OR 10 mg/kg OF AMPHETAMINE

Tissue	Amphetamine Dose	Time Post-Injection	Amphetamine Concentration* (μg/g) lean	*obob*
Hypothalamus	3 mg/kg	1.5 hr	0.76 ± 0.04 (n=3)	1.66 ± 0.13† (3)
Telencephalon	3 mg/kg	1.5 hr	0.93 ± 0.06 (3)	1.91 ± 0.16† (3)
Hypothalamus	10 mg/kg	1.5 hr	4.10 ± 1.00 (10)	9.06 ± 1.47† (10)
Telencephalon	10 mg/kg	1.5 hr	5.34 ± 1.31 (10)	11.01 ± 1.42† (10)
Hypothalamus	10 mg/kg	4.0 hr	0.54 ± 0.07 (5)	0.91 ± 0.26† (3)
Telencephalon	10 mg/kg	4.0 hr	0.67 ± 0.11 (5)	1.10 ± 0.29† (3)
Hypothalamus	10 mg/kg	6.5 hr	0.17 ± 0.03 (4)	0.43 ± 0.15† (4)
Telencephalon	10 mg/kg	6.5 hr	0.20 ± 0.03 (4)	0.48 ± 0.14† (4)

*Mean ± S.D.
†Differs from respective lean value, $p<0.05$.

chemical data for each specific experiment were converted to a percent of the average value of the saline-treated lean control for that experiment. These values were then correlated with the animals average food intake for hours 0–3 over the last three days of the experiment. The Pearson product-moment correlation coefficient between the hypothalamic NE levels and food intake was 0.63 ($p<0.001$) for all animals (n=57). In comparison, the correlation between hypothalamic DA levels and food intake was only 0.16 ($p>0.10$).

Brain Amphetamine Levels

As a result of their different body weights the *obob* and lean mice received different total amounts of amphetamine. If the amphetamine was not distributed equally to all tissue this could result in different brain concentrations of the drug in the two groups. To assess this possibility hypothalamic and telencephalic concentrations of amphetamine were determined in lean and *obob* mice by administering (^{3}H)-amphetamine. A comparison of brain amphetamine levels in mice treated chronically (11 days, 10 mg/kg) with mice treated acutely indicated that there were no significant differences in either hypothalamic (lean chronic = 4.0±1.5 μg/g, lean acute = 4.2±0.6 μg/g; *obob* chronic = 8.6±1.9 μg/g, *obob* acute = 9.3±1.2 μg/g) or telencephalic (lean chronic = 54±1.6 μg/g), lean acute = 5.3±1.2 μg/g; *obob* chronic = 11.0±1.4 μg/g, *obob* acute = 11.0±0.6) amphetamine concentrations in the two groups at 1.5 hours post-injection. These results suggest that there was little or no effect of chronic amphetamine treatment on the delivery of amphetamine to the various brain areas, a finding which is in agreement with the results of other investigators [7]. Consequently the data were combined and are presented along with the other acute treatment data in Table 6.

At 1.5 hours following drug treatment the hypothalamic (and telencephalic) levels of amphetamine in *obob* mice were about double the level in lean mice. This ratio was almost equivalent for the 3 mg/kg (*obob*/lean=2.18) and the 10 mg/kg (*obob*/lean=2.20) doses, and approximates the ratio between the body weights of *obob* and lean mice in these studies (*obob*/lean=2.6 for 3 mg/kg study and 2.4 for 10 mg/kg study). These results suggest that during the period of maximum anorexia the concentration of amphetamine in the hypothalamus (and telencephalon) of the obese mice was approximately twice that in the lean mice. A similar difference was found for the later time points as well. The magnitude of the difference was probably a result of the larger absolute dose of amphetamine administered to the obese animals.

TABLE 7

PLASMA GLUCOSE LEVELS IN LEAN AND *obob* MICE AFTER 6 DAYS OF AMPHETAMINE TREATMENT

Drug Treatment	Glucose Levels* (mg/100 ml plasma) lean	*obob*
Saline	162 ± 13 (n=6)	520 ± 89† (3)
Amphetamine (3 mg/kg)	141 ± 15‡ (5)	165 ± 15‡ (4)
Amphetamine (10 mg/kg)	152 ± 13 (6)	143 ± 37‡ (4)

*Mean ± S.D.
†Differs from the respective lean value, $p<0.01$.
‡Differs from the respective saline value, $p<0.05$.

Effects of "Equalized" Brain levels of Amphetamine on Food Intake and Brain Catecholamines

Significant differences in the brain concentration of amphetamine were found between lean and obese mice when the drug dose was calculated on a body-weight basis. To compensate for this difference a study was conducted in which an attempt was made to "equalize" the brain concentrations of amphetamine in the lean and obese mice. Analysis of the drug distribution data revealed that within all experiments the most consistent factor relating to the differences in brain levels was the total amount of drug administered. This

TABLE 8

BODY WEIGHTS OF LEAN AND *obob* MICE AFTER AMPHETAMINE TREATMENT (MEAN ± S.D.)

Study	Group	Treatment (mg/kg)	n	Days of Treatment	Body Weight (g) Day 1	Body Weight (g) Last Day	Change in Body Weight
Experiment 1	lean	Saline	5	6	23.6 ± 1.2	24.2 ± 1.3	+0.6 ± 0.32*
	obob	Saline	5	6	50.3 ± 1.4	51.0 ± 1.6	+0.7 ± 0.34*
	lean	Amph (3)	5	6	23.7 ± 0.7	24.3 ± 0.7	+0.6 ± 0.12†
	obob	Amph (3)	5	6	51.1 ± 3.2	51.4 ± 2.8	+0.3 ± 0.80
	lean	Amph (10)	5	6	22.8 ± 1.1	22.7 ± 1.6	−0.1 ± 1.09
	obob	Amph (10)	5	6	51.9 ± 1.9	50.6 ± 2.7	−1.3 ± 0.95*
Experiment 2	lean	Saline	7	11	23.2 ± 1.1	22.8 ± 1.1	−0.4 ± 0.58
	obob	Saline	7	11	47.7 ± 3.0	47.3 ± 3.3	−0.4 ± 0.78
	lean	Amph (3)	8	11	23.5 ± 1.4	23.0 ± 1.5	−0.5 ± 0.60
	obob	Amph (3)	8	11	49.4 ± 3.1	47.8 ± 3.3	−1.6 ± 0.71†
Experiment 3	lean	Saline	8	6	25.8 ± 2.3	25.9 ± 2.3	+0.1 ± 0.62
	obob	Saline	5	6	60.3 ± 3.8	60.5 ± 3.5	+0.2 ± 0.41
	obob	Amph (5)	5	6	61.2 ± 4.4	59.1 ± 4.1	−2.1 ± 0.44†
	lean	Amph (10)	7	6	25.7 ± 1.2	24.4 ± 1.0	−1.3 ± 0.54†
	obob	Amph (10)	5	6	62.0 ± 2.2	58.4 ± 2.8	−3.6 ± 0.81†
Experiment 4	lean	Saline	8	14	25.2 ± 2.0	26.2 ± 1.5	+1.0 ± 1.10*
	obob	Saline	8	14	59.2 ± 3.2	59.5 ± 4.0	+0.3 ± 1.25
	lean	Amph (10)	7	14	25.0 ± 1.6	23.4 ± 4.0	−1.6 ± 1.24*
	obob	Amph (10)	7	14	57.8 ± 2.7	51.9 ± 2.0	−5.9 ± 1.51†

*Significant change in body weight, $p<0.05$.
†Significant change in body weight, $p<0.01$.

in turn was related to the body weights. Since the ratio of the body weights of the obese and lean animals used in this study (*obob*/lean=2.6 on the first day of drug treatment and 2.3 on the last day) was about the same as the body weight ratios of the animals used in the ^{3}H-amphetamine study (see above), the obese animals were divided into two groups with one group receiving the same weight-determined dose as lean animals (10 mg/kg) and the other obese group receiving one-half of this dose (5 mg/kg).

The effects of this treatment on food intake are presented in Fig. 4A. Amphetamine treatment again produced a significant decrease in food intake during the first three hours of treatment as compared to saline-treated controls, $F(2,25)=134$, $p<0.001$. Direct comparison between the amphetamine-treated groups indicated that *obob* mice treated with 5 mg/kg of amphetamine ate significantly more than lean and *obob* mice treated with 10 mg/kg of amphetamine, $F(1,25)=7.3$, $p<0.05$, while the latter two groups did not differ from each other, $F(1,25)=0.24$, $p>0.10$. Inspection of Figure 4A also indicates, however, that on the first day of treatment *obob* mice treated with 5 mg/kg of amphetamine ate an amount nearly identical to that of lean mice treated with 10 mg/kg of amphetamine.

The 5 mg/kg amphetamine treatment produced a significant reduction in hypothalamic NE levels (72% of saline-treated *obob* control). When compared to the 10 mg/kg treatment groups, hypothalamic NE levels were slightly higher in the 5 mg/kg group, although the difference was not statistically significant, $t(12)=1.6$, $p>0.05$ (Table 3). However, analysis of the individual data indicated that hypothalamic NE values for 3 of the 4 *obob* mice were higher than the highest lean value.

Plasma Glucose Levels

Plasma glucose levels were determined in a group of lean and *obob* mice maintained on the restricted food schedule and treated with either saline or 3 or 10 mg/kg of amphetamine for 6 days. Although the saline-treated *obob* mice were hyperglycemic compared to saline-treated lean mice (Table 7), amphetamine treatment eliminated this hyperglycemia in *obob* mice, producing plasma glucose levels which were not significantly different from those of saline-treated lean mice. The low dose of amphetamine also produced a small but significant decrease in plasma glucose in the lean mice (−13%, $t(9)=2.5$, $p<0.05$).

Effects of Amphetamine Treatment on Body Weight

The effects of the amphetamine treatment on body weight were determined by comparing each animal's body weight on the first day of drug treatment with its weight on the last day of drug treatment. When analyzed in this manner the saline-treated mice showed either no change (Table 8, Experiments 2 and 3) or a slight gain in body weight (Experiments 1 and 4) over the experimental period. In lean mice the low dose of amphetamine (3 mg/kg) did not significantly reduce body weight (Experiments 1 and 2), while in *obob* mice there was a slight reduction in body weight (1.6 g, −3%) in Experiment 2 (but not in Experiment 1) using this dose.

The major effects on body weight were found in *obob* mice treated with the high dose of amphetamine (10 mg/kg). This dose produced a significant reduction in the body weights of the *obob* mice in every study (Experiments 1, 3, and 4), with the greatest weight loss (5.9 g, −10%) occurring with the longest treatment period (14 days; Experiment 4).

Lean mice treated with the high dose of amphetamine showed significant reductions in body weight in 2 of the 3 studies (Experiments 3 and 4), with the greatest loss (1.6 g, −6%) occurring with the longest treatment period (14 days).

DISCUSSION

Mice with the *obob* mutation develop a syndrome which includes numerous physiological and behavioral abnormalities. Among these abnormalities are hyperglycemia, hyperphagia, and increased hypothalamic NE levels [6,31]. The increased hypothalamic NE levels are particularly interesting because this neurotransmitter is believed to be involved in the regulation of feeding. In this respect the intrahypothalamic application of NE can either elicit or suppress feeding depending upon the site of infusion [24,32].

In the current study we examined the effect of pharmacological activation of the noradrenergic system on the food intake of the *obob* mouse. In order to study these effects the mice were acclimated to a feeding schedule which restricted food access to 6 hours per day. Under this schedule the *obob* mice maintained the hyperphagic and hyperglycemic characteristics typical of *obob* mice fed ad lib. Thus, the schedule allowed expression of major characteristics of the syndrome yet reduced the feeding period to a time frame in which it was possible to study the effect of pharmacological treatment on the feeding behavior. Manipulation of the catecholamine systems was accomplished by administration of amphetamine, a catecholamine-releasing and anorectic agent.

Amphetamine treatment was found to produce a dose-dependent decrease in food intake in both lean and *obob* mice. While saline-treated *obob* mice ate significantly more than saline-treated lean mice, lean and *obob* mice treated with doses of amphetamine calculated on a body-weight basis decreased their food intake to amounts which were not significantly different from each other. Amphetamine treatment also reduced the elevated hypothalamic NE content of *obob* mice to a level which was not significantly different from that found in similarly treated lean mice. Since amphetamine acts in part as a releaser of NE [2,9], it may be that a drug-induced release of NE contributed to the inhibition of feeding. If this were the case, then the results also suggest that the degree of inhibition of feeding is, in part, related to the amount of NE released, because higher doses of amphetamine produce a greater suppression of food intake and a larger decrease in hypothalamic NE levels. However, since the biochemical determinations were made only after chronic amphetamine treatment, it is possible that the decreased levels were actually a result of decreased synthesis produced by the chronic treatment. This appears unlikely since the biochemical data collected at the 5.0 hour sacrifice time, in comparison to the results at the 1.5 hour point, indicated that hypothalamic NE levels had either returned to or were approaching saline-treated control levels. The results of other investigators also indicate that following chronic amphetamine treatment, brain NE synthesis is either unchanged or increased [7, 16, 22, 38], but not decreased.

A comparison between the effects of amphetamine upon the behavioral and neurochemical variables indicated that the highest correlation was between food intake and hypothalamic NE content. Drug-induced changes in other neurochemical measures were at best only inconsistently related to the behavioral changes. Other studies, however, have implicated some of these other catecholamines in the anorectic effects of amphetamine [12, 18, 23]. A review of this work (e.g., [23]) suggests that the effects of catecholamines on ingestive behavior are complicated, and that in the normal rat the anorectic effect of amphetamine may be mediated by more than one neurochemical system. It is necessary to note, however, that several studies have implicated NE as a major neurotransmitter in mediating the anorectic actions of amphetamine [1,26]. In the current study the focus was on the effects of amphetamine upon a neurochemical and behavioral abnormality which has been identified in a genetic mutation. It is, therefore, possible that the results reflect a more easily detected effect of the amphetamine upon this specific neurochemical abnormality, and that this effect may have masked more subtle changes in other system.

Another important aspect of the current study is the observation of the relationship between brain amphetamine content and the effects on food intake and hypothalamic NE levels in lean and *obob* mice. When amphetamine was administered on a body-weight basis, brain amphetamine levels during the period of maximum anorexia were about twice as high in *obob* as in lean mice. This was the case for both acute and chronic amphetamine treatment. Since the obese animals started with higher baseline levels of both hypothalamic NE and food intake than the lean animals, a larger release of NE and a greater suppression of food intake was required in the *obob* mice to decrease these values to the same level to which they had been reduced in the lean animals. Although this was accomplished when the drug was administered on a body-weight basis, the actual brain concentrations of amphetamine were twice as high in *obob* as in lean mice. Thus, if the assessment of brain amphetamine levels had not been made it would have been easy to conclude that the *obob* mice were more sensitive to the amphetamine treatment. When brain amphetamine levels were adjusted to more similar levels in the two groups, the *obob* mice ate significantly more than lean mice over the total treatment period, and had slightly higher brain amine levels. Indeed, preliminary data indicate that in *in vitro* preparations of hypothalamic tissue it may be more difficult to obtain stimulated release of NE from tissue from *obob* mice than from lean mice [8]. This indicates that if inferences are to be made about the drug's site of action and potency, then attention must be paid to the drug's distribution to specific tissue sites when the experimental groups differ in measures such as body weight.

To determine whether tolerance to the anorectic effects of drug treatment would develop, the mice were treated with a relatively high dose of amphetamine (10 mg/kg) for a 14-day period (Experiment 4). It was found that lean mice did show a progressive increase in food intake over the treatment period but that *obob* mice did not. The failure of the *obob* mice to develop tolerance may have been a result of their higher brain levels of amphetamine, as the amphetamine distribution data indicated that after 11 days of chronic treatment the hypothalamic amphetamine levels were still twice as high in *obob* as in lean mice. It is also possible, however, that this failure to demonstrate some tolerance represents an increased period of sensitivity to the drug's anorectic properties. If so, this suggests that under certain circumstances an anorectic agent such as amphetamine might be useful in reducing food-intake on a long-term basis. Additional work is required to evalute this possibility.

In the current study it was shown that amphetamine treatment would both suppress feeding and decrease hypo-

thalamic NE levels in *obob* mice. Chronic amphetamine treatment also produced a pronounced decrease in plasma glucose levels in *obob* mice, essentially abolishing the hyperglycemia. The mechanism underlying the reversal of the hyperglycemia is not clear, but may be related to the decrease in food intake of the treated mice. The decreases in plasma glucose levels were not, however, dose-dependent in either lean or obese mice, and in this respect did not correspond to the effects of amphetamine treatment on either food intake or hypothalamic NE levels. This suggests that the relationship of the behavioral and neurochemical variables to the glucose levels is not simple.

There are other features of the obesity syndrome of the *obob* mutant which could be related to the results obtained in this study. The *obob* mutant is hypoactive and amphetamine could produce an interaction between activity and brain NE which would produce the pattern of neurochemical results reported. A straight forward, direct relationship of this kind, however, seems unlikely for several reasons. The hypoactivity of the *obob* mouse is secondary to the obesity [41], while the elevated hypothalamic NE levels are not [36]. Furthermore, Yen and Acton [40] reported that when the locomotor stimulating effect of amphetamine is compared in lean and *obob* mice the *obob* mice show a greater increase in activity compared to baseline levels, but that total activity is still less. Unpublished work in our laboratories has essentially replicated this finding. Thus, it would appear that the hypoactivity and elevated hypothalamic NE levels are independent events, and that both lean and *obob* mice respond to amphetamine treatment with increases in locomotor activity.

The experiments reported in the current study were conducted over an extended period of time. Even so, there was substantial inter-experiment consistency for many of the variables examined (e.g., control food intake levels). One exception to this finding was the variability in the absolute values (μg/g) of the neurochemical results for Experiments 1, 2 and 3. In spite of the inconsistencies in absolute values between experiments, when the data are examined using the saline-treated lean control values in each specific experiment as a reference point there is substantial consistency in the neurochemical data across experiments. In this respect the saline-treated *obob* mice always had significantly increased hypothalamic NE levels in comparison to the saline-treated lean mice, and amphetamine treatment produced dose-dependent decreases in hypothalamic NE levels across all experiments. This emphasizes the importance of including the saline-treated control group in all experiments in order to provide some kind of standard reference point over time.

Some recent studies have explored the relationship of the elevated hypothalamic NE levels to the hyperphagia of the *obob* mutant. Feldman and Blalock [15] did not find any effect of monoamine oxidase inhibition on body weight gain in either lean or *obob* mice. Batt *et al.* [3] reported a potentiation of the hyperphagia of *obob* mice after administration of the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine, a treatment which blocks NE synthesis and reduces hypothalamic and telencephalic NE content in *obob* mice [37]. Lorden [29] made central catecholamine neuron lesions in *obob* mice and another obese mutation with elevated hypothalamic NE levels, the *dbdb* mouse, using the neurotoxin 6-hydroxydopamine. Lorden found that although the lesions blocked further development of the obesity syndrome of *dbdb* mice, they produced only a transient weight loss followed by recovery and normal weight gain in *obob* mice. These results indicated that permanent removal of up to 60% of the hypothalamic NE system in *obob* mice had little effect on subsequent weight gain by this mutant. In the present study an attempt was made to activate, rather than eliminate, the hypothalamic NE systems in *obob* mice by the administration of amphetamine. The results indicated that amphetamine was effective in reducing both food intake and body weight in *obob* mice. During the period of anorexia amphetamine treatment also reduced the normally elevated hypothalamic NE levels of *obob* mice to amounts not significantly different from those of similarly treated lean mice. Although the possible involvement of other neurochemical systems and brain regions cannot be ruled out, the results provide tentative support for the position that the elevated hypothalamic NE levels in *obob* mice may be related to the hyperphagia of this mutant.

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