Pharmacology Biochemistry & Behavior, Vol. 5, pp. 617–620.

Effects of Food Restriction and Mutation on Central Catecholamine Levels in Genetically Obese Mice

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(Received 7 June 1976)

OLTMANS, G. A., J. F. LORDEN AND D. L. MARGULES. *Effects of food restriction and mutation on central catecholamine levels in genetically obese mice.* PHARMAC. BIOCHEM. BEHAV. 5(6) 617–620, 1976. – Norepinephrine (NE) levels in the hypothalamus and telencephalon of genetically obese mice with the OBOB and DBDB mutations are significantly higher than those of their lean littermates. Obese mice with the viable yellow mutation failed to show this increase. Restricting the diets of OBOB animals to prevent excessive weight gain does not affect NE levels in the hypothalamus, telencephalon or brainstem.

Genetic obesity Norepinephrine Dopamine Body weight Weight reduction in OBOB mice Food intake

RECENT work indicates that catecholamine levels in the brain are altered in some cases of genetic obesity. Lorden *et al.* [13] reported increased levels of hypothalamic and telencephalic norepinephrine (NE) in two cases of genetic obesity, OBOB and DBDB mice, when the brains of these animals were compared to those of their lean littermates. Cruce *et al.* [8] found significant differences in the catecholamine content of specific hypothalamic nuclei of the genetically obese Zucker fatty rat when compared with nonobese controls.

The abnormal catecholamine levels found in genetically obese rodents suggests that catecholamine neurons are directly involved in the production of the hyperphagia and obesity observed in these animals. Alternatively, the altered catecholamine levels may be a consequence of the obesity. For example, stress has been shown to affect brain NE content [23]. Thus, it is possible that stress associated with obesity could influence catecholamine levels in these animals.

The purpose of the present study was two-fold. The first objective was to determine if the differences in catecholamine levels observed between OBOB and DBDB mice and their lean littermates would be found in a third type of genetically obese mouse, the viable yellow. The second objective was to determine if prevention of obesity in the OBOB mutant would return central catecholamine content to control levels.

EXPERIMENT 1

Obesity is characteristic of viable yellow mice (A^{vy}), as it is of several other mutants [4,20]. However, there are differences in the obesity syndromes of the OBOB, DBDB, and A^{vy}. For example, the degree of obesity is more variable in viable yellow mice than in OBOB or DBDB mice and it is correlated with coat color [22]. To determine whether increased NE levels are generally characteristic of genetic obesity syndromes, central catecholamine levels were analyzed in viable yellow mice. This mutation is available on the C57BL/6J background strain as is the OBOB mutation. Thus, the A^{vy} mice make a good comparison group for the OBOB mice since any differences between the A^{vy} mice and the OBOB mice should not be related to unknown factors associated with strain.

Method

Four female and six male viable yellow mice (C57BL/6J-A^{vy}) were used. Controls for these mice were 5 females and 6 males of the C57BL/6J strain. All mice were obtained at 5 weeks of age from the Jackson Laboratories, Bar Harbor, ME, USA. The mice were housed in group cages and maintained on Purina rat chow and water. A 12 hr light-dark cycle was in effect in the colony room.

The mice were sacrificed at 7 months of age by decapitation and their brains dissected into hypothalamic and telencephalic sections as previously described [13]. The hypothalamic sections were analyzed fluorimetrically for NE and the telenecephalic sections, for NE and dopamine (DA) by a modified method of Hogan [11]. Data were analyzed by *t*-tests.

Results and Discussion

The body weights of the viable yellow mice for both males and females differed significantly from those of their control group at the time of sacrifice (Table 1). In this study the differences in the body weight of the viable

TABLE 1

HYPOTHALAMIC AND TELENCEPHALIC CATECHOLAMINE LEVELS IN VIABLE YELLOW MICE (C57BL/6J-A^{vy})

Section	N	Mean Body Wt (g ± SD)	Mean Brain Section Wt (mg ± SD)	Mean Catecholamine Levels† Norepinephrine (μg/g ± SD)	Dopamine (μg/g ± SD)
Hypothalamus					
Male obese	6	39 ± 3.6*	18.4 ± 1.7	1.50 ± 0.09	
Male lean	6	28 ± 2.6	16.7 ± 2.5	1.63 ± 0.14‡	
Female obese	4	34 ± 6.6*	18.4 ± 3.0	1.59 ± 0.20	
Female lean	5	21 ± 1.1	17.2 ± 2.1	1.46 ± 0.14	
Telencephalon					
Male obese	6		231.3 ± 9.6	0.37 ± 0.02	1.43 ± 0.13
Male lean	6		237.6 ± 19.8	0.36 ± 0.03	1.38 ± 0.14
Female obese	4		227.7 ± 9.1	0.38 ± 0.03	1.66 ± 0.14
Female lean	5		229.4 ± 4.0	0.36 ± 0.02	1.51 ± 0.07

*Differs from lean control, $p<.01$.
†Expressed as μg of amine/g fresh weight of brain.
‡Based on N = 4.

yellow mice were small in comparison to the differences in body weight reported for OBOB or DBDB mice and their lean littermates [13]. At 2 months of age OBOB mice maintained on a standard diet of Purina rat chow and water weigh approximately 12–16 g more than their nonobese littermates, and at 5 months of age, weigh 33g more [13]. In contrast at 7 months of age the viable yellow mice in the current experiment weighed an average of 12 g more than controls.

The differences in body weight observed in the viable yellow mice and their controls were not reflected in the amine levels. No significant differences were found between obese and lean mice in hypothalamic NE, telencephalic NE or telencephalic DA in either male or female mice (Table 1). Thus, elevated NE levels do not necessarily accompany genetic obesity. Obesity in the viable yellow mouse varies according to coat color, but no attempt was made to classify animals according to coat color in the present study. However, no significant correlation between body weights and amine levels was found. It is possible that analysis of smaller sections of tissue may have revealed local differences in catecholamine levels.

No differences in brain weights between the viable yellow mice and their controls were noted for either the hypothalamic or telencephalic sections. Thus, there is presently no evidence of neurological abnormality in the viable yellow mouse, either in terms of catecholamine content or brain weight. This is in contrast to previously reported data for the OBOB and DBDB mice [13,16]. In both of these mutations the telencephalons of the obese mice weighed 8–11% less than the telencephalons of the lean littermates.

EXPERIMENT 2

The syndrome exhibited by OBOB mice is complex and includes a number of abnormalities in addition to hyperphagia and obesity. Some aspects of the syndrome can be brought within normal limits by body weight reduction. For example, peripheral insulin resistance, hyperglycemia, and adrenal hypertrophy can be reduced by weight reduction [2, 7, 10]. In addition, the unusually low levels of locomotor activity observed in the OBOB can be increased to normal by weight reduction [17]. On the other hand, ovarian atrophy occurs regardless of changes in body weight [16]. Furthermore, the insensitivity of the hypothalamus of the OBOB to goldthioglucose lesions [1] is actually increased by weight reduction [19].

Thus, it is difficult to predict what effects weight reduction might have on central catecholamine levels in these mice. It is possible that the large and rapid weight gain observed in the OBOB, or some variable associated with weight gain, is responsible for the elevated NE levels observed in these animals. To determine if the weight gain of the OBOB mice was producing changes in central catecholamines the OBOB animals were placed on a restricted diet. This diet maintained their body weight near normal levels prior to analysis of catecholamine content.

Method

Female C57BL/6J-ob (OBOB) mice obtained from the Jackson Laboratories with their lean littermates were used. The mice were 5 weeks old on arrival. Each animal was housed individually and given ad lib access to Purina rat chow and water. Food intake was measured every 24 hr and special care was taken to collect spillage. After three days, half of the obese animals (N = 6) were placed on a restricted diet which consisted of a 24 hr allotment of food equal to that consumed by the lean littermates. The other six obese mice and the lean littermates (N = 9) received ad lib access to food. This feeding schedule was followed for 39 days. Body weights were taken every 7 days. At the end of this period, the animals were sacrificed as in Experiment 1 and NE and DA levels were determined as described previously [13]. Data were analyzed by an analysis of variance with specific comparisons made by *t*-tests.

Results

Body weights and the average daily food intake of obese

and lean animals with ad lib access to food are presented in Table 2. Obese animals ate about 27% more than their lean littermates on a daily basis. The body weights of the obese animals were already significantly higher than for the controls upon arrival in the laboratory. However, there were no differences in the weights of obese animals placed on restricted diets and those given ad lib access to food. The food restriction was successful in controlling the weight of the OBOB mice. At the end of the experiment the average weight of the 6 mice placed on the restricted diet was the same as it had been at the beginning of the experiment. In contrast, the OBOB mice with ad lib access to food gained an average of 14 g. The lean littermates gained an average of 4 g.

TABLE 2

AVERAGE DAILY FOOD INTAKE AND MEAN BODY WEIGHT OF RESTRICTED AND AD LIB FED MICE (C57BL/6J-ob)

Group	N	Average Daily Food Intake (g ± SD)	Mean Body Weight (g ± SD) At 36 Days of Age	At 75 Days of Age
Obese restricted	6		22 ± 2*	22 ± 2*
Obese ad lib	6	5.3 ± 0.3*	23 ± 3*	37 ± 3*
Lean ad lib	9	4.0 ± 0.2	14 ± 1	19 ± 2

*Differs from lean ad lib, $p<0.01$.

In addition to controlling the weight of the OBOB animals the food restriction regimen produced other marked behavioral changes. Food deprived animals were noticeably more aggressive and irritable than either the lean littermates or the OBOB mice with ad lib food access. When touched the food restricted animals frequently attempted to jump out of the cage, squealed, and tried to bite the experimenter. When placed in a cage with another mouse, the food-restricted OBOB invariably attacked the other mouse. This behavior was never observed in either the lean littermates or the ad lib fed OBOB group, and is surprising for the usually non-aggressive C57BL/6J mouse [3]

The results of the chemical analysis are presented in Table 3. Food restriction had no effect on brain catecholamine content. Hypothalamic levels of NE were significantly higher in both the food restricted and the ad lib access groups of OBOB mice than in the lean littermates (+42% and +35%, respectively), but were not significantly different from each other. Similar results were found with telencephalic and brainstem NE levels. Both food restricted and ad lib access OBOB mice had significantly higher levels of telencephalic and brainstem NE than did lean littermates. No significant differences in DA levels were observed. Thus, it appears that restricting the amount of food available to the OBOB mouse and thereby preventing the development of extreme obesity does not return brain NE content to levels found in lean littermates.

DISCUSSION

The results confirm earlier reports of elevated NE levels in genetically obese mice (OBOB) [13,14]. It does not appear, however, that a similar increase in central catecholamines occurs in another type of genetically obese mouse, the viable yellow. The results also indicate that maintaining the weight of the OBOB mouse at lean littermate levels does not reduce brain NE levels. Thus, the obesity does not appear to directly produce the elevated NE levels in OBOB mice.

The relationship between abnormal catecholamine levels in OBOB mice and the obesity and hyperphagia is not clear. It has been suggested that obesity in these mice may be due to a defect in the hypothalamus [4]. Many similarities exist between the obesity syndrome of the OBOB mouse and the syndrome produced by ventromedial hypothalamic lesions in rats. In both cases there is evidence of hyperinsulinemia [21] and hyperglycemia [16], as well as the more obvious increased adiposity [4,20] and hyperphagia [18]. Additional evidence of an abnormality in hypothalamic function in the OBOB is provided by studies indicating that the OBOB is less susceptible to gold thioglucose lesions than the normal mouse [1,19]. However, the OBOB appears to respond normally to ventromedial [6] or lateral [5] hypothalamic lesions.

On the basis of what is currently known about the obesity syndrome in OBOB mice, it is possible that elevated NE levels could be functioning in one of at least two alternative ways to effect the behavioral and physiological conditions of these animals. One possibility is via the hypothalmico-hypophyseal system with consequent effects on pituitary function. In fact, sterility in the OBOB mouse appears to be a result of a failure in hypothalamic control of the pituitary [4]. Alternatively, it is known that direct application of NE to the hypothalamus elicits food

TABLE 3

CENTRAL CATECHOLAMINE LEVELS IN FOOD RESTRICTED OR AD LIB FED OBOB MICE AND THEIR LEAN LITTERMATES

Group	N	Mean Amine Levels† (NE) Hypothalamus (μg/g ± SD)	(NE) Telencephalon (μg/g ± SD)	(NE) Brainstem (μg/g ± SD)	Dopamine Telencephalon (μg/g ± SD)
Obese restricted	6	2.16 ± .19*	0.43 ± 0.04*	0.71 ± 0.08*	2.03 ± 0.29
Obese Ad lib	6	2.05 ± .35*	0.43 ± 0.03*	0.73 ± 0.16*	2.03 ± 0.29
Lean Ad lib	9	1.52 ± .25	0.36 ± 0.02	0.53 ± 0.02‡	2.20 ± 0.14

*Differs from lean ad lib, $p<0.01$.
†Expressed as μg of amine/g, fresh weight of brain.
‡Based on N = 8.

intake in sated rats [9,12]. Thus, the elevated NE levels in the hypothalamus of the OBOB could act directly to produce hyperphagia in these animals. However, elevated levels of NE do not necessarily mean increased synthesis and release or decreased reuptake of the amine. In fact there does not appear to be any difference between OBOB mice and their lean littermates in either hypothalamic or telencephalic NE turnover [14]. However, no evidence is yet available comparing release, reuptake, or density of innervation of NE neurons in OBOB mice and their lean littermates.

Finally, it is possible that the elevated NE levels in OBOB mice are a compensatory response of noradrenergic neurons whose receptors are defective or subsensitive to NE. This possibility would be compatible with reports that NE provides part of a satiety message in the brain [15].

ACKNOWLEDGEMENTS

This work was supported in part by NIH Fellowships 1F22AM01663 and 1F22NS02339 and by NIMH Grant MH19438–05.

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