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Bidirectional Effects of Clonidine on Carbohydrate Intake in Genetically Obese (*ob/ob*) Mice

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CURRIE, P. J. AND L. M. WILSON. *Bidirectional effects of clonidine on carbohydrate intake in genetically obese* (ob/ob) *mice.* PHARMACOL BIOCHEM BEHAV **38**(1) 177–184, 1991.—Hypothalamic noradrenergic mechanisms contribute to altered caloric intake in genetically obese (C57BL/6J, *ob/ob*) mice. Noradrenergic mechanisms, principally in the paraventricular hypothalamus and of the α_2 subtype, have also been implicated in the macronutrient intake regulation of nonpathological models. Accordingly, this study assessed the extent to which clonidine, an α_2 agonist, altered macronutrient intake in genetically obese (*ob/ob*) and lean (C57BL/6J, +/?) mice. Following adaptation to a 6-h feeding regimen, mice were injected intraperitoneally with either clonidine (0.1, 0.5 mg/kg) or 0.15 M NaCl (Experiment 1) or 0.025 mg/kg clonidine or saline (Experiment 2) 30 min prior to simultaneous access to separate sources of carbohydrate, fat, and protein. Clonidine doses of 0.1 mg/kg or greater reduced total energy intake and intake of carbohydrate and fat ($p<0.005$) in all mice (Experiment 1). However, 0.025 mg/kg clonidine selectively increased ingestion of carbohydrate in obese mice by 212% of vehicle-injected values ($p<0.001$) without altering intake in lean mice (Experiment 2). These results implicate an α_2 receptor mechanism in genetic obesity.

ob/ob Genetic obesity Clonidine Macronutrient selection α-Noradrenergic Meal-feeding Carbohydrate intake C57BL/6J *Mus musculus*

THE genetically obese (*ob/ob*) mouse is characterized by abnormal behavioural, physiological, and hormonal states, including hyperphagia, impaired thermogenesis, increased adiposity, hyperinsulinemia (6, 7, 43, 52, 54) and exaggerated glycemic responses to stress (25). Although the mechanism underlying these effects remains unknown, an abnormality in central (CNS) function may contribute to the hyperphagia of the *ob/ob* mouse (8,46). Evidence of CNS abnormalities in the *ob/ob* include decreased neuronal size in several brain regions (2), altered dendritic orientation in lateral and ventromedial hypothalamic nuclei (3), decreased levels of cholecystokinin in the cerebral cortex (55), and increased cortical cholecystokinin receptors (19). The *ob/ob* has increased hypothalamic α_1-noradrenergic receptor density, although no apparent differences in α_2 receptor density and affinity are known to exist between obese and lean mice (8,46). No significant differences exist in either receptor number or affinity for α-adrenergic receptors in the cortex, or for dopaminergic or muscarinic receptors in the cortex or corpus striatum (46). Therefore, multiple abnormalities in neural systems putatively involved in the control of feeding exist in this mutant.

Biochemical abnormalities have also been cited as evidence of a CNS defect in the *ob/ob* mouse. Obese mice have higher levels of pituitary dopamine (35,36), serotonin (5-HT) (16), and β-endorphin (18,39) than do lean mice. Hypothalamic norepinephrine (NE) levels are increased in *ob/ob* mice (14), particularly in the paraventricular (PVN) and the ventromedial nuclei (45), areas which have been implicated in the α-noradrenergic and serotonergic regulation of satiety (4, 21, 26, 28, 29).

Postsynaptic α_2-noradrenergic receptors have been shown to mediate feeding induced by PVN microinjections of NE and clonidine in rats, an effect which is blocked by selective α_2 antagonists, including yohimbine and rauwolscine (17,27). Callahan et al. (8) have reported that peripherally administered yohimbine and rauwolscine significantly reduced 3- and 6-h food intake in both *ob/ob* and lean mice; however, *ob/ob* mice were more sensitive to the anorectic effect than were lean mice. Although clonidine increased 1-h food intake in *ob/ob* mice at doses which did not affect food intake in lean mice, again suggesting an increased sensitivity in the *ob/ob*, higher doses of clonidine suppressed intake in both obese and lean mice. Given that the *ob/ob* has increased PVN levels of NE, its hyperphagia may be attributed, at least in part, to an impaired α-adrenergic satiety control mechanism.

Studies examining the neuropharmacology of feeding in rats have frequently utilized a single, nutritionally complete diet with little concern for its nutritional composition (5). However, central and peripheral pharmacological manipulations alter macronutrient selection, suggesting that specific brain neurotransmitters may

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function to balance the proportion of carbohydrate, protein and fat consumed (24,32). For example, NE and clonidine not only increase total food intake (30, 32, 37, 40, 41, 49, 51, 56), but also selectively potentiate carbohydrate ingestion (29, 33, 53), which implicates noradrenergic neurons innervating the PVN of the rat in regulating carbohydrate selection, and in mediating the stimulating action of clonidine on carbohydrate ingestion.

Although hypothalamic catecholamines purportedly modulate total energy intake and specific macronutrient selection, research has yet to examine neurochemical regulation of macronutrient intake in an obese pathological model, such as the *ob/ob* mutant. Given the elevated NE levels in the *ob/ob*, it is possible that a genetically determined abnormality in the hypothalamic noradrenergic system may allow an increase in the amount of neurotransmitter reaching the postsynaptic membrane. It follows that increased PVN-NE would augment postsynaptic α_2 receptor stimulation which, in turn, may promote hyperphagia. The present research, therefore, examined α_2 noradrenergic effects on macronutrient selection in the *ob/ob*, in a self-selection feeding paradigm, in which mice were given access to separate sources of each of the three macronutrients, carbohydrate, protein, and fat. The α_2 adrenergic agonist clonidine was administered to assess its effects on specific macronutrient selection in both *ob/ob* and lean mice. Although clonidine alters total food intake and especially carbohydrate ingestion in the rat (29, 33, 53), its effects on macronutrient intake in the mouse, and, in particular, in the *ob/ob*, are unknown [cf. (8)].

EXPERIMENT 1

METHOD

Animals

Obese (C57BL/6J, *ob/ob*, n = 42) and lean (C57BL/6J, +/?, n = 42) adult male mice, purchased from the Jackson Laboratory, Bar Harbor, ME, 14 weeks old at the start of the experiment, were individually housed and tested in hanging wire cages (24 × 18 × 18 cm). Mice were maintained at 23°C on a 12-h light-dark cycle (lights on 0730 h). All mice were adapted to the colony regimen for at least 7 days before the start of the experiment and were maintained on water and standard mouse chow ad lib. [The chow was Wayne F-6 Rodent Blox, consisting of 24% protein, 6.5% fat, 45.4% carbohydrate (nitrogen-free extract), 3.7% crude fibre, 7.9% ash, and 12.5% moisture, which yields a calculated metabolizable energy of 3.1 kcal/g.]

Diets and Drugs

Three single energy source diets were presented simultaneously to each animal. Diets were presented in circular aluminum containers with a stainless steel cover with four 1-cm holes to allow access to the macronutrient and eliminate food spillage. The carbohydrate ration was composed of 43.9% dextrin (ICN Pharmaceuticals), 43.9% starch (St. Lawrence Starch Ltd.), 4% minerals (ICN), 3% vitamins (ICN), 5% fibre (ICN) and 0.2% choline (ICN). The protein component consisted of 86.3% vitamin-free casein (ICN), 4% minerals, 3% vitamins, 5% fibre, 1.5% methionine (ICN), and 0.2% choline. The fat diet was composed of 70.5% lard (Tenderflake), 10% essential fatty acids (Mazola Corn Oil), 8% minerals, 6% vitamins, 5% fibre, and 0.5% choline. Calculation of caloric density was based on caloric coefficients of 3.7 kcal/g for carbohydrate and protein, and 7.7 kcal/g for fat.

Clonidine-HCl (Sigma), a relatively specific α_2 agonist, was administered intraperitoneally (IP) at either 0.1 mg/kg or 0.5 mg/kg doses. Clonidine (CLON) doses were selected from published reports indicating an effect on food intake in *ob/ob* mice and in rats with no overt signs of locomotor depression or illness (8,41). The drug was dissolved in an isotonic saline (0.15 M NaCl) vehicle (VEH) at 0.5 ml/100 g body weight. Drugs were prepared daily and coded blind to the experimenter.

TABLE 1

MEAN (± SE) CALORIC INTAKE OF 6-h MEAL-FED AND 24-h AD LIB-FED OBESE AND LEAN MICE

		Carbohydrate	Protein	Fat	Total
Obese	6-h	1.94(0.54)	0.71(0.11)	4.90(0.28)	7.55(0.63)
	24-h	2.27(0.48)	1.73(0.26)	10.91(0.75)	14.91(1.19)
Lean	6-h	1.57(0.29)	1.28(0.14)	8.15(0.58)	11.02(0.50)
	24-h	2.94(0.71)	2.45(0.42)	10.97(0.74)	15.46(0.37)

Design and Procedure

Mice were initially given a brief adaptation of 3 days unlimited access to carbohydrate, protein, and fat, and were subsequently adapted to 6-h (0900–1500 h) daily access to the 3 macronutrients for one week. Water was available ad lib. Other work from our lab had previously determined that macronutrient intake stabilized one week after mice had been introduced to the 6-h regimen (10). Macronutrient and water intakes were monitored daily during the adaptation period, and body weights were monitored throughout the entire study to ensure that mice were never below 80% of free-feeding pretest weights. Table 1 shows the 24-h free-feeding and 6-h restricted-feeding baseline measures of caloric intake prior to drug treatment. Although both obese and lean mice ingested fewer kilocalories of energy when placed on the 6-h regimen, compared with 24-h intakes, obese mice on the 6-h regimen tended to consume fewer kilocalories than food-restricted lean mice, a finding also reported for obese mice on stricter meal-feeding schedules (22).

Obese and lean mice were assigned to one of three treatment groups: VEH control, 0.1 mg/kg CLON, and 0.5 mg/kg CLON, with a total of 14 *ob/ob* and 14 +/? mice in each treatment condition. Groups were matched for body weight within a phenotype and across treatment conditions. Following one week on the regimen, all mice received a VEH injection 30 min before presentation of preweighed amounts of carbohydrate, fat, and protein for two consecutive days. On the third day, mice received a single, appropriate body weight dose of either VEH or CLON according to group assignment 30 min before food access. Total intake and individual macronutrient intakes were assessed 1, 3, and 6 h (8,33) following initial food presentation by weighing food containers to the nearest 0.01 g on a Mettler PB-300 digital balance.

Independent variables included phenotype (*ob/ob*, +/?), drug dose (mg/kg), and sampling time (1, 3, 6 h), with dependent measures of total intake, carbohydrate, fat, and protein intake. Diet intakes were measured in grams, converted to kilocalories and analyzed as a percentage of intake of the previous vehicle day (% VEH day intake). That is, each mouse's intake measures were assessed in comparison to its total or respective macronutrient energy intake on the day prior to drug treatment, where VEH was administered to all mice regardless of treatment condition.

RESULTS

A 2 × 3 × 3 (Phenotype × Treatment × Sampling Time) ANOVA with repeated measures on the third variable was per-

TABLE 2

MEAN (±SE) BODY WEIGHTS (g) OF OBESE AND LEAN MICE

Phenotype	Clonidine Dose	Experiment 1*	Experiment 2†
ob/ob	Vehicle	49.57 (1.71)	53.38 (1.08)
	0.025 mg/kg		55.03 (1.77)
	0.100 mg/kg	49.37 (1.78)	
	0.500 mg/kg	49.05 (1.56)	
+/?	Vehicle	25.07 (0.39)	26.44 (0.48)
	0.025 mg/kg		27.08 (1.08)
	0.100 mg/kg	24.74 (0.77)	
	0.500 mg/kg	24.94 (0.67)	

*$F(1,78) = 556.57$, $p<0.0001$, for Phenotype main effect.
†$F(1,24) = 445.84$, $p<0.0001$, for Phenotype main effect.

formed on measures of total energy intake, carbohydrate, fat, and protein intake expressed in kilocalories, and as a percentage of the previous vehicle-injection day's values. Body weights (g) were analyzed using a 2×3 (Phenotype × Treatment) ANOVA. A Type 1 error rate of 0.05 was maintained, and post hoc Tukey tests (20) were performed on all group mean differences.

Analysis of body weights indicated that obese mice weighed approximately twice as much as lean mice (see Table 2), $F(1,78) = 556.57$, $p<0.0001$. However, body weights of mice within a given phenotype did not differ across treatment conditions.

The ANOVA examining energy intake in kilocalories indicated a significant main effect for phenotype, $F(1,78) = 32.42$, $p<0.0001$, treatment, $F(2,78) = 20.61$, $p<0.0001$, and sampling time, $F(2,156) = 529.46$, $p<0.0001$. As Table 3 shows, mice administered 0.5 mg/kg and 0.1 mg/kg CLON consumed significantly fewer kilocalories (means = 4.70, 5.82) than mice treated with VEH (mean = 8.16). Although *ob/ob* mice consumed fewer calories (mean = 4.95) than +/? mice (mean = 7.52), all mice increased caloric intake across sampling time. A significant Phenotype × Sampling Time interaction suggested that obese mice, across treatment conditions, consumed significantly fewer kilocalories in comparison to +/? mice, at 1, 3, and 6 h following diet presentation, $F(2,156) = 10.23$, $p<0.0001$. However, obese and lean mice administered 0.5 mg/kg CLON did consume similar amounts of energy (means = 4.11, 5.29), although *ob/ob* mice administered 0.1 mg/kg CLON or VEH consumed fewer calories (means = 4.77, 5.95) than similarly treated +/? mice (means = 6.86, 10.36), $F(2,78) = 4.58$, $p<0.01$.

Given that these and previous data (see Table 1) indicated that obese mice consumed less energy on a 6-h restricted feeding schedule compared to lean controls, a more accurate assessment of the effect of clonidine on energy intake would include a comparison of intake measures to the vehicle injection day prior to

TABLE 3

MEAN (±SE) CUMULATIVE CALORIC INTAKE AS A FUNCTION OF PHENOTYPE, CLONIDINE DOSE, AND HOURS OF DIET ACCESS (EXPERIMENT 1)

Clonidine Dose	Phenotype	1 h	3 h	6 h
Total Energy				
Vehicle	Obese	3.27 (0.41)	5.60 (0.58)	8.99 (0.83)
	Lean	6.27 (0.37)	10.62 (0.64)	14.20 (0.87)
0.1 mg/kg	Obese	1.91 (0.37)	4.74 (0.58)	7.67 (0.82)
	Lean	3.37 (0.43)	6.86 (0.53)	10.37 (0.52)
0.5 mg/kg	Obese	1.15 (0.54)	3.59 (0.63)	7.59 (0.71)
	Lean	1.12 (0.25)	5.32 (0.64)	9.54 (0.99)
Carbohydrate				
Vehicle	Obese	0.67 (0.09)	1.15 (0.12)	1.95 (0.22)
	Lean	1.21 (0.13)	2.22 (0.99)	3.19 (0.30)
0.1 mg/kg	Obese	0.33 (0.07)	0.85 (0.14)	1.44 (0.17)
	Lean	0.59 (0.08)	1.47 (0.17)	2.46 (0.25)
0.5 mg/kg	Obese	0.27 (0.10)	0.68 (0.11)	1.20 (0.16)
	Lean	0.29 (0.08)	1.06 (0.20)	2.10 (0.28)
Fat				
Vehicle	Obese	2.06 (0.26)	3.49 (0.39)	5.65 (0.53)
	Lean	4.36 (0.30)	7.16 (0.50)	9.26 (0.66)
0.1 mg/kg	Obese	1.32 (0.33)	3.24 (0.44)	5.24 (0.63)
	Lean	2.47 (0.38)	4.68 (0.45)	6.70 (0.53)
0.5 mg/kg	Obese	0.62 (0.39)	2.28 (0.48)	5.31 (0.48)
	Lean	0.65 (0.25)	3.66 (0.56)	6.35 (0.77)
Protein				
Vehicle	Obese	0.54 (0.18)	0.97 (0.27)	1.39 (0.35)
	Lean	0.69 (0.11)	1.24 (0.22)	1.75 (0.28)
0.1 mg/kg	Obese	0.26 (0.04)	0.65 (0.10)	0.99 (0.15)
	Lean	0.31 (0.04)	0.71 (0.08)	1.21 (0.12)
0.5 mg/kg	Obese	0.27 (0.06)	0.63 (0.09)	1.08 (0.18)
	Lean	0.18 (0.02)	0.59 (0.08)	0.99 (0.15)

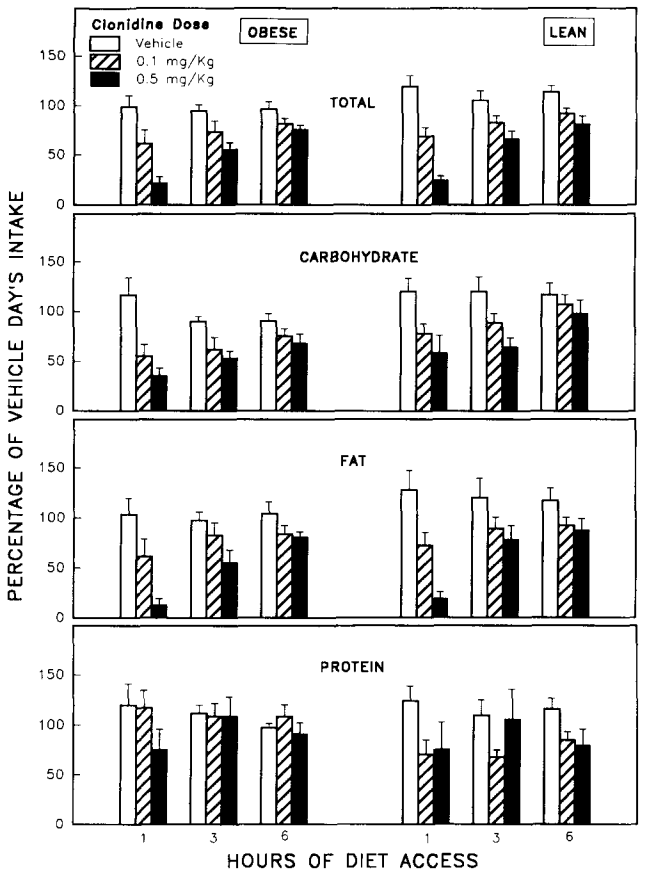

FIG. 1. Mean (+SEM) cumulative energy intake (total) and cumulative intake of carbohydrate, fat, and protein, expressed as a percentage of the preceding vehicle injection day's intake, in obese (left panel) and lean (right panel) mice as a function of clonidine dose [0.0 (open bar), 0.1 (hatched bar), 0.5 (solid bar) mg/kg body weight IP] at 1, 3, and 6 hours after the simultaneous presentation of separate sources of carbohydrate, fat, and protein.

drug administration, indicating the extent to which energy intake is suppressed and permitting comparisons between lean and obese mice when each animal serves as its own control.

As Fig. 1 (top panel) shows, total energy intake expressed as a percentage of VEH day intake decreased following clonidine treatment, $F(2,78)=32.72$, $p<0.0001$. Obese mice treated with 0.5 mg/kg and 0.1 mg/kg CLON did not differ significantly in percentage VEH day intake, although both groups demonstrated a significant suppression of intake when compared to VEH-treated *ob/ob* mice. Lean mice treated with 0.5 mg/kg CLON showed a suppression of caloric intake when compared to +/? mice administered 0.1 mg/kg CLON, and both groups differed significantly from VEH-treated lean controls. Both *ob/ob* and lean mice administered 0.5 mg/kg CLON had lower percentage VEH day intakes at 1 h than did 0.1 mg/kg CLON-treated mice, which, in turn, differed significantly from VEH-treated mice, $F(4,156)=12.75$, $p<0.0001$. Obese mice in all treatment groups differed significantly from each other 1 h following initial diet presentation. At 3 h following diet presentation, only *ob/ob* mice administered 0.5 mg/kg CLON differed significantly from VEH-treated *ob/ob* controls. Similar results were apparent for lean mice, except that the intake of 0.5 mg/kg CLON-treated +/? mice remained significantly suppressed from the intake of VEH controls throughout the remainder of the test period.

As Table 3 shows, obese mice ingested fewer kilocalories of carbohydrate (mean = 0.95) than lean mice (mean = 1.62), $F(1,78)=30.55$, $p<0.0001$. Overall, mice administered 0.5 mg/kg and 0.1 mg/kg CLON ingested similar amounts of carbohydrate (means = 0.93, 1.19) but significantly less than vehicle-injected mice (mean = 1.73), $F(2,78)=14.94$, $p<0.0001$. Obese mice administered 0.5 mg/kg CLON consumed significantly fewer kilocalories of carbohydrate (mean = 0.72) than vehicle-injected obese mice (mean = 1.26), although *ob/ob* mice administered 0.1 mg/kg CLON did not differ significantly from either group (mean = 0.87). In contrast, +/? mice given either 0.5 mg/kg or 0.1 mg/kg CLON ingested less carbohydrate (means = 1.15, 1.51) than vehicle-treated +/? mice (mean = 2.21). Although cumulative carbohydrate intake increased across sampling time for all mice, $F(2,156)=301.43$, $p<0.0001$, a significant Phenotype × Sampling Time interaction indicated that *ob/ob* mice consumed less carbohydrate than +/? mice 1, 3, and 6 h following diet presentation, $F(2,156)=20.51$, $p<0.0001$.

Following clonidine treatment, carbohydrate intake as a percentage of vehicle-injection day's intake decreased for both *ob/ob* and lean mice, $F(2,78)=11.55$, $p<0.0001$ (see Fig. 1, second panel from top). Percentage of VEH day intake was lower for *ob/ob* mice administered 0.5 mg/kg and 0.1 mg/kg CLON 1 h following diet presentation, although this difference was not maintained by 3 or 6 h. Similarly, carbohydrate intake for lean mice treated with 0.5 mg/kg and 0.1 mg/kg CLON was lower than intake for their VEH controls 1 h following diet presentation, $F(4,156)=4.18$, $p<0.003$, but not on the 3- and 6-h measure.

Clonidine also reduced fat intake in both *ob/ob* and lean mice, expressed as either cumulative caloric intake, $F(2,78)=13.90$, $p<0.0001$ (see Table 3) or percentage of vehicle injection day's intake, $F(2,78)=15.23$, $p<0.0001$ (see Fig. 1, third panel from top). Cumulative fat intakes were lower for obese (mean = 3.25 kcal) than lean mice (mean = 5.03 kcal), $F(1,78)=27.15$, $p<0.0001$. A significant Phenotype × Treatment interaction, $F(2,78)=4.42$, $p<0.02$, indicated that fat intake did not differ between *ob/ob* and +/? mice administered 0.5 mg/kg or 0.1 mg/kg CLON, although fat intake was greater for vehicle-injected +/? mice than for vehicle-injected *ob/ob*s. Although cumulative fat intake increased across hours of diet access, $F(2,156)=362.18$, $p<0.0001$, *ob/ob* mice consumed less fat than did +/? mice, $F(2,156)=5.36$, $p<0.006$.

Fat intake analysed as a percentage of vehicle-injection day's intake indicated lower intake for obese (mean = 75.89% of previous day's intake) than for lean (mean = 95.04%) mice, $F(1,78)=3.98$, $p<0.04$. A significant Treatment × Sampling Time interaction, $F(4,156)=11.82$, $p<0.0001$, was probed separately for obese and lean mice. At 1 h, percentage VEH day intake was lower for *ob/ob* mice administered 0.5 mg/kg CLON in comparison to *ob/ob* mice treated with 0.1 mg/kg CLON, both of which differed significantly from *ob/ob* VEH controls. At 3 h, only the higher dose of clonidine suppressed intakes of *ob/ob* mice, although this difference no longer held at 6 h. Lean mice showed similar effects on 1-h measures. At 3 h both CLON-treated lean groups ingested a lower percentage of their total intake as fat than they had at a comparable time on their preceding vehicle injection day. Six hours following diet presentation, only lean mice treated with 0.5 mg/kg CLON still consumed significantly less fat than controls.

The ANOVA examining cumulative protein intake indicated that overall mice administered 0.5 mg/kg and 0.1 mg/kg CLON consumed significantly fewer kilocalories (means = 0.62, 0.69, respectively) than vehicle-injected mice (mean = 1.09), $F(2,78)=5.63$, $p<0.005$. Although protein intake in clonidine-injected obese mice (at either dose) did not differ from intakes of vehicle-

TABLE 4

MEAN (±SE) CALORIC INTAKE AS A FUNCTION OF PHENOTYPE, CLONIDINE DOSE, AND HOURS OF DIET ACCESS (EXPERIMENT 2)

Clonidine Dose	Phenotype	1 h	3 h	6 h
Total Energy				
Vehicle	Obese	3.18 (0.36)	5.08 (0.58)	9.10 (0.86)
	Lean	6.66 (0.47)	11.17 (0.73)	14.23 (1.03)
0.025 mg/kg	Obese	5.03 (0.45)	8.43 (1.13)	12.25 (1.37)
	Lean	6.07 (0.59)	10.84 (1.26)	13.95 (1.46)
Carbohydrate				
Vehicle	Obese	0.67 (0.05)	1.14 (0.15)	1.78 (0.21)
	Lean	1.17 (0.12)	2.05 (0.18)	2.81 (0.24)
0.025 mg/kg	Obese	1.37 (0.19)	2.06 (0.30)	2.85 (0.38)
	Lean	1.38 (0.22)	2.56 (0.34)	3.46 (0.53)
Fat				
Vehicle	Obese	2.12 (0.24)	3.19 (0.28)	6.08 (0.33)
	Lean	4.81 (0.34)	7.79 (0.41)	9.56 (0.53)
0.025 mg/kg	Obese	3.28 (0.43)	5.46 (0.75)	8.15 (0.95)
	Lean	4.16 (0.47)	7.07 (0.94)	8.85 (1.03)
Protein				
Vehicle	Obese	0.39 (0.20)	0.75 (0.35)	1.25 (0.56)
	Lean	0.69 (0.19)	1.33 (0.37)	1.86 (0.48)
0.025 mg/kg	Obese	0.38 (0.17)	0.90 (0.38)	1.24 (0.48)
	Lean	0.53 (0.08)	1.21 (0.25)	1.64 (0.30)

injected obese mice, protein intake of +/? mice receiving the 0.5 mg/kg clonidine dose did differ from that of vehicle-injected lean mice. Clonidine treatment did not affect protein intake in either *ob/ob* or lean mice, when intakes were analysed as percentages of vehicle-injection day intakes (see Fig. 1, bottom panel).

DISCUSSION

The results indicate that the α_2 agonist, clonidine, reduces energy intake in *ob/ob* and lean mice. These results are partially consistent with those of Callahan et al. (8), in which 0.5 mg/kg CLON decreased food intake in *ob/ob* and +/? mice with 6-h daily access to a single composite food source. However, Callahan et al. (8) also reported that 0.1 mg/kg clonidine increased 1-h food intake in obese mice, a dose that in the present study reduced intake in both *ob/ob* and +/? mice, albeit only at 1 h for *ob/ob*s and throughout the 6-h test for leans. Given that Callahan et al. (8) examined clonidine's effects on the intake of standard laboratory chow, while the current study assessed macronutrient intake, the anorectic effect of clonidine in our study might be attributed, in part, to the differences in the test diets.

This inconsistency emerges elsewhere in reports of clonidine's effects on food intake in mice and rats. Although some researchers have found clonidine to be anorectic in mice (8), others have reported either no effect (41), an initial, dose-dependent decrease (50), or an increase in food intake in the rat (29, 30, 32, 37, 40, 49, 51) following clonidine administration. These apparent differences in the effects of clonidine may depend on the dose of clonidine administered, on interspecies differences in clonidine's mechanism of action, on the frequency of drug administration, on the interval following clonidine administration at which intake is measured (50), on differences in the feeding regimens of test animals, or on differences in test diets.

Although clonidine doses in the present experiment did not appear to produce any overt signs of psychomotor depression in our mice, they did suppress total energy intake as well as both carbohydrate and fat intake, when analysed as either absolute caloric values or as percentages of the preceding vehicle injection day's values, and protein intake, only when data were analysed as absolute values. This pattern represented a more generalized suppression of intake and a less selective effect of clonidine on individual macronutrient ingestion than previous research had suggested (29, 33, 53). The doses used in this experiment were comparable to those tested by others (8) with *ob/ob* and lean mice. However, even our lowest dose was 4 times higher than the single IP dose with which Leibowitz et al. (29) had potentiated carbohydrate intake of 6-h schedule-fed rats by 267% over saline values. Therefore, a second experiment extended the dose-effect curve for clonidine and macronutrient intake in *ob/ob* and lean mice.

EXPERIMENT 2

Procedures identical to those of Experiment 1 were followed, except that fresh groups of *ob/ob* and lean mice, 16 weeks of age, received IP injections of either 0.025 mg/kg body weight clonidine (n=7) or saline vehicle (n=7) following adaptation to the diet and feeding schedule.

RESULTS

A 2×2×3 (Phenotype × Treatment × Sampling Time) ANOVA with repeated measures on the third variable was performed on measures of total energy, carbohydrate, fat, and protein intake expressed in kilocalories, and as percentages of each mouse's previous vehicle injection day's intakes. A Type 1 error rate of 0.05 was maintained, and post hoc Tukey tests were performed on all group mean differences.

Body weights (g) were analyzed using a 2×2 (Phenotype × Treatment) ANOVA. As in Experiment 1, obese mice weighed significantly more than lean mice, although body weights of mice within a particular phenotype did not differ across treatment conditions (see Table 2), $F(1,24)=445.84$, $p<0.0001$.

Cumulative caloric measures for total energy, carbohydrate,

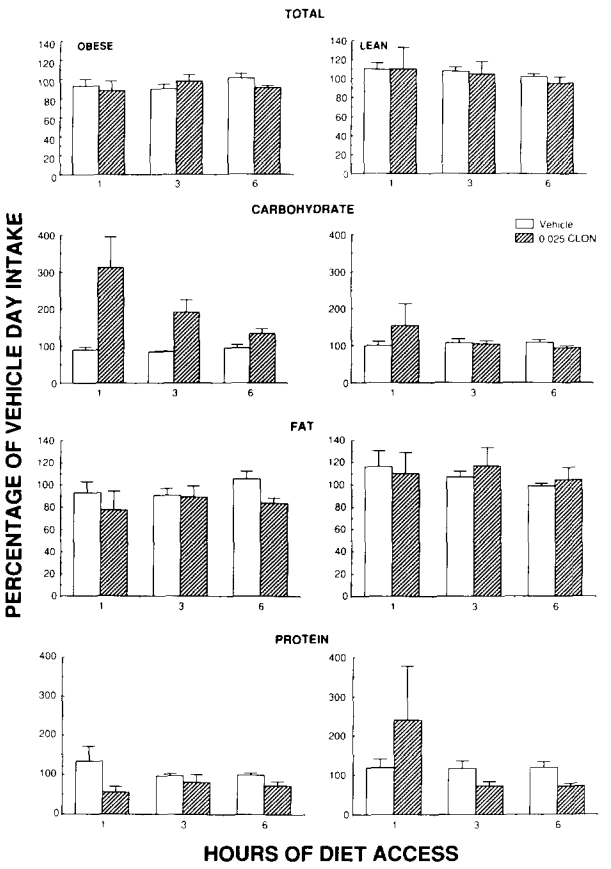

FIG. 2. Mean (+SEM) cumulative energy intake (total) and cumulative intake from carbohydrate, fat, and protein, expressed as a percentage of the preceding vehicle injection day's intake in obese (left panel) and lean (right panel) mice as a function of clonidine dose [0.0 (Vehicle) (open bar) or 0.025 CLON (slanted line bar) mg/kg body weight IP] at 1, 3, and 6 hours after the simultaneous presentation of separate sources of carbohydrate, fat, and protein.

fat, and protein intake are presented in Table 4. Although clonidine did not alter total energy intake or ingestion of protein, the ANOVA examining fat intake in kilocalories indicated a significant main effect for phenotype, $F(1,24) = 17.00$, $p<0.0004$, and a significant Phenotype × Treatment interaction, $F(1,24) = 5.02$, $p<0.03$. Treatment with 0.025 mg/kg clonidine increased the intake of fat in *ob/ob*s (mean = 5.63) in comparison to that of vehicle-treated *ob/ob* mice (mean = 3.79), without significantly altering intake in similarly treated +/? mice (means = 6.69, 7.38). The ANOVA of carbohydrate ingestion indicated a significant main effect for phenotype, $F(1,24) = 5.58$, $p<0.03$, and treatment, $F(1,24) = 7.28$, $p<0.01$. However, the treatment effect appears to be largely attributed to the impact of clonidine on obese mice (means = 2.10, 1.19 for clonidine and vehicle-treated *ob/ob* mice), although intake in lean mice also showed an increase with clonidine (means = 2.47, 2.01). This description becomes even more evident upon comparison of mean intake values for obese and lean mice at 1 h and 3 h (see Table 4).

However, when each mouse is used as its own control, as in the percentage of vehicle intake analyses, which allows for a more cautious interpretation of the results, 0.025 mg/kg clonidine selectively increased the ingestion of carbohydrate in obese mice by 212% of their previous day's vehicle-injected values without altering intake in lean mice [Phenotype × Treatment interaction: $F(1,24) = 5.38$, $p<0.03$] or significantly affecting fat or protein intake in either phenotype (see Fig. 2). Although total food intake for *ob/ob* and lean mice treated with 0.025 mg/kg CLON did not differ significantly from control values, there was a tendency for fat and protein intake to be reduced compared to mice's preceding vehicle injection day values, particularly in *ob/ob* mice. The reductions, however, were not statistically significant. This finding suggests that total caloric intake in clonidine-treated *ob/ob* mice remained unaltered, despite potentiated carbohydrate ingestion, because of the nonsignificant decreases in fat and protein intake.

GENERAL DISCUSSION

The α_2 agonist, clonidine, elicited a dose-dependent, bidirectional effect on carbohydrate intake in *ob/ob* mice. While doses of 0.1 mg/kg clonidine or greater reduced total energy intake, by suppressing intake of carbohydrate and fat, in particular, in both *ob/ob* and lean mice, a 0.025 mg/kg dose selectively increased carbohydrate ingestion in *ob/ob* mice only, leaving intakes unaffected in lean mice. Clonidine also increased cumulative fat consumption in *ob/ob* mice only. When mice were used as their own controls, however, the apparent potentiation of fat intake was eliminated. These results support the role of an α_2 noradrenergic receptor mechanism in the regulation of macronutrient intake in the *ob/ob* mouse.

Recent studies have indicated that clonidine, when administered peripherally or centrally, potentiates feeding in satiated rats in a manner similar to that observed following injection of NE into the PVN (17, 29, 37, 51, 57). In fact, the most consistent research finding is an increase in food intake following clonidine treatment (8, 17, 29, 49, 51, 56). Our results partially confirm these findings: Although total intake was not increased, clonidine did potentiate carbohydrate ingestion in *ob/ob* mice. However, consistent with some reports of either no effect or an anorectic effect following clonidine treatment (8, 41, 56), our results suggest that clonidine can elicit a dose-dependent bidirectional effect on food intake. Specifically, at low doses, clonidine treatment results in no effect (lean mice) or an increase in carbohydrate intake (*ob/ob* mice), whereas at higher doses, clonidine suppresses total caloric intake in both *ob/ob* and lean mice.

Obese mice typically consume a greater proportion of daily caloric intake from fat when given simultaneous access to carbohydrate, fat, and protein sources on either ad lib (42,47) or meal-feeding (10) regimens. The present study suggests that clonidine may alter feeding patterns through its influence on the selection of specific macronutrients. Clonidine potentiated carbohydrate intake in obese mice (Experiment 2), and this clonidine-induced change is consistent with previous findings of similar alterations in diet following central and peripheral clonidine administration (13, 29, 33, 53). Further, the selective carbohydrate effect following PVN administration of NE (29, 32, 33, 53) has led to the hypothesis that the PVN may be a primary site in the mediation of clonidine-induced hyperphagia and carbohydrate selection. In the *ob/ob*, additional information on this issue may be gained from assessing the ability of α_2 antagonists to eliminate the robust and selective increase in carbohydrate intake reported here. Although yohimbine, a selective α_2 blocker, is anorectic in meal-feeding mice, with *ob/ob* mice responding to lower doses than lean controls (8), preliminary findings in our lab have shown that yohimbine can antagonize the anorectic effect of higher doses of clonidine (11).

Several factors may have contributed to the heightened sensitivity of *ob/ob* mice to α_2-noradrenergic stimulation. For example, hypothalamic α_2 receptors, especially in the PVN, are up-

regulated by corticosterone and down-regulated following adrenalectomy (23). The efficacy of norepinephrine to increase total caloric intake and carbohydrate intake, in particular, through an α_2-noradrenergic mechanism, depends on intact adrenocortical function (31). The *ob/ob* mouse has elevated corticosterone levels across the 24-h day (48) and displays enhanced sensitivity to glucocorticoid-induced food intake (38). Their chronically elevated plasma corticosterone levels (44,48), which appear as early as 17 days postpartum (12), could, therefore, permit enhanced α receptor number, affinity, or both, to determine this mutant's response to low doses of clonidine. If interphenotypic corticosterone differences influence this α receptor subtype in mice as they do in rats, then a substrate for the altered clonidine sensitivity of *ob/ob* mice may be identified. The *ob/ob* mouse has 58% more hypothalamic α_1 receptors than does its lean control (46), determined by the binding of [^{3}H]-WB-4101, a highly selective α_1 antagonist. Comparable binding assays for α_2 receptors have demonstrated no differences in α_2 receptor density or affinity between *ob/ob* and lean mice (8). However, both α_1 and α_2 densities were determined in pooled samples, following unspecified feeding regimens, which may have masked significant intrahypothalamic differences between groups. In another model of genetic obesity, the Zucker fatty rat, both α_1 and α_2 receptor density is elevated, principally in the PVN, compared to inbred lean controls (15).

Previous research suggested that a 0.2 mg/kg clonidine dose may have sedative effects in rats (41), with no change in their total intake after clonidine injection secondary to decreased arousal. This implies that the potentiating effect of clonidine on food intake is only found within a narrow dose range, which is consistent with the narrow therapeutic range that characterizes clonidine's antihypertensive properties (9). However, sedative effects of clonidine at the doses used in our study have not been reported in either *ob/ob* or lean mice (8). Further, given that the paraventricular and medial hypothalamic regions have been implicated in satiety control, involving α-adrenergic and serotonergic systems (21,28), where NE inhibits, and 5-HT facilitates, satiety, if clonidine acts only on the α_2 receptor, then treatment would be expected to increase food intake. However, given the reduction in total energy and macronutrient intake in Experiment 1 for both strains, clonidine may have facilitated satiety by acting on a serotonergic mechanism. Although clonidine doses used in the present study were selected on their ability to selectively affect the α_2 receptor (8), Anden et al. (1) have shown that clonidine decreases 5-HT turnover at doses of 30 and 100 μg. Therefore, it is possible that our clonidine doses (viz., 0.025–0.5 mg/kg) affected more than the α_2 receptor, although we cannot eliminate nonspecific causes of feeding suppression at higher clonidine doses.

In summary, the hyperphagia of the *ob/ob* may result from its altered noradrenergic profile (8,45). Although the precise nature of the noradrenergic abnormality remains unknown, an increase in endogenous hypothalamic NE has been localized to the PVN (45). In the current study the specificity of the clonidine-induced potentiation of carbohydrate intake to the *ob/ob* is consistent with previous reports of alterations in long-term feeding patterns by chronic PVN infusion of NE or clonidine, resulting in increased daily food intake and enhanced body weight gain (32,34). More specifically, it is possible that an aberrant hypothalamic, and most likely PVN, noradrenergic system may be involved in the development and maintenance of genetic obesity. This could involve a number of neurochemical mechanisms, including abnormal release mechanisms of the presynaptic neuron, modified storage properties, altered reuptake mechanisms, or enhanced postsynaptic receptor activity. All of these could be orchestrated by endogenous glucocorticoids, opioid peptides, glucose, and insulin, each of which displays abnormal functioning in the genetically obese mouse.

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