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Yohimbine Attenuates Clonidine-Induced Feeding and Macronutrient Selection in Genetically Obese (*ob/ob*) Mice

PAUL J. CURRIE,*[1] AND LINDA M. WILSON†

*Section of Biopsychology, Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1R8
†Department of Psychology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

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CURRIE, P. J. AND L. M. WILSON. *Yohimbine attenuates clonidine-induced feeding and macronutrient selection in genetically obese (ob/ob) mice.* PHARMACOL BIOCHEM BEHAV 43(4) 1039–1046, 1992.—Biochemical abnormalities in the hypothalamus of the genetically obese (C57Bl/6J, *ob/ob*) mouse, including increased levels of endogenous norepinephrine (NE) in the paraventricular nucleus (PVN) and reduced medial hypothalamic NE metabolism, have been cited as evidence of a CNS defect contributing to altered caloric intake in this genetic strain. In the current study, the α_2-antagonist yohimbine (YOH) and the α_2-agonist clonidine (CLON) were administered systemically to 6-h meal-feeding obese and lean mice. Yohimbine (3–5 mg/kg, IP) significantly reduced total energy intake and intake of carbohydrate and fat, in both phenotypes, without altering protein intake. In contrast, CLON (25 μg/kg, IP) potentiated feeding, resulting in a shift in macronutrient selection toward a significant increase in the proportional intake of carbohydrate. Obese mice, however, showed an enhanced behavioral response to CLON injection. Pretreatment with 1 mg/kg YOH, a dose that alone did not significantly alter energy intake or diet selection, blocked CLON's stimulatory effect on feeding and carbohydrate preference. These results are consistent with a role for α_2-noradrenergic receptors in appetite regulation of *ob/ob* and lean mice and suggest that disturbances in this system may be involved in the development of genetic obesity.

α_2-Noradrenergic receptors Yohimbine Clonidine Macronutrient selection Feeding Genetic obesity C57Bl/6J *ob/ob*

THE genetically obese (*ob/ob*) mouse exhibits insulin resistance, glucose intolerance, increased adiposity, abnormal diurnal feeding, and excessive food intake (2,5,6,16,28,39,44). Similarly, electrolytic lesion of the paraventricular nucleus (PVN) produces altered plasma insulin levels, disturbances in diurnal feeding, hyperphagia, increases in body weight gain, and ultimately obesity (1,23,40,43). Together, these findings suggest that the genetic aberration in the *ob/ob* is of hypothalamic origin. More direct evidence of hypothalamic abnormalities in the obese mouse include increased α_1-noradrenergic receptor density (33), decreased neuronal size in hypothalamic nuclei (3), and altered dendritic orientation in the lateral and ventromedial areas (4). The *ob/ob* also has reduced metabolism of hypothalamic norepinephrine (NE) (27) and increased NE in the PVN (14), a nucleus implicated in the α-noradrenergic control of feeding (22,23).

Norepinephrine injected into the hypothalamic PVN is known to elicit feeding in satiated rats and enhance feeding in hungry rats, an effect blocked by selective α_2-receptor antagonism (15,21,22,26,45). The α_2-agonist clonidine (CLON) has been shown to stimulate feeding when injected systemically in a number of species or directly into the PVN of the rat (22,29,30,37,38,41,47). Moreover, long-term feeding patterns are altered by chronic PVN infusion of NE or CLON, producing a potentiation of daily food intake associated with an increase in meal size and body weight gain (21,25,26,42). In addition, administration of CLON or NE appears to selectively potentiate carbohydrate ingestion relative to protein and fat intake (8,22,41,46), suggesting that specific brain neurotransmitters may control appetite for specific macronutrients.

Research examining α_2-noradrenergic effects on feeding in the *ob/ob* has shown that the α_2-receptor antagonists yohimbine (YOH) and rauwolscine significantly reduce food intake of standard rodent chow in 6-h meal-feeding *ob/ob* and lean mice, with *ob/ob* mice showing an increased sensitivity to neuropharmacological treatment (7). Clonidine increases food intake in *ob/ob* mice at doses that do not appear to affect food intake in lean mice, but higher doses of this agonist suppress feeding in both phenotypes (7). Although few studies have examined specific and selective effects on macronutrient

[1] Requests for reprints should be addressed to Dr. P. J. Currie, Section of Biopsychology, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario M5T 1R8, Canada.

intake in the genetically obese mouse, it appears that anorectic doses of CLON reduce total energy intake primarily through suppression of carbohydrate and fat consumption (9,12). Lower doses of CLON (<0.1 mg/kg), however, appear to selectively increase the ingestion of carbohydrate (10,11). While the specificity of the CLON-induced potentiation of carbohydrate intake in the *ob/ob* is consistent with previous reports of alterations in macronutrient preference in the rat following NE or CLON treatment, it remains possible that a genetically determined alteration in a hypothalamic noradrenergic feeding system may result in impaired satiety control in the *ob/ob*. For example, phasically elevated PVN NE could enhance postsynaptic α_2-receptor stimulation, which could, in turn, promote hyperphagia and appetite for specific macronutrient. In contrast, α_2-antagonists might be predicted to decrease macronutrient intake and attenuate feeding induced by selective α_2-agonists. To test these possibilities, in the current study the α_2-blocker YOH and the α_2-agonist CLON were administered to *ob/ob* and lean mice in an attempt to pharmacologically manipulate postsynaptic α_2-receptor stimulation. The effects on feeding and macronutrient selection were then determined.

METHOD

Animals

Genetically obese (C57Bl/6J, *ob/ob*, $n = 49$) and lean (C57Bl/6J, +/?, $n = 49$) adult, male mice (Jackson Laboratory, Bar Harbor, ME), aged 12 weeks at the start of the experiment, were individually housed and tested in hanging wire cages (24 × 18 × 18 cm). Mice were maintained under controlled light (lights on 0730–1930 h) and temperature (23°C). Body weights averaged 51.4 g for obese mice and 28.6 g for lean mice. All animals were adapted to the colony regiment for at least 2 weeks before the start of the experiment and were allowed free access to water and chow. [The rodent chow, Wayne F-6 Rodent Blox, consisted of 45.4% carbohydrate (nitrogen-free extract), 24% protein, 12.5% moisture, 7.9% ash, 6.5% fat, and 3.7% crude fiber, yielding 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 insert with four 1-cm holes permitting access to the macronutrient and minimizing food spillage. The carbohydrate diet (3.7 kcal/g) was composed of 43.9% dextrin (ICN Pharmaceuticals, Montreal, Quebec, Canada), 43.9% starch (St. Lawrence Starch Ltd., Port Credit, Ontario, Canada), 5% fiber (ICN), 4% minerals (ICN), 3% vitamins (ICN), and 0.2% choline (ICN). The fat ration (7.7 kcal/g) consisted of 70.5% lard (Tenderflake), 10% corn oil (Mazola), 8% minerals, 6% vitamins, 5% fiber, and 0.5% choline. The protein component (3.7 kcal/g) was composed of 86.3% vitamin-free casein (ICN), 5% fiber, 4% minerals, 3% vitamins, 1.5% methionine (ICN), and 0.2% choline.

Yohimbine HCl (Sigma Chemical Co., St. Louis, MO), an α_2-noradrenergic antagonist, was administered IP at doses ranging from 1–5 mg/kg. Clonidine HCl (Sigma), an α_2-agonist, was administered at a dose of 25 μg/kg IP. Yohimbine and clonidine doses were selected from published reports indicating an effect on food intake in mice with no overt signs of locomotor depression or malaise (7,11). Drugs were dissolved in an isotonic saline vehicle (0.15 M NaCl) in a volume of 0.5 ml/100 g body weight.

Design and Procedure

Prior to drug injection, all mice were given a 1-week period to acclimate to the test diets with unlimited access to carbohydrate, fat, and protein sources. Mice were subsequently adapted to a 6-h (0900–1500 h) meal-feeding regimen for 2 weeks. Water was always available ad lib. Previous work in this laboratory has shown that macronutrient intakes stabilize approximately 2 weeks after mice are introduced to the 6-h regimen (12). The purpose of the feeding schedule was to assess drug effects of total daily food intake (6-h feeding), as well as on shorter interval (1–3 h) food intake.

Following 2 weeks on the meal-feeding regimen, all mice received a vehicle (VEH) injection 30 min before presentation of preweighed amounts of carbohydrate, fat, and protein, for several consecutive days, to adapt to the test procedure. On the subsequent test day, obese ($n = 7$) and lean ($n = 7$) mice received a single appropriate body weight dose of YOH (1, 3, or 5 mg/kg), or saline, 30 min prior to diet access. Total energy intake and macronutrient intakes were assessed at 1, 3, and 6 h following initial food presentation. In a similar paradigm, other mice were pretreated with YOH and then injected with CLON in an attempt to block CLON's reported stimulatory effect on feeding in *ob/ob* mice (7,10). Separate groups of obese ($n = 7$) and lean ($n = 7$) mice received either YOH (1 mg/kg) or VEH 30 min prior to injection of CLON (25 μg/kg). A control group received two consecutive saline injections separated by a 30-min period. Again, macronutrient intakes were assessed throughout the 6-h feed.

Independent variables included phenotype (*ob/ob*, +/?), drug treatment, and test interval (1, 3, and 6 h postinjection), with dependent measures of total energy intake, carbohydrate, fat, and protein intake. Diets were measured to the nearest 0.01 g using a Mettler (Fisher Scientific, Winnipeg, Manitoba, Canada) PB-300 digital balance and converted to kilocalories. Data were analyzed using two- and three-way analyses of variance (ANOVA) for repeated measures. Posthoc Tukey tests (17) were performed on all group mean differences. The alpha level was set at $p < 0.05$.

RESULTS

Baseline Macronutrient Selection Patterns

Macronutrient intakes for 6-h meal-feeding obese and lean mice, prior to drug treatment, are shown in Table 1. Both obese and lean mice self-selected more kcal from fat, and consequently a higher proportion of energy from this macronutrient, than from the carbohydrate or protein diets, $F(1, 96) = 10.02$, $p < 0.001$. However, obese mice ingested more fat, but fewer kcal of the carbohydrate, than lean mice. Protein intakes did not differ significantly between phenotypes.

Effects of Yohimbine on Total Energy Intake and Diet Selection

A 2 × 4 × 3 (phenotype × treatment × test interval) ANOVA with repeated measures on the third variable was performed on measures of total energy intake, in addition to carbohydrate, fat, and protein intakes express in kcal. Macronutrient intakes were also examined as a percentage of intake for saline-treated mice (calculated by dividing the drug score by the saline score) using similar ANOVA. Yohimbine re-

TABLE 1

MEAN (±SE) CALORIC INTAKE AND PERCENT MACRONUTRIENT INGESTED IN 6-h MEAL-FEEDING OBESE AND LEAN MICE

	Carbohydrate	Fat	Protein	Total
Obese				
kcal	2.20 (0.21)*	8.14 (0.62)*	1.75 (0.49)	12.09 (0.95)
%	18.2 (4.31)*	67.3 (4.93)*	14.5 (4.06)	
Lean				
kcal	3.35 (0.57)	6.90 (0.46)	1.67 (0.29)	11.92 (0.84)
%	28.1 (4.92)	57.9 (5.11)	14.0 (3.88)	

*$p < 0.05$ vs. intake in lean mice.

duced total caloric intake, in a dose-dependent manner, in both *ob/ob* and lean mice, $F(3, 48) = 3.04$, $p < 0.03$, with the exception of the lowest dose, which did not produce a statistically significant change in the amount of food consumed in either phenotype (see Fig. 1). The suppression in intake was particularly evident at the highest dose tested, reducing caloric intake in obese and lean mice by approximately 65% of vehicle-injected controls. The reduction in overall energy intake although maintained at 3 h was no longer apparent 6 h postinjection.

Analysis of macronutrient intakes showed that while YOH significantly decreased the ingestion of carbohydrate and fat the α_2-antagonist had little effect on protein intake and did not alter intake of this macronutrient at any of the doses or time intervals tested. Carbohydrate intake was reduced at 3- to 5-mg/kg doses and in both obese and lean mice by over 40% of controls. While intake was suppressed in lean mice largely at 1 h, obese mice showed a decrease in carbohydrate intake across the entire 6-h test, $F(6, 96) = 3.98$, $p < 0.02$. The reduction in fat intake was also dose dependent, $F(3, 48) = 7.58$, $p < 0.003$. Injection of 5 mg/kg YOH decreased 1-h fat intake by at least 70% in both phenotypes. Although YOH tended to suppress 3-h intakes as well, this effect was not maintained on the 6-h measure. Again, however, a dose of 1 mg/kg YOH did not significantly alter carbohydrate or fat intake in either obese or lean mice.

Effects of Yohimbine Pretreatment on Clonidine-Induced Feeding and Macronutrient Selection

Caloric intakes and percent concentration scores (calculated by determining the percent of each diet eaten relative to total intake and indicating diet preference) were analyzed using a 2 × 3 × 3 (phenotype × treatment × test interval) ANOVA. Intakes expressed as a percentage of control (saline-injected obese and lean mice) were also examined using similar statistical procedures. Figure 2 shows the effects of CLON, administered after either saline or YOH injection, on macronutrient caloric intakes. The α_2-agonist increased feeding and overall caloric intake in obese mice over the initial 3 h of testing. Intake in lean mice also increased but only on the 1-h measure, $F(4, 72) = 5.78$, $p < 0.0004$. Yohimbine pretreatment, at a dose that alone did not produce a statistically significant change in total energy intake compared to controls, blocked CLON's potentiating action, suggesting competitive pharmacological antagonism.

Clonidine also differentially altered macronutrient intake, potentiating carbohydrate and fat ingestion, without altering protein consumption. Carbohydrate intake was dramatically enhanced 1 h postinjection in obese mice to over 240% of saline values, $F(4, 72) = 3.37$, $p < 0.01$, and remained elevated on the 3-h measure. Intake of this diet was also increased in lean mice, although the effect was less pronounced (approximately 150% of controls) and was only found at 1 h. Yohimbine pretreatment blocked the potentiating effect of CLON on carbohydrate intake in both phenotypes, resulting in intakes similar to controls. Fat intake was enhanced in obese and lean mice, $F(4, 72) = 2.55$, $p < 0.03$. However the modest effect on fat intake appeared only on the 1-h intake measure. No effect on fat intake was found if mice were pretreated with YOH.

The increased consumption of carbohydrate by obese mice after CLON injection, in particular at 1 h, resulted in a significant increase in the percent concentration or proportion of carbohydrate ingested (from 17.9% to 30.8%) relative to the other nutrients, $F(4, 72) = 10.91$, $p < 0.001$. The effect was also present but less pronounced in lean mice (see Table 2). Again, CLON had little effect on the caloric intake of protein, resulting in a decrease in the proportion of protein consumed. Although caloric intake of fat was enhanced, the effect was considerably smaller than the potentiation of carbohydrate intake and, consequently, a decline in the proportion of calories ingested from fat was obtained. Again, the effect was most pronounced in the *ob/ob*. However, pretreatment with YOH eliminated the CLON-induced shift in nutrient selection and preference. Mice injected with CLON did not show an increased preference for carbohydrate if YOH was administered prior to CLON.

DISCUSSION

The results of the current study indicate that the α_2-antagonist YOH can elicit a dose-dependent decrease in overall feeding, as well as macronutrient selection in both obese and lean mice. The lowest dose tested, however, failed to significantly reduce caloric intake. Yohimbine reduced carbohydrate and fat ingestion but had little effect on protein intake. In fact, intake of carbohydrate for obese mice was suppressed across the entire 6-h test. The general reduction in energy intake observed is consistent with previous reports that have shown that IP injection of YOH can decrease feeding of standard rodent chow in *ob/ob* and lean mice, with obese mice showing an increased sensitivity to drug treatment (7). Further, it appears that the anorectic effects of YOH were not the result of a drug-induced generalized malaise. This is confirmed by previous research that has shown that effective anorectic doses of YOH, including those used in the current study, do not produce any effects on water intake in water-

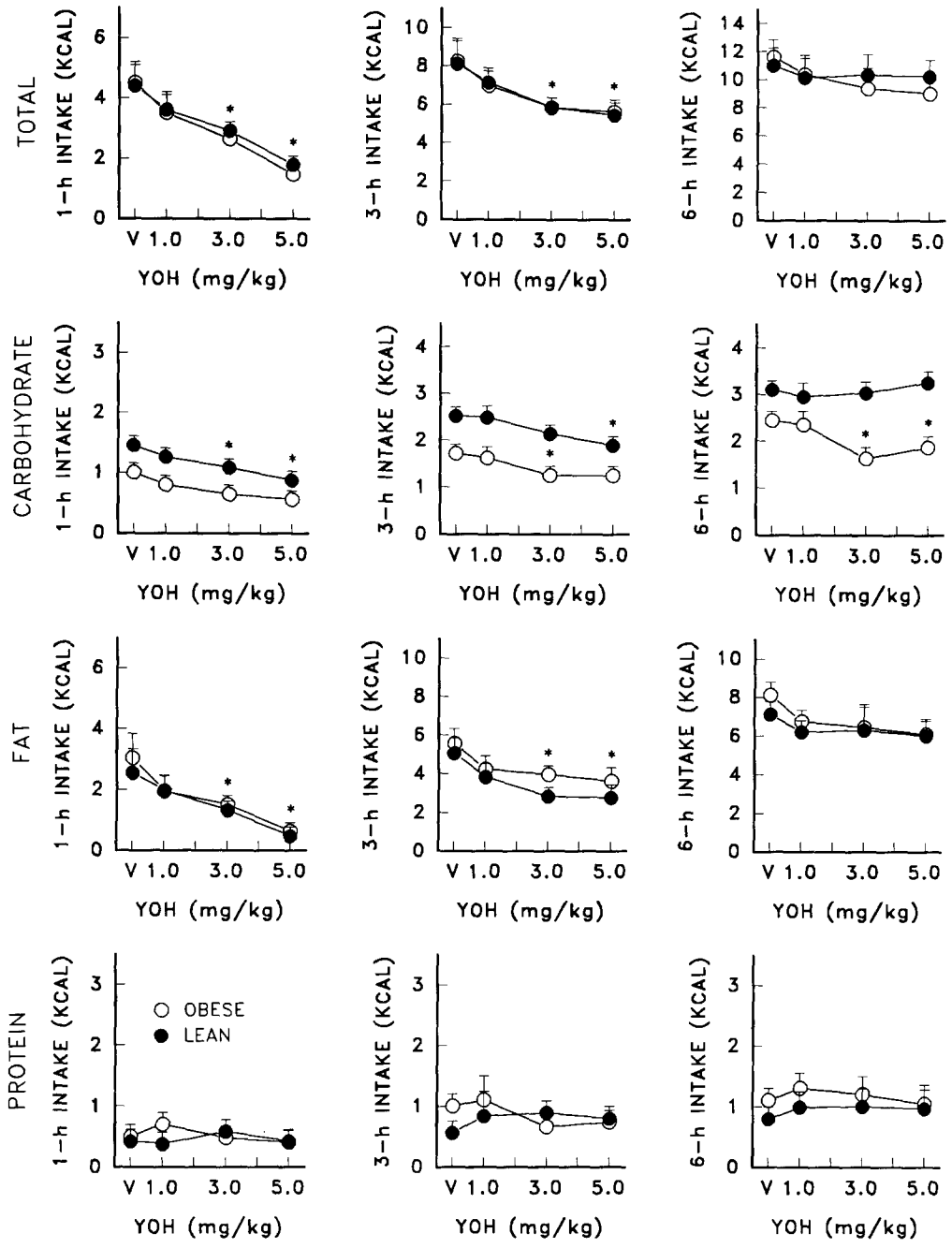

FIG. 1. Effects of yohimbine (YOH) on total caloric intake and macronutrient selection in genetically obese and lean mice. Intakes (kcal) were assessed at 1, 3, and 6 h following systemic drug injection. Yohimbine (3.0 and 5.0 mg/kg) reduced total energy intake and intake of carbohydrate and fat in both phenotypes, compared to vehicle (V), but did not alter protein ingestion. The 1-mg/kg YOH dose did not significantly alter feeding or macronutrient intake. *$p < 0.05$ vs. vehicle control of similar phenotype.

deprived mice and do not elicit any obvious sign of distress or illness (7,9). In addition, studies in rats suggest that doses administered in the current study were below those that produce behavioral disruption (32). Together, these observations suggest that the anorectic effects of YOH on energy intake result from selective interaction with the α_2-receptor.

In contrast, treatment with the α_2-agonist CLON increased feeding and overall caloric intake in both obese and lean mice, potentiating carbohydrate and fat intake, without altering protein consumption. Again, the shift in the pattern of nutrient selection toward a substantial increase in carbohydrate resulted in a significant increase in the percent concentration or proportion of carbohydrate ingested relative to total energy intake. Although caloric intake of fat was enhanced, the effect

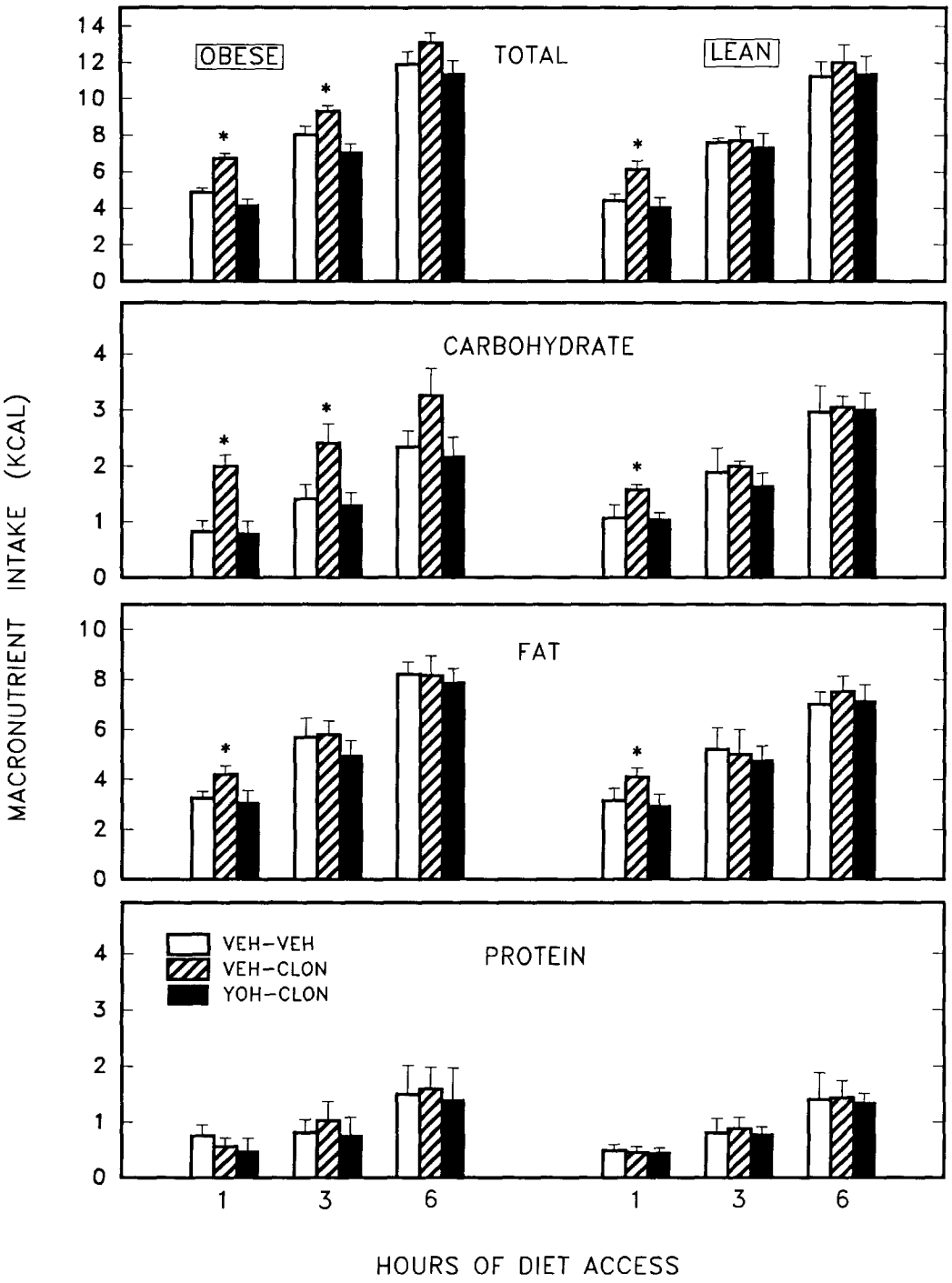

FIG. 2. Effects of yohimbine (YOH) pretreatment on alterations in macronutrient selection induced by clonidine (CLON). Mice received two IP injections of vehicle (VEH), vehicle paired with CLON (25 μg/kg), or YOH (1 mg/kg) followed by CLON (25 μg/kg), Intakes (kcal) were assessed 1, 3, and 6 h postinjection. Clonidine increased total energy, carbohydrate, and fat intake in obese and lean mice. However, pretreatment with YOH antagonized CLON's potentiating effects on feeding and macronutrient selection. *$p < 0.05$ vs. vehicle control of same phenotype.

TABLE 2

PERCENT CONCENTRATION OF CARBOHYDRATE, FAT, AND PROTEIN INGESTED AFTER YOHIMBINE AND CLONIDINE INJECTION

	Hours of Diet Access		
	1	3	6
Obese			
Carbohydrate			
VEH-VEH	17.9 (3.6)	19.5 (4.1)	19.7 (5.3)
VEH-CLON	30.8 (4.9)*	27.6 (4.3)	24.8 (5.7)
YOH-CLON	19.2 (3.9)	18.9 (6.1)	19.1 (4.5)
Fat			
VEH-VEH	68.9 (4.1)	68.4 (5.9)	67.7 (6.3)
VEH-CLON	60.8 (3.2)*	61.3 (6.7)	62.2 (7.2)
YOH-CLON	68.7 (4.5)	68.7 (5.9)	68.5 (6.6)
Protein			
VEH-VEH	13.2 (0.7)	12.1 (1.5)	12.6 (2.6)
VEH-CLON	8.4 (0.9)*	11.1 (1.2)	12.1 (2.0)
YOH-CLON	12.1 (1.1)	12.4 (2.7)	12.4 (1.9)
Lean			
Carbohydrate			
VEH-VEH	21.1 (1.0)	25.2 (1.7)	26.9 (2.5)
VEH-CLON	26.6 (1.4)*	25.8 (1.9)	25.8 (4.7)
YOH-CLON	22.6 (1.8)	23.4 (2.4)	26.4 (3.9)
Fat			
VEH-VEH	65.8 (4.5)	62.4 (5.1)	60.2 (6.1)
VEH-CLON	63.5 (4.9)	62.8 (5.9)	61.2 (6.3)
YOH-CLON	66.2 (5.2)	64.8 (6.1)	60.9 (7.4)
Protein			
VEH-VEH	13.1 (1.1)	12.4 (1.4)	12.9 (1.8)
VEH-CLON	9.9 (1.0)*	11.4 (2.1)	13.0 (1.6)
YOH-CLON	11.2 (2.1)	11.8 (1.8)	12.7 (2.3)

*$p < 0.05$ vs. vehicle control of same phenotype.

was considerably less dramatic than the potentiation of carbohydrate, resulting in a decline in the percent concentration of fat. A similar reduction in the proportion of protein consumed was also found. Both obese and lean mice demonstrated an increased preference for carbohydrate following CLON administration, but *ob/ob* mice appeared to show a heightened sensitivity to CLON treatment, in particular with respect to enhanced carbohydrate and total energy intakes. However, yohimbine pretreatment, at a dose that alone did not alter energy intake or diet selection, blocked the CLON-induced potentiation of feeding and macronutrient intake in both phenotypes.

Recent studies have shown that central and systemic injections of CLON can potentiate feeding in satiated rats in a manner similar to that observed following injection of NE into the PVN (15,22,30,41). The present study extends these findings to another species, the genetically obese mouse, and suggests that CLON can increase feeding in this pathologic model. While the increased sensitivity of the *ob/ob* to systemic CLON treatment could result from a greater absolute amount of drug administered (on a mg/kg basis), or reflect altered storage and release of CLON from fat depots or impaired drug clearance in comparison to lean mice, preliminary findings in our lab suggest that the *ob/ob* also shows an increased sensitivity to the orexigenic effects of centrally administered CLON (13). Further, the enhanced behavioral response of the *ob/ob* to central CLON injection has been found in free-feeding mice, suggesting that the increased pharmacological sensitivity of the *ob/ob* is not simply a response to the meal-feeding regimen employed in the current study.

Although obese mice consume a greater proportion of daily caloric intake from fat when given simultaneous access to carbohydrate, fat, and protein sources, on either ad lib or meal-feeding regimens (12,35), the present study suggests that CLON can alter eating patterns through its influence on specific macronutrients in *ob/ob* mice. Clonidine potentiated carbohydrate intake in obese and lean mice and previous reports have shown similar alterations in diet selection following administration of this α_2-agonist in the rat (22,41). The selective carbohydrate effect following PVN microinjection of NE or CLON suggests that the PVN is a primary site in the mediation of CLON-induced hyperphagia. Further, discrete electrolytic lesions of the PVN abolish feeding and carbohydrate preference resulting from peripheral CLON administration, while neurotoxin lesions of the PVN, which reduce PVN NE levels, fail to alter peripheral CLON-induced feeding (41). This indicates that CLON may be acting via PVN postsynaptic α_2-receptors to potentiate carbohydrate intake rather than by altering presynaptic release of NE from nerve terminals in this nucleus. Moreover, the specificity of the CLON-induced potentiation of feeding in the *ob/ob* is consistent with previous reports of alterations in long-term feeding patterns by chronic PVN infusion of NE or CLON, resulting in increased daily food intake and body weight gain in rats (25,26).

The heightened sensitivity of the *ob/ob* to CLON-induced feeding suggests that this monoaminergic receptor system may be involved in the development and/or maintenance of genetic obesity. In partial support of this hypothesis, recent work has shown that genetically obese Zucker rats have an increased concentration of PVN α_2-receptors compared to lean rats (18). While differences in PVN α_2-receptor binding for obese and lean mice have not yet been demonstrated, facilitated release of PVN NE or enhanced postsynaptic α_2-receptor activity could promote hyperphagia and macronutrient-specific appetite. Abnormal NE synthesis, storage, or release could in turn be influenced by endogenous glucocorticoids, opioid peptides, glucose, and insulin. For example, it is now believed that PVN NE acts in close association with two circulating hormones, the adrenal glucocorticoid corticosterone and the pancreatic hormone insulin. Feeding elicited by PVN NE microinjection is abolished by adrenalectomy and attenuated by dissection of vagal afferents to the pancreas in the rat (24,34). Circulating corticosterone appears to upregulate α_2-receptors (19), and the concentration of α_2-receptors in the PVN is positively correlated with circulating glucose concentrations (20). In addition to hyperinsulinemia and hyperglycemia, the *ob/ob* has elevated corticosterone levels and displays enhanced sensitivity to glucocorticoid-induced food intake (6,31,36,39,44). Although the nature of the relationship between the increased levels of corticosterone, insulin, and glucose to the hypothalamic α_2-noradrenergic feeding system of the *ob/ob* remains to be determined, it is possible that such increases may have an exaggerated impact on PVN α_2-receptors and NE neurotransmission and subsequently affect feeding behavior.

In summary, our data demonstrate an anorectic effect of systemic YOH on overall feeding and specific macronutrient selection patterns in genetically obese and lean mice. Clonidine, in contrast, potentiated feeding and nutrient intake, with obese mice exhibiting an enhanced carbohydrate preference. Pretreatment with YOH, however, resulted in competitive α_2-noradrenergic receptor antagonism and blocked CLON's stimulatory effect on feeding. These findings are therefore consistent with a general role for α_2-receptors in appetite regulation of mice, as has been previously shown in the rat. More importantly, they suggest that disturbances in this system may contribute to the abnormal feeding pattern of *ob/ob* mice.

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