Pharmacology Biochemistry & Behavior, Vol. 20, pp. 591–599, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Yohimbine and Rauwolscine Reduce Food Intake of Genetically Obese (*obob*) and Lean Mice

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Received 20 June 1983

CALLAHAN, M. F., M. BEALES AND G. A. OLTMANS. *Yohimbine and rauwolscine reduce food intake of genetically obese (obob) and lean mice.* PHARMACOL BIOCHEM BEHAV 20(4)591–599, 1984.—Multiple behavioral and neurochemical abnormalities are found in the genetically obese mouse, *obob*, including hyperphagia, elevated hypothalamic norepinephrine (NE) levels, and increases α-1 receptor density. The obese mutant also responds abnormally to neuropharmacological agents. In the current study the α-2 receptor blockers yohimbine and rauwolscine were administered to food-restricted (6-hour food access) *obob* and lean mice. Yohimbine and rauwolscine significantly reduced the 3- and 6-hour food intake of both *obob* and lean mice. The *obob* mice were, however, more sensitive to this anorectic effect than lean mice. Effective anorectic doses of yohimbine did not affect water intake in water-deprived lean mice, suggesting a specific effect of the drug upon food intake. Low doses (50 and 100 μg) of the α-2 agonist clonidine increased the 1-hour food intake of *obob* mice, but did not affect the food intake of lean mice. No differences were found between *obob* and lean mice in the number of α-receptors in the hypothalamus. The results suggest that modification of NE release by manipulation of α-2 receptor can alter food intake, and that the *obob* mutant is particularly sensitive to this effect.

Obesity Anorexia Food intake *obob* mice Genetically obese mice Yohimbine Rauwolscine Clonidine α-2 Receptor blockers α-2 Receptors

THE genetically obese mouse, *obob*, exhibits multiple physiological and behavioral abnormalities including hyperinsulinemia, hyperphagia, hypothermia, hypo-activity, and hyperglycemia [8,9]. Evidence from normal animals indicates that hypothalamic mechanisms contribute to the regulation of many of the conditions which are changed in the *obob* [26] and it has been suggested that a hypothalamic defect may be responsible for many of the altered states [9,11]. In support of this hypothesis, evidence of hypothalamic abnormalities in the *obob* is now available and includes increased numbers of hypothalamic α-1 receptors [29], increased hypothalamic norepinephrine (NE) levels [12,21], and decreased cell body size in several hypothalamic nuclei [5]. Although the relationship of the physiological and behavioral conditions to the hypothalamic changes has not been clearly established, the finding of altered hypothalamic noradrenergic systems does provide a link between the behavioral and neurochemical variables. In this respect, it has been shown that the intra-hypothalamic administration of NE can substantially modify the food intake of normal animals [6, 17, 22]. Thus, a defect in hypothalamic NE systems in the *obob* may lead to the hyperphagia exhibited by this mutant.

Although the precise nature of the noradrenergic abnormality is not known, it has been shown that the *obob* has a delayed and/or decreased depletion of hypothalamic NE in response to pharmacological manipulations [19,30]. These results, in combination with the observation of increased numbers of hypothalamic α-1 receptors, indirectly suggest possible problems in NE release or activity. In this respect, decreased NE release could lead to compensatory postsynaptic increases in α-1 receptors [41]. One way to increase NE activity is by administration of a releasing agent such as amphetamine. When this was done with *obob* mice their food intake was reduced [15]. In contrast, when α-methyl-para-tyrosine was administered to *obob* mice, the NE content [20], and presumably the synaptic availability of this transmitter, was reduced and the hyperphagia was potentiated [4].

Another approach to altering NE release is via manipulation of the presynaptic α-2 receptor, as it has been shown that activation of this receptor can inhibit NE release [16]. Interestingly, other work indicates that the alpha-2 agonist clonidine can increase food intake in normal rats [24,33]. Thus, α-2 antagonists might be predicted to decrease food intake. In the current studies the α-2 blockers yohimbine and rauwolscine, as well as the α-2 agonist clonidine, were administered to *obob* and lean mice in an attempt to pharmacologically manipulate the synaptic availability of NE. The effects on food intake were then determined. To more

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clearly assess any differential effects the drugs might have in *obob* and lean mice, α-2 receptor density and affinity were also determined.

METHOD

Subjects were male C57BL/6j-*ob* mice (*ob/ob*) and their lean littermate controls (*OB/?*) obtained from the Jackson Laboratory, Bar Harbor, ME. Mice were maintained on a reverse day-night cycle (lights off 0900–2100 hours) in group housing conditions (7–8 mice/cage) with ad lib water access. The mice were 2 to 6 months old at the time of the various experiments, but within any specific experiment the ages were the same. Any animals that became ill during the course of a study were excluded from the study. In all studies mice were randomly assigned to either drug or vehicle treatment.

For the yohimbine and clonidine studies the mice were acclimated to a six-hour food access schedule as described before [15]. Briefly, at 0930 hours the mice were removed from the group-housing cages, weighed, administered an IP injection of distilled-deionized water (0.1 ml/10 g body weight), and placed into individual cages. Thirty minutes later they were given a pre-weighed amount of food and food intake was determined one, three, and six hours later by reweighing the food (to the nearest 0.01 g). The mice were then returned to the group-housing cages (no food available). This process was repeated daily until food intake had stabilized (3 to 4 weeks). For drug testing the specific drug was substituted for the water injection.

For the rauwolscine study a different food deprivation and acclimation procedure was employed. For the first nine days of acclimation the mice were given six hours of food access and food deprived for 18 hours in their home cages under the group-housing conditions. On the next four days the mice were weighed, administered an IP injection of water, returned to their home cage, and given 6 hours of food access. For the next 10 days the animals were treated as in the yohimbine and clonidine studies (injected, given 6-hour food access in individual cages, and then returned to home cages). After the drug testing period had started the mice were occasionally given 48 hours of free access to food, and subsequent testing was conducted following reacclimation to the restricted 6-hour food access schedule. Using this procedure there were fewer problems with animals becoming ill as a result of the restricted food-access, and the animals rapidly readapted (usually within one to two days) to the 6-hour feeding schedule following a period of 48-hour food access.

The following drugs were studied for their effects on food intake: yohimbine-HCl (Sigma Chemical Co.), rauwolscine-HCl (Roth Chemie), and clonidine-HCl (Boehringer Ingelheim). At low doses yohimbine and rauwolscine are relatively specific α-2 receptor antagonists [2, 7, 37, 38] and clonidine is an α-2 agonist [36]. Maximum doses of rauwolscine and yohimbine were determined by preliminary studies which indicated that the doses used produced no overt signs of locomotor depression or illness. For yohimbine this dose was <6 mg/kg and for rauwolscine <15 mg/kg. Clonidine doses were selected on the basis of published reports indicating an effect on food intake [24]. Drugs were administered in a distilled-deionized water vehicle. On non-drug days all animals received vehicle injections in all experiments. Drug doses were calculated as the salt.

Four separate experiments were conducted. In all experiments injections were administered IP, and 4 groups of animals were run (*obob*-drug, *obob*-vehicle, lean-drug, lean-vehicle). In Experiment 1 yohimbine-HCl was administered to animals 2 months old at the beginning of the experiment. The drug concentrations were prepared so that the mice received a 0.1 ml injection for every 10 g of body weight (0.21–0.46 mg/ml in Experiment 1). The following injection schedule was used: Days 1 and 2, *obob* and lean=3.0 mg/kg; Days 8–11, *obob*=3.0 mg/kg, lean=4.6 mg/kg; Day 13, *obob*=2.1 mg/kg, lean=3.0 mg/kg. The differential doses administered on days 8–11 and day 13 were used as a means of compensating for the higher total drug dose the *obob* mice received when the drug was administered on a body-weight basis. Previous work comparing the effects of amphetamine in *obob* and lean mice indicates that this may be an important consideration [15]. Thus, this differential dose represents an equivalent total dose treatment in the lean and obese mice.

In Experiment 2 increasing doses of yohimbine-HCl were administered to 3 month old animals using the following schedule: Day 1, *obob* and lean=1.0 mg/kg; Day 3, *obob* and lean=2.0 mg/kg; Day 7, *obob* and lean=3.0 mg/kg; Day 11, *obob*=2.78 mg/kg, lean=5.0 mg/kg. Three and 6 hour water intake was also measured in this experiment by weighing the water bottles before and after food was made available.

In Experiment 3 rauwolscine-HCl was administered to animals 6 months old at the beginning of the experiment. Both lean and *obob* mice were administered 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, and 10.0 mg/kg doses in an ascending order. Drug injections were separated by 5 to 10 days except for the low doses (0.5, 1.0, 2.0), where injections were separated by 1 to 2 days.

In Experiment 4 clonidine-HCl was administered to the animals used in Experiment 2 following a drug-free period of 7 days. The same dose of clonidine was administered to lean and obese mice according to the following schedule: Day 1=500 μg/kg; Day 10=50 μg/kg; Day 14=100 μg/kg; Day 17=200 μg/kg.

In a separate study (Experiment 5) the effects of yohimbine on water intake in water-deprived mice were studied in a group of C57/BL-6J mice (Jackson Laboratories). Mice were given ad lib access to food but allowed only 6 hours access to water (food present). Water intake was measured at 3 and 6 hours by weighing the water bottles before and after water access. When the animals had acclimated to this procedure and water intake had stabilized, yohimbine-HCl was administered (3, 5 or 10 mg/kg) and the effect on fluid intake determined.

Receptor-Binding Studies

The status of the α-2 receptor was studied in the hypothalamus, telencephalon, and brainstem of lean and *obob* mice using the ligand ^{3}H-rauwolscine (New England Nuclear, Specific Activity=83.5 Ci/mmol) according to the procedure of Perry and U'Prichard [31] with minor modifications. In brief, fresh pooled tissue (8–9 hypothalami or 2 brainstems or 2 telencephalons) was homogenized in 40 ml of ice cold Tris-HCl buffer (0.05 M, pH=7.0 at 25°C) and centrifuged (35,000 g at 4°C). The pellet was resuspended in the Tris buffer as a 20 mg/ml solution and 5 mg of tissue (250 μl) were added to tubes containing ^{3}H-rauwolscine in concentrations ranging from 0.6 to 33.4 nM. Four tubes were run for each tissue at each of six concentrations and one-half of the tubes contained yohimbine-HCl (10^{-6}M) as a displacer to determine specific binding. The final incubation volume was

TABLE 1

EFFECT OF YOHIMBINE ON THE FOOD INTAKE OF *obob* AND LEAN MICE (EXPERIMENT 1)

Day	Yohimbine Dose (mg/kg)	Group	N	Food Intake (g) (Mean ± S.D.) 3 Hour	6 Hour
1	3.0	*obob*-drug	6	1.14 ± 0.50*†	2.59 ± 1.10*
		obob-vehicle	7	2.32 ± 0.26	4.16 ± 0.27
	3.0	lean-drug	5	1.88 ± 0.33	3.39 ± 0.71
		lean-vehicle	5	1.95 ± 0.26	3.54 ± 0.79
2	3.0	*obob*-drug	6	1.37 ± 0.25*†	3.54 ± 0.40*
		obob-vehicle	7	2.04 ± 0.22	4.15 ± 0.26
	3.0	lean-drug	5	1.96 ± 0.50	3.86 ± 1.03
		lean-vehicle	5	2.08 ± 0.39	3.89 ± 0.72
8	3.0	*obob*-drug	6	1.42 ± 0.28*†	3.29 ± 0.59*
		obob-vehicle	7	2.05 ± 0.35	3.92 ± 0.28
	4.6	lean-drug	4	1.87 ± 0.10*	3.98 ± 0.15
		lean-drug	5	2.08 ± 0.14	3.55 ± 0.66
9	3.0	*obob*-drug	6	1.40 ± 0.22*	3.51 ± 0.39
		obob-vehicle	7	1.91 ± 0.29	3.86 ± 0.29
	4.6	lean-drug	5	1.53 ± 0.54	3.29 ± 0.68
		lean-vehicle	5	1.89 ± 0.23	3.50 ± 0.67
10	3.0	*obob*-drug	6	1.11 ± 0.59*	2.87 ± 0.58
		obob-vehicle	7	1.83 ± 0.50	3.32 ± 0.45
	4.6	lean-drug	5	1.30 ± 0.75	2.41 ± 1.36
		lean-vehicle	5	1.64 ± 0.26	2.96 ± 0.49
11	3.0	*obob*-drug	6	1.57 ± 0.76	3.30 ± 1.38
		obob-vehicle	7	2.12 ± 0.58	3.78 ± 0.79
	4.6	lean-drug	5	2.17 ± 0.45	3.74 ± 0.89
		lean-vehicle	5	2.30 ± 0.93	3.65 ± 0.57
13	2.1	*obob*-drug	6	1.11 ± 0.55*†	2.68 ± 1.33
		obob-vehicle	7	1.74 ± 0.37	3.73 ± 0.81
	3.0	lean-drug	5	1.84 ± 0.35	3.53 ± 0.67
		lean-vehicle	5	2.06 ± 0.24	3.73 ± 0.51

*Differs significantly from vehicle-treated mice with same genotype, $p<0.05$.
†Differs significantly from drug-treated lean, $p<0.05$.

270 μl. Samples were incubated in the dark at 4°C for 90 minutes. The incubation was stopped by filtering through Whatman GF/B filters using a cell harvester (Brandel, Gaithersberg, MD). The filters were immediately rinsed 3 times with 4 ml of the ice cold Tris buffer. The receptor density (B_{max}) and dissociation constant (K_d) were determined using an inverted Rosenthal plot (bound vs. bound/free) and linear regression analysis. Three to four separate analyses were completed for each brain section. Tissue from lean and obese mice was processed concurrently in each assay.

Data were analyzed by an analysis of variance with follow-up comparisons made by Newman-Keuls test. In some cases specific comparisons were made by means of a *t*-test.

RESULTS

Food intake on the day immediately preceding drug treatment (when all animals received vehicle injection) never differed significantly between mice with the same genotype but assigned to the vehicle or drug-treatment groups. This was true for all experiments (1–4) and indicates comparable food intake in the animals assigned to the drug and vehicle groups in the absence of the drug treatment. For the yohimbine and rauwolscine studies the relative effects of drug treatment were similar for the 1-hour and 3-hour food intake periods. Consequently, data are given only for the 3-hour period in order to simplify presentation of these experiments.

Experiment 1

The effects of yohimbine on food intake are presented in Table 1. The initial treatment with yohimbine (3.0 mg/kg, Days 1 and 2) produced a significant decrease in the food intake of *obob* mice at both the 3- and 6-hour time points, but did not reduce the food intake of lean mice at either time. The drug-treated *obob* mice also ate significantly less than drug-treated lean mice at the 3-hour point. Vehicle-treated *obob* mice ate amounts larger than or comparable to those of vehicle-treated lean mice at the 3 hour point.

Following a 6 day drug-free period the *obob* mice were

TABLE 2

DOSE-RESPONSE ANALYSIS OF THE EFFECTS OF YOHIMBINE ON FOOD INTAKE IN *obob* AND LEAN MICE (EXPERIMENT 2)

Day	Yohimbine Dose (mg/kg)	Group	N	Food Intake (g) (Mean ± S.D.) 3 Hour	6 Hour
1	1.0	*obob*-drug	8	1.72 ± 0.19	3.66 ± 0.19
		obob-vehicle	7	1.63 ± 0.22	3.50 ± 0.43
	1.0	lean-drug	8	1.86 ± 0.32	3.51 ± 0.36
		lean-vehicle	7	1.94 ± 0.22	3.40 ± 0.37
3	2.0	*obob*-drug	8	1.34 ± 0.28	3.12 ± 0.17
		obob-vehicle	7	1.62 ± 0.33	3.44 ± 0.62
	2.0	lean-drug	8	1.53 ± 0.24	3.10 ± 0.25
		lean-vehicle	7	1.74 ± 0.32	3.27 ± 0.51
7	3.0	*obob*-drug	8	1.21 ± 0.19*	2.66 ± 0.26*
		obob-vehicle	7	1.56 ± 0.20	3.46 ± 0.64
	3.0	lean-drug	8	1.32 ± 0.10	2.70 ± 0.15
		lean-vehicle	7	1.58 ± 0.37	2.98 ± 0.51
11	2.8	*obob*-drug	8	1.29 ± 0.15*	2.60 ± 0.26*
		obob-vehicle	7	1.77 ± 0.21	3.31 ± 0.29
	5.0	lean-drug	8	1.25 ± 0.45*	2.24 ± 0.78*
		lean-vehicle	7	1.79 ± 0.24	2.96 ± 0.37

*Differs significantly from vehicle-treated mice with same genotype, $p<0.05$.

again administered the 3.0 mg/kg dose, while lean mice were administered a larger mg/kg dose (4.6). This differential dose resulted in the two groups receiving an equivalent total amount of drug (see the Method section). This time both lean and *obob* mice significantly reduced their food intake for the initial 3-hours of food access, but drug-treated *obob* mice still ate significantly less than drug-treated lean mice. After 6-hours of food access only the *obob* mice showed a significant reduction in food intake compared to their vehicle-treated controls.

To determine if tolerance would develop to the anorectic effects of yohimbine, the differential dose treatment was continued for an additional three days (Days 9–11). With this schedule drug-treated *obob* mice ate less than vehicle-treated *obob* mice during the initial three hours of food access on days 9 and 10, but did not differ significantly from controls after 6 hours of food access or at either time on Day 11. Treatment of lean mice with the 4.6 mg/kg dose of yohimbine did not produce a significant suppression of their food intake compared to the vehicle-treated lean controls when the drugs were administered on consecutive days.

Following a one day drug-free period, the lean mice were administered a 3.0 mg/kg dose of yohimbine and the *obob* mice a 2.1 mg/kg dose, which also produced equal total doses in lean and *obob* mice. Once again the *obob* mice showed a significant reduction in 3-hour food intake compared to both vehicle-treated *obob* mice and drug-treated lean mice, while drug-treated lean mice did not differ significantly from vehicle-treated lean mice.

In summary, the results of Experiment 1 indicate that yohimbine appears to have an anorectic effect in lean and *obob* mice. This anorectic effect is more pronounced in the *obob* mice, as the lean mice showed a significant reduction in food intake on only one of the testing occasions at the doses being studied, while *obob* mice consistently reduced their food intake in response to the drug treatment. It also appears that some tolerance to the anorectic effects of yohimbine can develop with repeated drug administration.

Experiment 2

Since the results of Experiment 1 suggested an increased sensitivity to the anorectic effects of yohimbine on the part of *obob* mice, in Experiment 2 a dose-response curve was run in a different group of animals to determine where the threshold might be. Water intake was also measured in this experiment.

Neither a 1.0 nor 2.0 mg/kg dose of yohimbine produced a significant effect on food intake (Table 2) in either lean or *obob* mice. Once again, however, the 3.0 mg/kg dose significantly reduced the food intake of *obob*, but not lean, mice after 3 and 6 hours of food access. Administration of a higher dose of yohimbine (5.0 mg/kg) to lean mice did reduce their food intake, and a similar suppression was found in *obob* mice given a comparable total body dose (2.78 mg/kg). The effects of drug treatment on water intake paralleled those on food intake (Table 3).

The results of this experiment confirmed the findings of Experiment 1 and indicated that on a body weight basis the threshold dose for an anorectic effect of yohimbine in *obob* mice was lower than that for lean mice.

Experiment 3

In this experiment the effects of another α-2 blocking drug, rauwolscine, were studied to determine if the anorectic effects seen with yohimbine were unique to that drug. Rauwolscine is a structural isomer of yohimbine but has more specificity for the α-2 receptor than yohimbine [38]. The characteristics of the food restriction schedule were also modified in this experiment (see the Method section). As in

TABLE 3

EFFECT OF YOHIMBINE ON WATER INTAKE OF *obob* AND LEAN MICE (EXPERIMENT 3)

Day	Yohimbine Dose (mg/kg)	Group	N	Water Intake (ml) (Mean ± S.D.) 3 Hour	6 Hour
1	1.0	*obob*-drug	8	2.02 ± 0.45	4.20 ± 0.35
		obob-vehicle	7	1.87 ± 0.41	3.94 ± 0.65
	1.0	lean-drug	8	2.78 ± 0.33	5.43 ± 0.57
		lean-vehicle	7	3.23 ± 0.69	5.49 ± 1.00
3	2.0	*obob*-drug	8	1.60 ± 0.47	3.72 ± 0.36
		obob-vehicle	7	1.94 ± 0.70	4.23 ± 1.35
	2.0	lean-drug	8	2.43 ± 0.36	5.02 ± 0.76
		lean-vehicle	7	2.73 ± 0.50	4.89 ± 0.88
7	3.0	*obob*-drug	8	1.50 ± 0.34*	3.21 ± 0.49*
		obob-vehicle	7	2.02 ± 0.45	4.40 ± 1.15
	3.0	lean-drug	8	2.09 ± 0.25	4.21 ± 0.67
		lean-vehicle	7	2.50 ± 0.64	4.44 ± 0.61
11	2.8	*obob*-drug	8	1.69 ± 0.42*	3.42 ± 0.60*
		obob-vehicle	7	2.52 ± 0.31	4.47 ± 0.58
	5.0	lean-drug	8	1.99 ± 0.66*	3.90 ± 1.23
		lean-vehicle	7	2.80 ± 0.21	4.62 ± 0.43

*Differs significantly from vehicle-treated mice with same genotype, $p<0.05$.

Experiments 1 and 2, a comparison of the food intakes of drug- and vehicle-treated groups on the day prior to drug-treatment (when all groups received a vehicle injection) indicated that in all instances there were no significant differences in the food intake of these groups for either lean or obese mice.

Compared to vehicle-treated controls, rauwolscine treatment significantly decreased 3- and 6-hour food intake in both lean and *obob* mice, but the dose required to achieve this effect differed in the two groups. In Fig. 1 food intake in drug-treated lean and *obob* mice for the initial 3 hours of food access is expressed as a percent of the food intake of their respective vehicle-treated controls. For *obob* mice, three-hour food intake was significantly decreased by doses of rauwolscine as low as 1.0 mg/kg, while a 4.0 mg/kg dose was required to see a significant effect in lean mice. Six-hour food intake was significantly suppressed in *obob* mice with a 5.0 mg/kg dose, while only the 10 mg/kg dose decreased the 6-hour food intake of lean mice (Table 4).

The results with rauwolscine are comparable to the results found for yohimbine, and again suggest that the *obob* mice were more sensitive than lean mice to the anorectic effects of the drug treatment. Compared to yohimbine, the effects of rauwolscine on three-hour food intake were found to occur at a lower dose. If the anorectic effects are related to α-2 blockade, this finding would be consistent with other reports indicating that rauwolscine has a greater α-2 blocking potency than yohimbine [38].

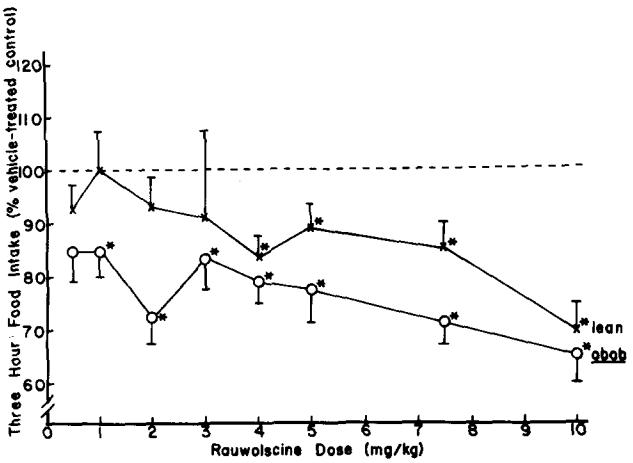

FIG. 1. Rauwolscine treatment significantly reduced food intake in *obob* and lean mice in comparison to vehicle-treated controls. The threshold dose for this effect for *obob* mice (1.0 mg/kg) was one-fourth of that for lean mice (4.0 mg/kg). *Differs significantly from vehicle-treated mice with same genotype, $p<0.05$ (Mean±S.D.).

Experiment 4

In this experiment the α-2 agonist clonidine was administered to a group of animals also used in Experiment 2 following a drug-free period of 7 days. Although the data are conflicting [3,33], some reports indicate that clonidine can increase food intake. Because this effect may be brief [24] food intakes are reported for the 1 hour as well as the 3 and 6 hour measurements in this study.

The effects of clonidine on 1 hour food intake are presented in Fig. 2. At the lowest dose studied (50 μg/kg) clonidine produced a small increase in the one hour food intake of *obob* mice ($0.05<p<0.07$), but did not affect food intake in lean mice. A higher dose of clonidine (100 μg/kg) produced a significant increase in the one-hour food intake of *obob* mice (+40%, $p<0.01$), but again did not affect food

TABLE 4

EFFECT OF RAUWOLSCINE ON 6-HOUR FOOD INTAKE OF *obob* AND LEAN MICE (EXPERIMENT 3)

		6-Hour Food Intake (g) (Mean ± S.D.)			
		obob		lean	
Day	Rauwolscine Dose (mg/kg)	drug (n=8)	vehicle (n=7)	drug (n=8)	vehicle (n=7)
1	0.5	3.10 ± 0.38	3.56 ± 0.59	3.50 ± 0.31	3.60 ± 0.25
3	1.0	3.17 ± 0.48	3.50 ± 0.43	3.76 ± 0.61	4.00 ± 0.24
6	2.0	3.16 ± 0.81*	4.08 ± 0.51	3.62 ± 0.61	3.91 ± 0.35
17	3.0	3.21 ± 0.39	3.36 ± 0.47	3.32 ± 0.98	3.90 ± 0.66
23	4.0	3.31 ± 0.44	3.60 ± 0.67	3.46 ± 0.50	3.49 ± 0.24
32	5.0	3.21 ± 0.39*	3.93 ± 0.35	3.23 ± 0.46	3.46 ± 0.39
38	7.5	3.36 ± 0.35*	4.21 ± 0.45	4.16 ± 0.76	4.70 ± 0.72
46	10.0	2.15 ± 0.42*	3.22 ± 0.42	2.38 ± 0.22*	3.21 ± 0.30

*Differs significantly from vehicle-treated mice with same genotype, $p<0.05$.

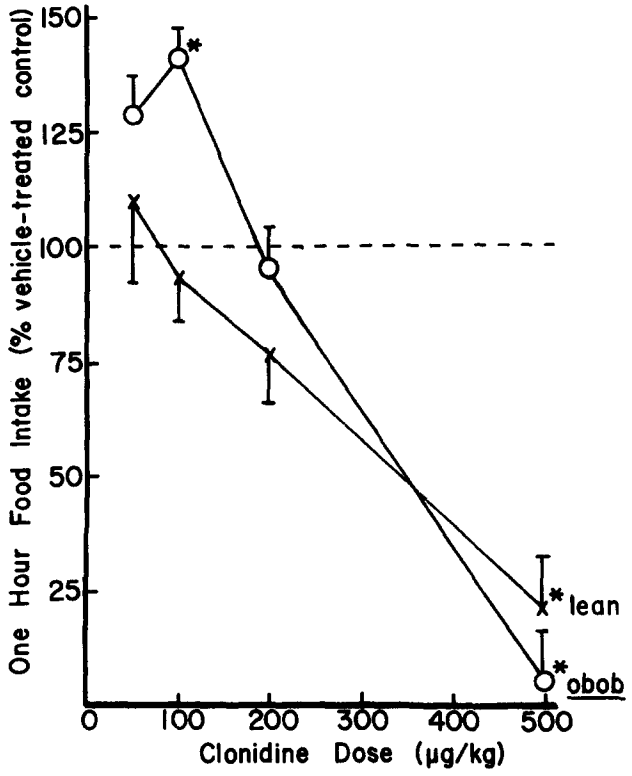

FIG. 2. At low doses clonidine produced an increase in the initial one-hour food intake of *obob* mice and did not affect the food intake of lean mice in comparison to vehicle-treated controls. Higher doses of clonidine reduced the food intake of both *obob* and lean mice. *Differs significantly from vehicle-treated mice with the same genotype, $p<0.05$ (Mean±S.D.).

TABLE 5

EFFECT OF CLONIDINE ON 3- AND 6-HOUR FOOD INTAKE OF *obob* AND LEAN MICE (EXPERIMENT 4)

Clonidine Dose (µg/kg)	Group	N	Food Intake (g) (Mean ± S.D.) 3 Hour	6 Hour
50	*obob*-drug	8	1.82 ± 0.41	3.93 ± 0.37
	obob-vehicle	7	1.72 ± 0.22	4.00 ± 0.60
	lean-drug	6	1.78 ± 0.48	4.30 ± 0.78
	lean-vehicle	5	1.85 ± 0.39	3.62 ± 0.74
100	*obob*-drug	8	1.76 ± 0.20	3.87 ± 0.33
	obob-vehicle	7	1.89 ± 0.18	4.14 ± 0.30
	lean-drug	6	1.64 ± 0.44	3.36 ± 0.72
	lean-vehicle	5	1.77 ± 0.53	3.31 ± 0.86
200	*obob*-drug	8	1.59 ± 0.22*	3.68 ± 0.43*
	obob-vehicle	7	2.35 ± 0.63	4.90 ± 0.86
	lean-drug	6	1.79 ± 0.22*	3.67 ± 0.47
	lean-vehicle	5	2.16 ± 0.28	4.28 ± 0.59
500	*obob*-drug	8	0.80 ± 0.25*	1.85 ± 0.31*
	obob-vehicle	7	1.73 ± 0.42	2.45 ± 0.50
	lean-drug	6	1.00 ± 0.24*	3.87 ± 0.75
	lean-vehicle	5	2.03 ± 0.10	3.81 ± 0.24

*Differs significantly from vehicle-treated mice with same genotype, $p<0.05$.

TABLE 6

EFFECT OF YOHIMBINE TREATMENT ON WATER INTAKE OF WATER-DEPRIVED MICE

Day	Yohimbine Dose (mg/kg)	Group	N	Water Intake (ml) (Mean ± S.D.) 3 Hour	6 Hour
1	0	Drug	6	1.69 ± 0.45	2.48 ± 0.55
		Vehicle	6	1.54 ± 0.61	2.83 ± 1.30
2	3.0	Drug	6	1.79 ± 0.36	2.81 ± 0.84
		Vehicle	6	1.36 ± 0.41	2.66 ± 0.94
3	5.0	Drug	6	1.28 ± 0.43	2.71 ± 0.84
		Vehicle	6	1.55 ± 0.61	2.81 ± 1.12
10	5.0	Drug	6	1.77 ± 0.68	2.80 ± 0.97
		Vehicle	6	1.73 ± 0.51	3.15 ± 0.76
12	5.0	Drug	6	2.08 ± 0.54	3.34 ± 0.90
		Vehicle	6	1.91 ± 0.67	3.15 ± 1.22
16	10.0	Drug	6	1.03 ± 0.36	2.97 ± 0.61
		Vehicle	6	1.88 ± 0.85	3.82 ± 1.17
18	10.0	Drug	6	1.10 ± 0.41*	2.82 ± 0.62*
		Vehicle	6	2.53 ± 0.57	3.94 ± 0.76

*Differs significantly from vehicle-treated group, $p<0.05$.

TABLE 7

RECEPTOR DENSITY (B_{max}) AND DISSOCIATION CONSTANT (K_d) VALUES FOR RAUWOLSCINE BINDING IN BRAIN AREAS OF *obob* AND LEAN MICE

Brain Area	Group	B_{max}†	K_d†
Hypothalamus	*obob*	29.5 ± 5.4 (4)*	12.8
	lean	31.6 ± 3.7 (3)	13.3
Telecephalon	*obob*	24.1 ± 4.1 (4)	9.0
	lean	22.3 ± 2.1 (4)	7.9
Brainstem	*obob*	20.4 ± 5.4 (4)	11.2
	lean	17.3 ± 3.4 (4)	9.7

*Mean ± S.D. (N).

†B_{max} values are expressed as fmol bound/mg tissue, K_d values are nanomolar.

intake in lean mice. Higher doses of clonidine either had no effect or significantly decreased 1 hour food intake in both lean and *obob* mice. The effects of clonidine treatment on 3 and 6 hour food intake are presented in Table 5. The lower doses of clonidine (50 and 100 μg/kg) had no effect on the food intake of either lean or *obob* mice, while the higher doses (200 and 500 μg/kg) decreased 3 hour food intake in both lean and *obob* mice, and decreased 6 hour food intake in *obob* mice.

These results indicate that clonidine can produce a dose-dependent biphasic effect on food intake. Of particular relevance to the current study is the finding that at low doses clonidine produced either no effect (lean mice) or a moderate increase (*obob* mice) in the food intake of animals on the restricted food access schedule. This suggests that drug treatment does not inevitably reduce food intake solely on the basis of a "novelty" effect.

Experiment 5

In this experiment the effects of yohimbine treatment on water intake in water-deprived C57/BL-6j (lean) mice were examined to evaluate the possibility that yohimbine's inhibitory effects on food intake were the result of a more general depressant effect on all ingestive behavior. After 18 hours of water deprivation, 3- and 6-hour water intakes of yohimbine- and vehicle-treated mice did not differ significantly following treatment with either 3.0 or 5.0 mg/kg yohimbine (Table 6). At 10.0 mg/kg, however, there was a significant reduction in the 3- and 6-hour water intake of yohimbine-treated mice on one of the 2 days that this dose was used. These results indicate that at higher doses yohimbine treatment can reduce fluid intake in water-deprived animals, but that the effect is inconsistent and requires doses higher than those needed to reduce food intake (about 5 mg/kg in lean mice, Experiments 1 and 2).

Receptor binding. No differences were found between lean and *obob* mice in α-2 receptors in the hypothalamus, brainstem, or telencephalon as determined from ^{3}H-rauwolscine binding studies. This was true for both the receptor density (B_{max}) and affinity (K_d) (Table 7).

DISCUSSION

The results of the current study indicate that the α-2 blocking agents, yohimbine and rauwolscine, can reduce food intake in mice. The results also suggest that the *obob* mutant may be more sensitive to this effect than its lean littermate. Although the basis of this potentiated anorectic effect is not clear, these results are consistent with other work indicating altered drug sensitivity in the *obob* mutant [10,23].

When drugs are administered on a mg/kg-basis, however, it is difficult to determine whether a response at a "lower" dose is the result of an increased sensitivity on the part of the obese animal or due to a higher drug level in the target organ as a result of the higher total dose of drug administered to the obese animal [15]. In the current studies an attempt was made to deal with this issue by occasionally administering differential body-weight based doses which resulted in the administration of equivalent total drug doses to the lean and obese mice. When this strategy was employed with yohimbine in Experiment 1, it was found that drug-treated obese animals still ate less than drug-treated lean animals (Table 1). With rauwolscine a direct comparison of equivalent total doses was not made, but the dose-response curve indicated that the obese animals showed a significant effect on food intake at one-fourth the dose required for the lean mice. This is substantially less than the differences in the body weights for the two groups (less than a two-fold difference) and is again indicative of an increased sensitivity to the drug effect on the part of the obese mutant.

A second concern which developed during the course of the study was that any drug treatment might inhibit the food intake of *obob* mice on a food-restriction schedule. Indeed, it had been previously shown that dissimilar agents such as amphetamine, fenfluramine, and naloxone could have an anorectic effect in food restricted mice [10, 15, 23]. In the

current study, however, treatment with low doses of clonidine increased the food intake of the *obob* mice, and did not alter the intake of the lean mice, indicating that reduced food intake was not an inevitable consequence of drug treatment.

At high doses, clonidine treatment reduced the food intake of both lean and *obob* mice. Work from other laboratories indicates that the effects of clonidine on the food intake of normal animals are complicated, and are both time-dependent and affected by dose and frequency of administration [3,24]. This may be related to the finding that at higher doses clonidine can stimulate α-1 as well as α-2 receptors [40]. Another possibility is that clonidine may affect both pre- and post-synaptic α-2 receptors depending upon the dose employed [39].

It also appears that the anorectic effects of the yohimbine and rauwolscine were not the result of a drug-induced generalized malaise or illness. This is inferred from the fact that in preliminary studies the doses employed in the current study did not produce any obvious signs of distress, and the finding that effective anorectic doses did not produce any effects on water intake in water-deprived animals. Both of these observations support the position that the anorectic effects were a specific drug action. In addition, studies in rats suggest that the doses administered in the current studies were below those which produce behavioral distruption [25], although cross-specie comparisons might be misleading.

The drugs employed in the current study were chosen for their ability to affect the α-2 receptor. None of these drugs are, however, absolutely specific for this receptor. Clonidine can decrease serotonin turnover [1], while yohimbine can affect dopamine and serotonin systems [32, 34, 35]. It is, therefore, interesting that the effects on food intake were found at relatively low doses, a condition which would favor selective interaction with the α-2 receptor [2, 38, 40]. A comparison of the effective threshold doses for eliciting the anorectic effect for yohimbine and rauwolscine (Experiments 2 and 3) also provides indirect support for an action on the α-2 receptors. In this respect, in the *obob* mice rauwolscine had an anorectic effect at 1.0 mg/kg, while yohimbine had an anorectic effect at 2.8 to 3.0 mg/kg. Since yohimbine and rauwolscine are structural isomors this difference is not due to a dose difference as a result of different molecular weights. The difference is, however, similar to their differential potency at the α-2 receptor in *in vitro* studies [7,38]. Thus, the combination of the low drug doses administered, plus the order of potency for the rauwolscine and yohimbine, suggest a relatively selective effect on the α-2 receptor.

Although it appears likely that yohimbine and rauwolscine produce their anorectic effect by an action on the presynaptic α-2 receptor (see Discussion below), an effect on post-synaptic α-2 receptors cannot be completely excluded. If this were the case, then the differential drug sensitivity found in *obob* and lean mice might be explained by differences in α-2 receptor density or affinity between the two groups. The failure to find such differences in the rauwolscine binding studies, however, indicates comparable α-2 status in the two groups and does not support a post-synaptic α-2 effect by this mechanism.

In contrast, if examined in conjuction with other published data, a drug effect upon presynaptic α-2 receptors could account for the enhanced behavioral effects seen in the *obob* mice. In this respect, endogenous hypothalamic NE levels are increased in the *obob* [12,21], and these increases are found predominately in the paraventricular nucleus, ventromedial hypothalamus, and lateral hypothalamus [28], areas which have been shown to be involved in the regulation of feeding [13, 14, 18]. One possible explanation for the increased levels is a decreased neuronal firing rate and subsequent decreased release of NE. Since decreased transmitter availability has been shown to be associated with increases in receptor number [27,41], this explanation is consistent with the observation that α-1 receptor density is increased in the *obob*. Under the conditions of increased receptor number, administration of an agent which enhances NE release would produce increased neurotransmitter activity in a "supersensitive" area and could, therefore, elicit an enhanced behavioral effect.

This hypothesis is dependent upon showing that the α-2 blockers will enhance NE release, and that NE will inhibit feeding. Studies have now shown that yohimbine will increase the rate of disappearance of NE following α-methyl-para-tyrosine treatment, suggesting enhanced NE turnover and release [2,32]. In addition, preliminary results from this laboratory (unpublished data) indicate that rauwolscine treatment will increase NE release in *obob* and lean mice. Other work indicates that intra-hypothalamic infusion of α-agonists can reduce food intake [22]. Thus, two of the necessary criteria for the α-2 antagonists to produce their anorectic effect by enhancing NE release through blockade of the presynaptic α-2 receptors appear satisfied.

In summary, the results indicate an anorectic effect of α-2 antagonists in *obob* and lean mice. The effect appears to be specific for food intake, and *obob* mice demonstrate an enhanced sensitivity to the effect. The potentiated behavioral effect seen in the *obob* mutant may be a result of increasing NE release in an area where chronic reduction of NE release has induced supersensitivity. This in turn results in the more sensitive behavioral response.

ACKNOWLEDGEMENTS

This study was supported in part by NIH Grant RO1-NS15600. Clonidine-HCl was a generous gift of the Boehringer Ingelheim Company.

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