Peptides that Regulate Food Intake
Antagonism of opioid receptors reduces body fat in obese rats by decreasing food intake and stimulating lipid utilization

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Submitted 11 October 2002; accepted in final form 24 February 2003

Antagonism of opioid receptors reduces body fat in obese rats by decreasing food intake and stimulating lipid utilization. An analog of 3,4-dimethyl-4-(3-hydroxyphenyl)piperidines, LY255582, is a potent non-morphinan antagonist for µ-, κ-, and δ-receptors (Ki of 0.4, 2.0, and 5.2 nM, respectively). In the present study, we examined the effects of oral LY255582 treatment on caloric intake, calorie expenditure, and body composition in dietary-induced obese rats. Acute oral treatment of LY255582 produced a dose-dependent decrease in energy intake and respiratory quotient (RQ), which correlated with the occupancy of central opioid receptors. Animals receiving chronic oral treatment with LY255582 for 14 days maintained a negative energy balance that was sustained by increased lipid use. Analysis of body composition revealed a reduction in fat mass accretion, with no change in lean body mass, in animals treated with LY255582. Therefore, chronic treatment with LY255582 reduces adipose tissue mass by reducing energy intake and stimulating lipid use.

A LARGE BODY OF EVIDENCE implicates central opioidergic networks in regulating energy balance. Orexigenic effects of opioids were first reported by Martin et al. (23) studying morphine dependence in rats and were later confirmed by numerous groups (see Refs. 11, 34). Moreover, endogenous opioid peptides (β-endorphin, the enkephalins, and dynorphin) are elevated in obesity and by fasting (6, 9, 14, 21, 44). Numerous studies using antagonists to opioid receptors report reduced food intake and body weight in rodents (1, 17, 21, 22, 33, 38, 39). Interestingly, the inhibitory effects of opioid receptor antagonists on food intake and body weight appear most pronounced in obese animals or when animals are fed a highly palatable diet (12, 19). Similar inhibitory effects on appetite are observed clinically in humans treated with the opioid receptor antagonist naloxone, naltrexone, or nalmefene; however, body weight does not appear to be consistently affected by these treatments (3, 18, 26).

Trans-3,4-dimethyl-4-phenylpiperidines are high-affinity non-morphinan opioid receptor antagonists (25, 47). Several compounds of this chemical series exhibit subnanomolar in vitro receptor binding and antagonist affinity for the µ- and κ-subtypes, with lower affinity for the δ-receptor (24, 25, 37). Structure-activity studies indicate that N-substituted phenylpiperidines retain pure µ- and κ-opioid receptor antagonist activity in vivo, being potent inhibitors of morphine- and U50,488-mediated analgesia and bremazocine-induced diuresis (25, 47). Recently, we reported that 3,4-dimethyl-4-(3-hydroxyphenyl)piperidines exhibit sodium-dependent increased binding affinity at µ-, κ-, and δ-sites and functional (using GTP-γS binding) inverse agonist efficacy in cell lines expressing the cloned human δ-receptor (24). Thus compounds from this chemical series are potent, centrally active opioid receptor inverse agonists.

Analog from the trans-3,4-dimethyl-4-phenylpiperidine series, LY255582, has high binding affinity for µ (Ki = 0.41 nM), κ (Ki = 2.0 nM), and δ (Ki = 5.2 nM)-opioid receptors in vitro (25). LY255582 was found to potently inhibit consumption of standard laboratory chow in rats after central (17) and peripheral (38, 39) administration. Interestingly, LY255582 decreased feeding in meal-fed obese Zucker rats more effectively than ephedrine, amphetamine, fenfluramine, and the opioid antagonists naltrexone and nalmefene (38, 39). Moreover, LY255582 demonstrated efficacy with chronic dosing, reducing body weight gain in obese Zucker rats (38). Therefore, it appears that LY255582 produces sustained reductions in energy intake and body weight through a central mechanism in meal-fed genetically obese rats maintained on standard laboratory chow.

In the present study we examined LY255582 actions in ad libitum-fed dietary-induced obese Long-Evans rats.
rats maintained on an energy-dense palatable diet after oral dosing. In addition to measuring energy intake and body weight, we employed indirect calorimetry to study changes in calorie use and dual-energy X-ray absorptiometry to determine changes in body composition associated with reductions in body weight. We compared these changes to calorie-restricted animals maintained on the same diet, but pair-fed to the calorie intake of LY255582-treated animals. To better understand the dose-response relationship and the duration of brain exposure, we evaluated LY255582-mediated inhibition of [3H]diprenorphine ([3H]DPN) labeling of striatal homogenates by ex vivo receptor binding. Herein we report that LY255582 reduces energy intake and body fat mass through the occupancy of central opioid receptors.

METHODS

Animals. Male Long-Evans rats (394–434 g) maintained on a high-energy diet (40% calories from fat, 41% calories from carbohydrate, 19% calories from protein; TD95217 Harlan Teklad, Madison, WI) postweaning were purchased from Harlan Sprague Dawley (Indianapolis, IN). The animals were housed individually, maintained on the high-energy diet with free access to water, and experienced a 12:12-h light/dark photoperiod (lights from 0600 to 1800) at 27°C ambient temperature. All animal use in the study was conducted in accordance with approved institutional animal care and use protocols following National Institutes of Health guidelines (NIH Publication No. 86-23, 1985).

Compounds. Modification of the trans-3,4-dimethyl-4-phenylpiperidine nucleus led to the discovery of LY255582 [5(3R,4R)-1-(S)-3-hydroxy-3-cyclohexylpropyl]-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidine]. LY255582 is a single isomer with the indicated absolute configuration.

Indirect calorimetry. Twenty-four-hour energy expenditure (EE) and respiratory quotient (RQ) were measured by indirect calorimetry as described (5) using an open-circuit calorimetry system (Oxymax, Columbus Instruments). RQ is the ratio of the volume of CO2 produced (VCO2) to the volume of O2 consumed (VO2). EE was calculated as the product of calorific value of oxygen (CV) and VO2 per kilogram of body weight, where CV = 3.815 + 1.232 (RQ) (8). Total calories expended were calculated to determine daily fuel use. To calculate the proportion of protein, fat, and carbohydrate that is used during that 24-h period, we used Piet’s proposal (10) and formulas, as well as other derived constants (8). Locomotor activity was measured by counting the number of times an animal broke a new light beam during each period of 24 h in the calorimeter.

Body composition. Body composition was measured in vivo before the start of treatment and on day 11 of the treatment period by dual-energy X-ray absorptiometry (DEXA) using an Eclipse instrument (Norland) as described previously (35).

Experiment 1. LY255582 suspended in 10% Acacia (0.2, 0.6, 1, 3, 5, 10, or 20 mg/kg oral) or vehicle (1.0 ml/kg) was administered by oral gavage 30 min before data measurements. Twenty-four-hour EE and RQ were measured as described. Food consumption was measured by recording the food weight in the 24-h period after treatment.

Experiment 2. Animals were treated once daily with LY255582 in 10% Acacia (20 mg/kg oral) or vehicle for 14 days. Food consumed daily was recorded at 0800 over the experimental period. A pair-fed group was established by providing a vehicle-treated group with the average food consumed by animals treated with LY255582 on the previous day. On day 7 of the testing period, EE and RQ were measured. On day 11 of treatment, body composition was determined by DEXA. In addition, blood was collected from tail vein on day 11, and plasma levels of glucose, urea nitrogen, triglyceride, insulin, leptin, thyroid hormone, and corticosterone were measured. Plasma glucose, urea nitrogen, and triglyceride levels were determined using the Monarch Multi-analyzer, whereas RIA (Coat-A-Count) was used for insulin, leptin, thyroid hormone, and corticosterone measurements.

Ex vivo receptor binding. Animals were treated with LY255582 in 10% Acacia (0.1, 1, 10, or 20 mg/kg by mouth) or vehicle. The animals were then killed at 1, 3, 6, or 18 h after treatment. Striatum was then dissected from each animal and rapidly frozen on dry ice. Tissue was homogenized in 10 vol (wt/vol) of binding buffer [50 mM Tris·HCl (pH 7.4), 5 mM MgCl2, and 100 mM NaCl] using a Polytron (9,000 rpm, 2 min). Binding was initiated by the addition of the membrane homogenate (10 μl) to reactions containing binding buffer, and 0.8 nM [3H]DPN (50 Ci/mmol, Perkin Elmer/NEST, Boston, MA) specific binding was determined in the presence of 10 μM naltrexone. Reactions were incubated in quadruplicate at 4°C for 30 min. These incubation conditions were chosen to limit posthomogenization redistribution of the in vivo administered LY255582. Bound radioactivity was separated from free by rapid filtration through GF/A glass fiber filters (Wallac, Turku, Finland) presoaked in 0.05% polyethyleneimine using a 96-well Tomtec cell harvester. Filters were dried for 90 min at 60°C, imbedded with MultiLex A solid scintillant (Wallac, Turku, Finland), and counted using a Wallac Microbeta counter. As [3H]DPN binding is proportional to the number of available binding sites, prior in vivo drug administration reduces the number of binding sites available for ex vivo labeling.

Statistics. All data were expressed as means ± SE. Because of animal limitations, the acute LY255582 dose-response study consisted of four separate experiments, each with a vehicle control. The four studies were combined together and each dose group was compared with that dose group’s vehicle control using Tukey’s multiple comparison procedure. Because of different vehicle levels across studies, each dose response is shown as the percentage of its corresponding vehicle mean. Responses analyzed were RQ area under the curve (AUC), food consumption, and total carbohydrate, fat, and protein energy expenditure.

For chronic studies, a mixed-model, repeated-measures ANOVA was conducted to compare body weight profiles in the three groups over the 16 days (27) and to examine group differences by day. Overall effects were examined by comparing the AUC between each group. Bonferroni corrections were applied to the parallel profile tests, and Tukey’s multiple comparison procedure was used to compare AUC and the group means by day. The same model was used to compare food consumption over the 16 days. The pair-fed group was not included in the primary analysis, because, except for days 1, 5, and 16, food consumption in that group was definitionally equal to the LY255582 group’s average consumption on the previous day. Secondary analysis of food consumption on days 1, 5, and 16 were compared between all three groups. Overall effects were examined by comparing the total food consumption over the 16-day period among the three groups. Twenty-four-hour respiratory quotient (RQ) was examined by comparing the AUC over day 7 across the three groups using Tukey’s multiple-comparison procedure. All other com-
parisons were made using ANOVA with Student-Newman-Keuls post hoc test.

RESULTS

Experiment 1. The acute effect of LY255582 was studied in ad libitum-fed dietary-induced obese Long-Evans rats. LY255582 reduced 24-h calorie intake after oral administration (Fig. 1). Control animals treated with 10% Acacia vehicle (1 ml/kg by mouth) consumed 19.0 ± 1.2 g of food. Treatment with LY255582 reduced food intake at the 1 mg/kg dose and reached statistical significance at 5 mg/kg. Maximum inhibition of food intake was observed at the 10 mg/kg dose (P < 0.008). Further increases in dose did not result in further reductions in 24-h food intake.

Twenty-four-hour RQ was dose dependently reduced in LY255582-treated animals (Fig. 2A). Analysis of the mean decrease in RQ by an AUC estimate indicated statistically significant reductions at doses of 5 mg/kg (P < 0.05). Percent control total, carbohydrate, and lipid energy expenditures are shown in Fig. 2B. The 5, 10, and 20 mg/kg groups showed decreased carbohydrate use (P < 0.05), and the 5 and 20 mg/kg groups showed increased lipid use (P < 0.05). Total 24 h EE (Fig. 2B) was decreased by the 20 mg/kg dose but was not changed by lower concentrations of LY255582. Locomotor activity (data not shown) was not changed by any dose of LY255582.

In vivo antagonism determined by ex vivo receptor binding. Oral administration of LY255582 produced a dose-dependent reduction in [3H]DPN binding in rat striatum homogenates (Fig. 3A). An IC50 of 0.91 ± 0.05 mg/kg was calculated from the dose-response curve 1 h after oral administration of LY255582. The maximal inhibition of [3H]DPN binding produced 1 h after LY255582 administration was 77% of control. During the later time points, the degree of inhibition produced by LY255582 decreased; however, 20 mg/kg maintained a 60.7 percent reduction in [3H]DPN binding for 18 h. Ex vivo inhibition of [3H]DPN binding produced by LY255582 at 18 h correlated (r² = 0.92) with 24-h food intake (Fig. 3B).

Experiment 2. Daily treatment with LY255582 (20 mg/kg oral) reduced total food intake over 14 days compared with vehicle-treated controls (P = 0.001). The vehicle and LY255582 groups did not have parallel profiles (P = 0.001). The rate of change in food consumption differed on days 1–2, 7–8, and 14–15 between the two groups. Only on day 1 was there a statistically significant difference in food consumption (P < 0.001). The subanalysis of days 1, 15, and 16 using all three groups indicates that the pair-fed group food consumption was different from the LY255582-treated group (P = 0.003), but not vehicle, on day 1 (P = 1.000), the day before pair feeding to LY255582 treatment levels. Food consumption in pair-fed animals was significantly higher than both the vehicle and LY255582 groups on day 15 after the return to ad libitum feeding conditions (P < 0.001 both groups). There was no significant difference in food consumption by day 16 in any of the treatment groups (P > 0.358 all tests).

Body weight was consistently reduced by treatment with LY255582 over the 14-day period (Fig. 4B). Comparing LY255582 to vehicle, the rates of change in body weight between days 0–1 and 7–8 were unequal (P < 0.001 both comparisons). Similar differences in the rates of body weight change were observed with pair feeding compared with vehicle on days 1–2 (P < 0.001), 7–8 (P = 0.003), and days 14–15 (P < 0.001). Inconsistent differences in the rate of body weight loss comparing LY255582-treated and pair-fed groups were observed; the rates of change differed on days 1–2 (P < 0.001), days 10–12 (P < 0.039), and days 14–15 (P < 0.001). Both the LY255582 and pair-fed groups showed a statistically significant decrease in body weight from vehicle-treated controls on day 14 (P < 0.037 both comparisons). In addition, LY255582-treated animals had a statistically significant decrease in body weight on day 16 (P = 0.033).

Indirect calorimetry on day 7 revealed a marked difference in the pattern of metabolism (as represented by RQ) between pair-fed and LY255582-treated animals. Treatment with LY255582 produced a decrease in hourly measured RQ with the same pattern observed in vehicle-treated controls, whereas pair-fed rats exhibited an exaggerated RQ cycle (Fig. 5). Treatment with LY255582 or pair feeding produced a significant decrease (P < 0.001) in average 24-h RQ AUC compared with vehicle-treated ad libitum-fed controls (0.915 ± 0.007 vehicle, 0.873 ± 0.007 LY255582, 0.881 ± 0.001 pair-fed control). No difference was observed in the average RQ AUC of LY255582-treated animals compared with pair-fed rats (P > 0.05).

Analysis of body composition by DEXA on day 11 of the study revealed both similarities and differences between the treatment groups. Total body mass increased in all treatment groups over initial prestudy levels (Fig. 6). Total body mass was similarly reduced in both LY255582-treated and pair-fed animals com-
pared with vehicle-treated animals. Total fat mass was significantly reduced by treatment with LY255582 compared with pair-fed and vehicle-treated animals. Interestingly, the pair-fed animals accumulated fat mass at an intermediate level. Moreover, the reduced body mass exhibited by the pair-fed group appeared to come at the expense of lean body mass, although this difference did not reach statistical significance.

Blood was collected on day 11 of the study before feeding. No differences in plasma glucose, plasma urea nitrogen, or T3 were observed between the treatment groups. Both LY255582-treated (leptin = 9.3 ± 1.2 ng/ml, P = 0.008; insulin = 4.1 ± 0.5 ng/ml, P < 0.001) and pair-fed (leptin = 8.9 ± 0.9 ng/ml, P = 0.015; insulin = 3.7 ± 0.5 ng/ml, P < 0.001) animals exhibited reduced leptin and insulin levels compared with vehicle-treated (leptin = 17.7 ± 2.9 ng/ml, insulin = 7.6 ± 0.5 ng/ml) controls (Table 1). Corticosterone levels tended to increase in the pair-fed group on day 11 compared with vehicle (63% increase)- and LY255582 (38% increase)-treated animals; however, this difference did not reach statistical significance.

DISCUSSION

Opioid receptor antagonists inhibit spontaneous food intake and food intake stimulated by glucoprivation, food deprivation, neuropeptide Y, melanocortin 4 receptor antagonism with agouti-related peptide (AGRP), tail pinch stress, and electrical stimulation of the lateral hypothalamus (12, 13, 29, 34). Reduced consummatory behaviors appear to be most pronounced when animals are obese or fed a palatable cafeteria diet containing large amounts of a preferred macronutrient. A current hypothesis is that opioid antagonists produce their effects on food intake by preventing central reward mechanisms that occur when overeating a preferred or palatable diet. In this manner, opioidergic control of appetite for palatable energy-dense foods may share common neural substrates responsible for the development of nicotine, alcohol, and narcotic dependence and addiction (4, 15).

In this study we found that acute oral administration of the opioid receptor antagonist LY255582 was
very potent in decreasing 24-h spontaneous feeding in dietary-induced obese rats maintained on a palatable chow. These findings contrast with those of naloxone and naltrexone treatment, which require significantly higher doses or parenteral administration (20, 22, 33). The actions of LY255582 were mediated through occupancy of opioid receptors as demonstrated by ex vivo receptor binding. At the minimal effective dose (1 mg/kg by mouth) for reducing food intake, a significant reduction in the number of available binding sites for [3H]DPN was measured for up to 6 h. Both LY255582 and [3H]DPN have high affinity for κ-, κ-, and δ-receptors, so at present we cannot relate occupancy at a single receptor subtype to efficacy. At higher doses, LY255582 appeared to remain bound to opioid receptors in the striatum for prolonged periods. Moreover, inhibition of specific opioid receptor binding at 18 h correlated well with reductions in 24-h food intake. These data suggest that oral administration of LY255582 produces a brain exposure that is higher, and of greater duration, than that predicted from its low plasma exposure after oral dosing (42). Conversely, the long receptor occupancy could result from an active metabolite of LY255582, which this assay could not discriminate from the parent molecule.

To assess the ability of LY255582 to produce sustained effects on food intake and body weight, a 14-day study using once-a-day dosing was employed. A dose of 20 mg/kg was chosen for the chronic study, because this dose was found to reliably occupy >50% of the available [3H]DPN binding sites in the striatum for up to 18 h. Chronic treatment with LY255582 produced a sustained negative energy balance, leading to a decrease in body weight of dietary-induced obese rats maintained on a high-energy diet. LY255582 treatment maintained a negative energy balance by reducing daily food intake over the treatment period. These findings are consistent with previous work demonstrating long-term efficacy of LY255582 in inhibiting food intake and body weight gain in genetically obese Zucker rats (38, 39). Central intracerebroventricular administration of LY255582 was ~100 times more potent in reducing food intake compared with peripheral administration (17, 39), supporting a central mechanism of action.

The decreased food intake observed with chronic treatment of LY255582 produced a concomitant decrease in RQ, indicating a shift in fuel use from carbohydrate to fat. The RQ is a function of the ratio of fuels being used and, as such, is directly related to feeding status. Therefore, reduced food consumption will result in a decrease in the average RQ. This effect was observed with even modest reductions in food consumption observed on day 7 for LY255582-treated and pair-fed rats. A previous study reported reductions in RQ after chronic infusions of naltrexone via subcutaneous

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**Fig. 3.** Inhibition of specific [3H]diprenorphine ([3H]DPN) binding with LY255582 treatment measured ex vivo. A: dose and time dependence for the inhibition of [3H]DPN binding in homogenates of rat striatum after oral administration of LY255582. Two-way ANOVA revealed significant effects of both time and dose. Bars (means ± SE) highlighted with different stars are significantly different from each other and control (P < 0.05). B: linear regression analysis of the inhibition of [3H]DPN binding at the 18-h time point after LY255582 and the inhibition of 24-h food consumption. Each data point represents means ± SE of 4 animals. ccpm, Corrected counts per minute.
osmotic minipumps (22); however, those investigators measured a single resting RQ 1 wk after initiating the naltrexone infusion, after significant reductions in both food intake and body weight. Our study illustrates a pattern in RQ values that varies widely over a 24-h period.

Differences in the pattern of the RQ curve were observed between LY255582-treated and pair-fed groups, reflecting differences in the pattern of food intake. Under control conditions, RQ values recorded over a 24-h period will elevate during the dark photoperiod and gradually fall during the light photoperiod, reaching the lowest point just before the start of the dark photoperiod. Treatment with LY255582 reduced 24-h food intake and RQ, but produced a lower RQ curve with the same pattern observed with vehicle-treated controls. Conversely, pair-fed animals began the calorimetry session at a lower RQ and exhibited exaggerated elevations and decreases in RQ over the 24-h session. Of note was that after several days of food restriction, pair-fed rats began to eat their allotted food as soon as it was presented, whereas LY255582- and vehicle-treated animals exhibited a similar feeding pattern. The feeding-related rapid increase in RQ observed in pair-fed animals indicates the predominance of carbohydrate use for fuel. These data suggest that pair-fed animals seek to correct their negative energy balance by exhibiting deprivation-mediated feeding, whereas animals treated with LY255582 preserve a normal pattern of appetite and metabolism under re-
duced caloric intake. Similar effects on energy balance and lipid use have been observed in leptin-treated animals compared with vehicle and pair-fed controls (5). An intriguing finding is that the pair-fed animals in the former study exhibited a similar reduced RQ at the start of the calorimetry session with an exaggerated 24-h RQ curve, although the total 24-h calorie intake was the same between the leptin-treated and pair-fed groups. This indicates that with chronic treatment, LY255582- and leptin-treated rats feed normally but at reduced levels, whereas pair-fed animals eat quickly as soon as food is available. At present, the difference in RQ at the start of the calorimetry session observed with pair-feeding compared with compound treatment is unknown, but may reflect differences in compensatory responses of central orexigenic neuropeptides (i.e., neuropeptide Y, AGRP, and melanin-concentrating hormone) to food deprivation. Alternatively, the differences in RQ may reflect a disruption of anticipatory or learned feeding behavior in animals no longer feeding under ad libitum conditions.

Interestingly, cessation of LY255582 treatment did not produce rebound overeating as observed in the pair-fed animals. Instead, when LY255582 treatment was discontinued, food intake tended to return to vehicle-treated levels over the next several days. This observation contrasted with pair-fed animals, which consumed significantly more food than either vehicle-

Fig. 5. Twenty-four-hour RQ and energy use measured during day 7 of treatment with LY255582 (20 mg/kg oral single dose per day) in DIO Long-Evans rats (n = 5 animals per group). RQ was measured using indirect calorimetry in an open-circuit calorimeter. RQ was calculated as the ratio of the VCO2 to the VO2. Symbols represent means ± SE, n = 5 animals per group. Compound was dissolved in 10% Acacia (vehicle) and administered in a volume of 1.0 ml/kg.

Fig. 6. Body composition changes after 11 days of treatment with LY255582 or pair feeding as measured by dual-energy X-ray absorptiometry (n = 5 animals per group). LY255582-treated rats gained less fat mass on the high-energy diet than either vehicle-treated or pair-fed vehicle-treated animals. The apparent lower lean mass seen with pair fed animals was not statistically significant. #P < 0.05 compared with vehicle and pair-fed, *P < 0.05 compared with vehicle using ANOVA with Student-Newman-Keuls post hoc test.

Fig. 5
or LY255582-treated animals, consistent with rebound binge overeating. Rebound overeating has been reported after cessation of chronic treatment with the melanocortin receptor agonist MTII (31), dexfenfluramine and phentermine combination (FEN/PHEN) (36), and leptin (5), but not with an analog of ciliary neurotrophic factor (CNTFAx15) (16). Treatment with LY255582 or CNTFAx15 appears to alter the animal’s response to a defended body weight such that compensatory orexigenic signals and stress responses are not triggered. Whereas the molecular mechanism of LY255582-mediated action is unknown, CNTFAx15 appears to act at least in part through activation of leptinlike neuronal circuits within the hypothalamus (16). Of note was that the body weight of LY255582-treated animals began to return to normal within 24–48 h after cessation of treatment, paralleling the return of food intake to control levels, whereas CNTFAx15-treated animals continued to exhibit weight loss for several days after treatment termination, although food intake was returning to control levels (16). Therefore, LY255582 and CNTFAx15 likely regulate different body weight signals encoded within the central nervous system.

Analysis of body composition revealed a different pattern of weight loss produced by LY255582 compared with caloric restriction. Although both treatments produce a sustained negative energy balance leading to weight loss, chronic treatment with LY255582 reduced body mass by selectively decreasing fat mass while preserving lean body mass. These findings contrasted to that observed in pair-fed animals, which exhibited a reduced total body mass comprised of both fat and lean mass. Pair-fed animals had a significantly larger fat mass than LY255582-treated animals and began to demonstrate a loss of lean body mass compared with those treated with LY255582. The alterations in lean mass, however, did not reach statistical significance. Therefore, treatment with LY255582 or leptin (5) reduces body weight while sparing lean mass. Studies with additional opioid receptor antagonists and compounds targeting other orexigenic and anorexigenic pathways will be necessary to determine if this effect is specific for LY255582 and leptin, or if it is generalized to any pharmacological agent producing weight loss without cachexia.

Interestingly, whereas treatment with naltrexone or naloxone produces a consistent reduction in food intake after acute administration (see Refs. 12, 34), the effects of chronic naltrexone or naloxone administration on body weight are equivocal. Several studies have reported sustained reductions in food intake and body weight in rodents after chronic administration of naltrexone or naloxone (1, 21, 22, 33). However, others failed to observe long-term alterations in food intake or body weight with naloxone or naltrexone treatment (28, 30, 32, 39). Similar inconsistent results have been observed in humans. Consistent reductions in food consumption after acute treatment of humans with the opioid receptor antagonists naloxone, naltrexone, or nalmefene have been reported (2, 7, 40, 43, 45, 46). Subjective ratings of reward-related aspects of food palatability appear to be most related to the inhibitory effects of opioid antagonists on appetite in humans (46). Unfortunately, chronic treatment with opioid antagonists failed to produce consistent reductions in body weight clinically (3, 18, 26). The reason for the disparity between the acute effects of opioid receptor antagonists and chronic effects is unknown. One possible explanation may be derived from rodent studies where naloxone and naltrexone appear to consistently reduce body weight in normal weight, genetically obese, or rapidly growing animals, but have limited efficacy in animals with dietary-induced obesity (20, 22, 33). Therefore, the etiology of the obese phenotype may play a particularly important role in determining if intervention with an opioid receptor antagonist will be efficacious. Alternatively, the improved efficacy demonstrated by LY255582 over other opioid antagonists may relate to its subnanomolar potency (24), producing a slower rate of dissociation from opioid receptors (41) that in vivo results in a long duration of occupancy at multiple opioid receptor subtypes. Moreover, improved in vivo activity may result from the inverse agonist efficacy of LY255582 (24), which further distinguishes this compound from other broad opioid receptor antagonists such as naltrexone. Future studies focusing on the pharmacological and molecular basis for the enhanced chronic efficacy of LY255582 over other opioid receptor antagonists are warranted. Collectively, our findings suggest that reducing endogenous opioidergic tone using an opioid antagonist similar to LY255582 may be clinically useful in treating obesity.

The authors thank J. Jacobs, C. Roundtree, and D. Burkhart for valuable technical assistance. Additional recognition and thanks to Drs. P. Emmerson and M. Tscho for expert opinions and feedback on preparing this manuscript.

REFERENCES


Table 1. Blood chemistry taken on day 11 reveals reduced leptin and insulin levels in animals treated with LY255582

<table>
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<th>Ad-Libitum Control</th>
<th>LY255582 (20 mg/kg)</th>
<th>Pair-Fed Control</th>
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<tr>
<td>Glucose, mg/dl</td>
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<td>23.6 ± 14.7</td>
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Values represent the mean ± SE of 5 rats per group. UN, urea nitrogen. *P < 0.05 vs. control using ANOVA with Student-Newman-Keuls post hoc test.


