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Long-Term Treatment of Obese Zucker Rats With LY255582 and Other Appetite Suppressants

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SHAW, W. N. *Long-term treatment of obese Zucker rats with LY255582 and other appetite suppressants.* PHARMACOL BIOCHEM BEHAV **46**(3) 653–659, 1993. – LY255582, administered subcutaneously, decreased food intake and body weight gain of fed obese Zucker rats during the entire 30-day period of treatment. No tolerance to these biologic effects of LY255582 could be demonstrated. *d*-Amphetamine and naltrexone, administered subcutaneously, and *d,l*-fenfluramine and salbutamol, administered orally, decreased food intake for no more than 6 to 12 days, in contrast to the long-lasting effects of LY255582. Salbutamol suppressed the appetite of obese rats for 3–4 days only. After an additional 12 days of treatment, weight gain decreased significantly accompanied by no appetite suppression. Thus, there is a difference in the duration of action of the opioid antagonist, LY255582, when compared to amphetamine, fenfluramine, naltrexone, and salbutamol, on food intake and body weight gain of obese rats.

Obese Zucker rats Amphetamine Fenfluramine Naltrexone Salbutamol Appetite suppression Weight loss

IN many studies of anoretic agents to control body weight gain in laboratory animals and in man, amphetamine has played a pivotal role in the realization that appetite suppressants have limitations when administered chronically, which detracts eventually from their utility (7,22). Thus, with the discovery and the development of any new antiobesity agent, especially a new appetite suppressant, the issue of drug tolerance must be examined along with potential side effects and the mechanism of action, in order to determine the potential usefulness in chronic studies in man.

The discovery of the existence of opioid receptors in the early 1970s was followed by the development and the characterization of endogenous ligands that interact with opioid receptors in the brain. In 1974, naloxone, an opioid antagonist, was reported to decrease food consumption in food-deprived rats (8). This finding was extended to other species (10), including man (1). In the ensuing years, a prodigous amount of research has been conducted in an attempt to determine and define the role of opioid receptors in feeding behavior and has led to the study of various substances that interact with opioid receptors (14–16).

In 1978, compounds belonging to a *trans*-3,4-dimethyl-4-phenylpiperidine series were found to have potent opioid antagonist activity (24). Subsequently, certain members of this chemical series were shown to decrease food consumption of meal-fed obese Zucker rats following a single subcutaneous dose (13,20). On chronic subcutaneous administration to meal-fed obese Zucker rats, two compounds from this phenylpiperidine series (LY88329 at 5 mg/kg three times daily, and LY117413 at 1 mg/kg twice daily) were shown to decrease daily food intake and to decrease body weight gain of growing obese rats (20). Also, LY117413 in older, nongrowing meal-fed obese Zucker rats caused weight loss (20). There was no evidence of tolerance to these drug effects during the 40 plus day duration of these studies. Another compound of this series, LY255582 (administered chronically once daily to meal-fed obese Zucker rats at a dose of 0.31 mg·kg for 69 days), also decreased food intake and body weight gain (21). Again, there was no evidence of tolerance to the effects of LY255582. The effect of the chronic subcutaneous administration of LY255582 on food consumption and body weight gain of fed obese Zucker rats is described in this report. We have compared its effect on these parameters with that of *d*-amphetamine, *d,l*-fenfluramine, naltrexone, and salbutamol in this animal model for obesity.

METHOD

Animals

Pathogen-free, 60-day-old obese Zucker rats were obtained from Charles River Laboratories (Raleigh, NC; male and female) and from the Diabetes Animal Support Facility, Indiana University School of Medicine (Indianapolis, IN; female only). Stock used to establish both colonies were originally

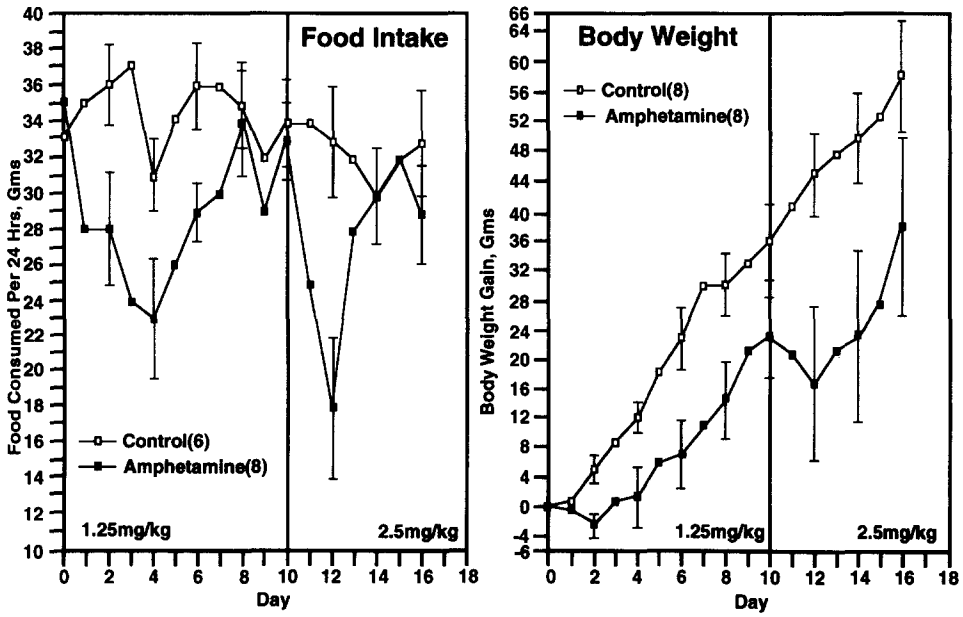

FIG. 1. The effect of *d*-amphetamine on the food intake and body weight gain of obese Zucker rats. Each group consisted of eight 90-day-old fed obese Zucker rats (four female, four male). The mean initial body weight of the control group was 467 ± 26 g; that of the amphetamine-treated group was 444 ± 22 g. Amphetamine was administered subcutaneously twice daily (0800 and 1600) at 1.25 and 2.5 mg/kg. The data are the daily mean values with the mean ± SEM values at intervals.

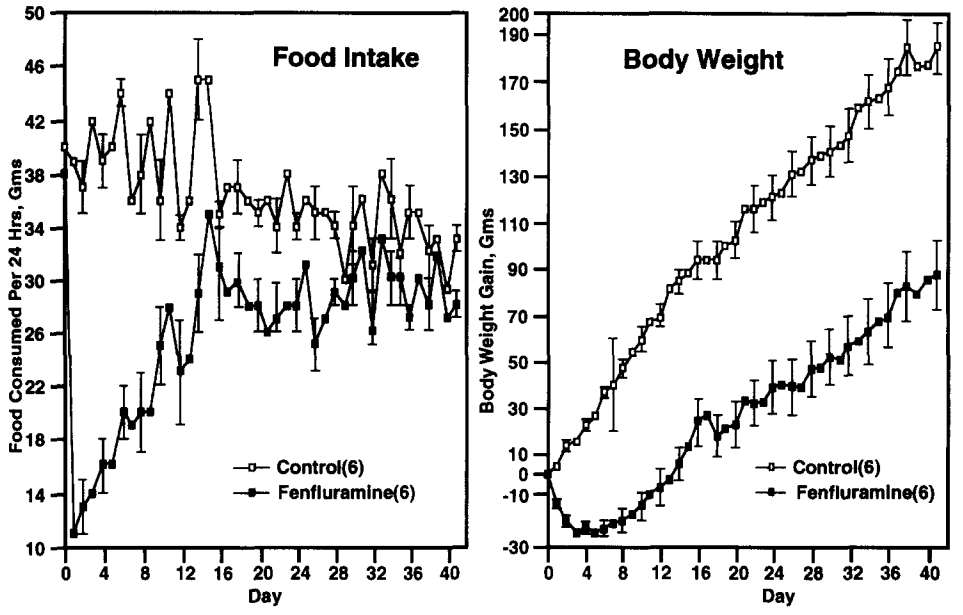

FIG. 2. The effect of *d,l*-fenfluramine on the food intake and body weight gain of obese Zucker rats. Each group consisted of six 80-day-old fed obese Zucker rats (three female, three male). The mean initial body weight of the control group was 405 ± 11 g; that of the fenfluramine-treated group was 393 ± 13 g. Fenfluramine was administered orally once daily (0800) at 10 mg/kg. The data are the daily mean values with the mean ± SEM values at intervals.

obtained from Dr. L. Zucker (Harriet Bird Memorial Laboratories, Stowe, MA). Obesity in the rat is inherited as a Mendelian recessive trait (23). These rats are characterized as normoglycemic, hyperphagic, hypertriglyceridemic, and hyperinsulinemic. The obese rats were given Teklad mouse/rat pellets (Harlan, Madison, WI) containing 44% carbohydrate, 6% fat, 24% protein, and 5% crude fiber. All rats were allowed to eat ad lib and had access to water at all times. Each rat was housed in a plastic shoebox cage in an animal room where ambient temperature was maintained about 75°F. Room lights were controlled to provide a light period from 0700 to 1700 and a dark period from 1700 to 0700 each day. The obese rats were maintained as described for about 3–4 weeks before being placed in a study.

Body weight and food consumption were measured once every 24-h period just prior to drug administration. Each study consisted of two groups of rats. One served as controls and were given vehicle only by the same route and at the same frequency of administration of the drug. The other group was given the drug by the route and frequency indicated. The statistical significance of the changes in food intake and body weight was determined using the unpaired two-tailed Student's *t*-test.

Compounds

The compounds used in this study were *d*-amphetamine sulfate, *d,l*-fenfluramine hydrochloride, naltrexone hydrochloride (Sigma Chemical Co.), salbutamol sulfate (Allen and Hamby Ltd.), and LY255582 (Lilly Research Laboratories). Amphetamine sulfate, *d,l*-fenfluramine hydrochloride, naltrexone hydrochloride, and salbutamol were dissolved in normal saline, and doses were calculated as the total salt. LY255582 [(*R*)-*cis*-(A)-cyclohexyl-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidine propanolol] was dissolved in acidified 15% ethanol. The vehicle and vehicle with drug were administered to the control and treated obese rats in a similar volume per 100 g body weight. In each study, drug solutions were prepared daily.

RESULTS

Amphetamine was administered subcutaneously to a group of 90-day-old obese Zucker rats. The drug was administered twice daily (0800 and 1600 h) at two different doses. Figure 1 shows the effect on food consumption and body weight gain. At the initial subcutaneous dose of 1.25 mg/kg, food consumption was decreased for only the first 7 days. On day 10, the amphetamine dose was increased to 2.5 mg/kg and food consumption was again significantly decreased for only 2 days. The initial anoretic effect of amphetamine at 1.25 mg/kg was sufficient to produce a cessation in body weight gain for 4 days. As a result, the growth curve of the treated rats was shifted to the right of that of the control rats, and total weight gain remained significantly less than the control. From day 4 to day 10, the amphetamine-treated group gained weight at the same rate as the control group. When the subcutaneous amphetamine dose was increased to 2.5 mg/kg, body weight gain was decreased for 2 days only. From days 12 to 16, the rate of body weight gain was the same as that of the control group. A similar response was shown in previous study using meal-fed obese Zucker rats in which amphetamine was administered subcutaneously twice daily for 37 days at 3 mg/kg (Shaw, unpublished observation). Food consumption and body weight gain were decreased for only the first 14 days of the study. After 37 days at 3 mg/kg, the subcutaneous amphetamine dose was raised to 6 mg/kg. It produced no further appetite suppression or effect on weight gain.

d,l-Fenfluramine was administered chronically to fed obese Zucker rats once daily at 0800 at an oral dose of 10 mg/kg

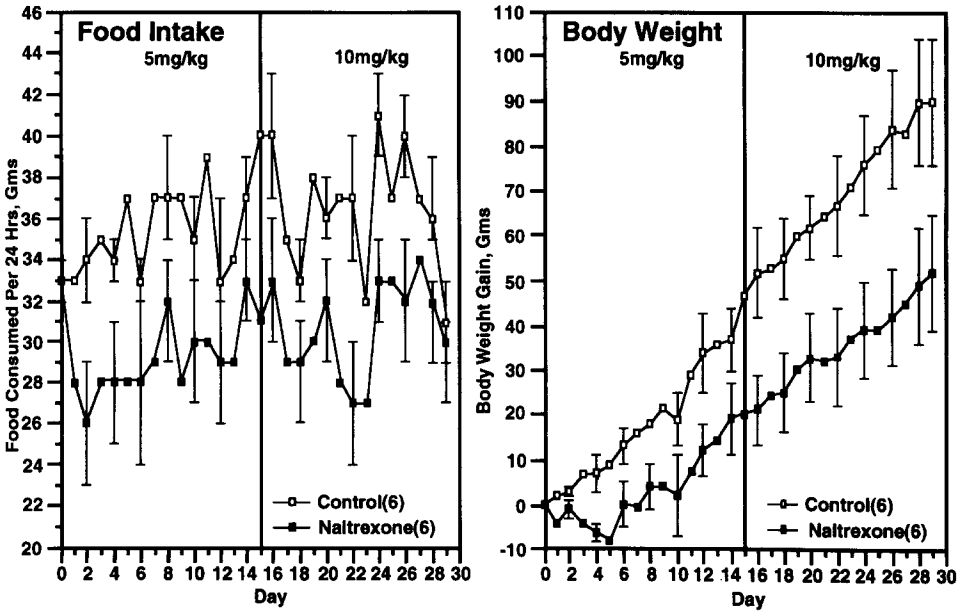

FIG. 3. The effect of naltrexone on food intake and body weight gain of obese Zucker rats. Each group consisted of six 89-day-old fed obese Zucker rats (three female, three male). The mean initial body weight of the control group was 507 ± 20 g; that of the naltrexone-treated group was 523 ± 16 g. Naltrexone was administered subcutaneously at 5 and 10 mg/kg twice daily (0800 and 1600). The data are the daily mean values with mean ± SEM values at intervals.

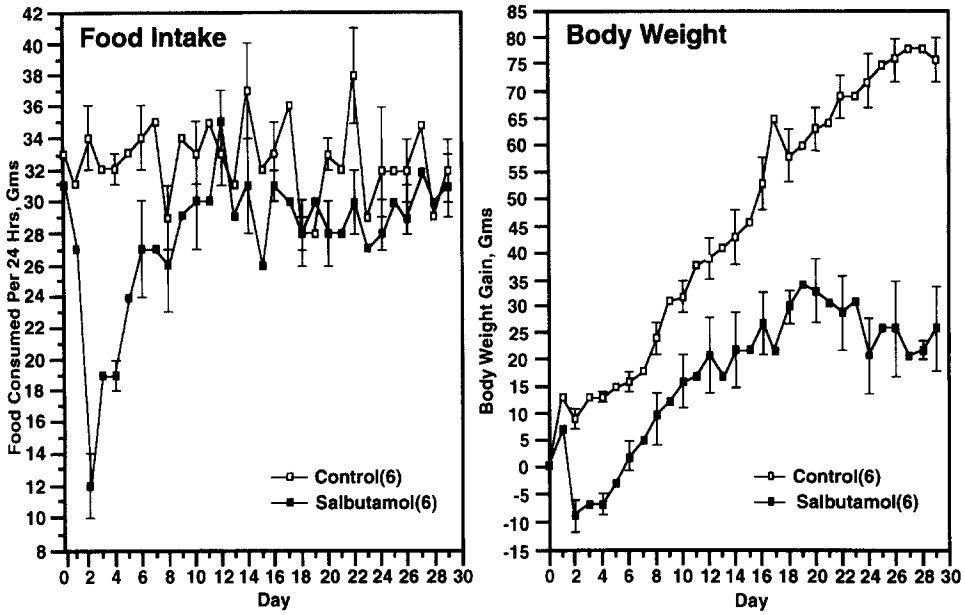

FIG. 4. The effect of salbutamol on food intake and body weight gain of obese Zucker rats. Each group consisted of six 88-day-old fed obese Zucker rats (three female, three male). The mean initial body weight of the control group was 400 ± 26 g; that of the salbutamol-treated group was 383 ± 29 g. Salbutamol was administered orally twice daily (0800 and 1600) at the dose of 20 mg/kg. The data are the daily mean values with mean ± SEM values at intervals.

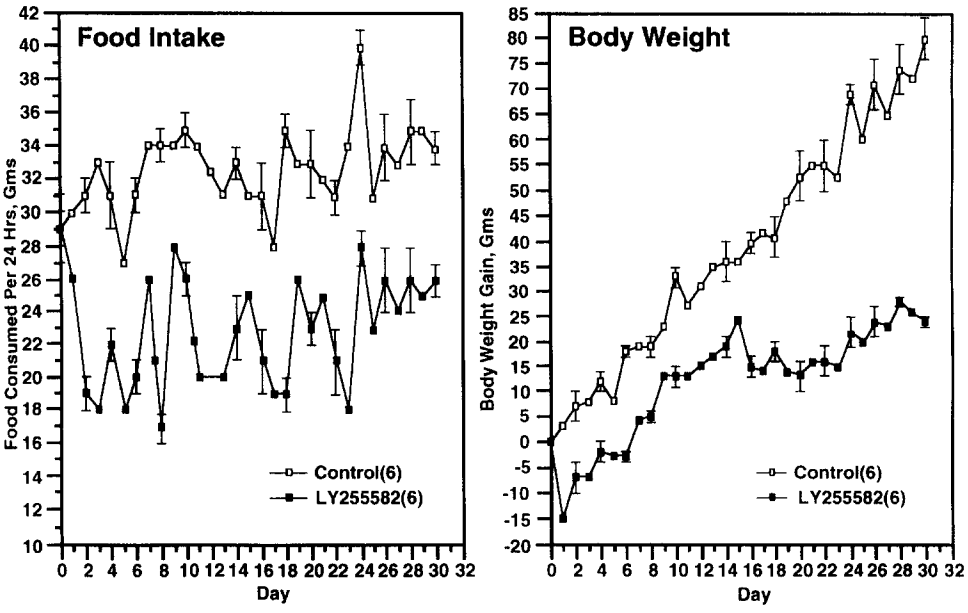

FIG. 5. The effect of LY255582 on food intake and body weight gain of obese Zucker rats. Each group consisted of six 80-day-old fed obese Zucker rats (three female, three male). The initial mean body weight of the control group was 386 ± 14 g; that of the LY255582-treated group was 388 ± 7 g. LY255582 was administered subcutaneously once daily (1600) at a dose of 15 mg/kg. The data are the daily mean values with mean ± SEM values at intervals.

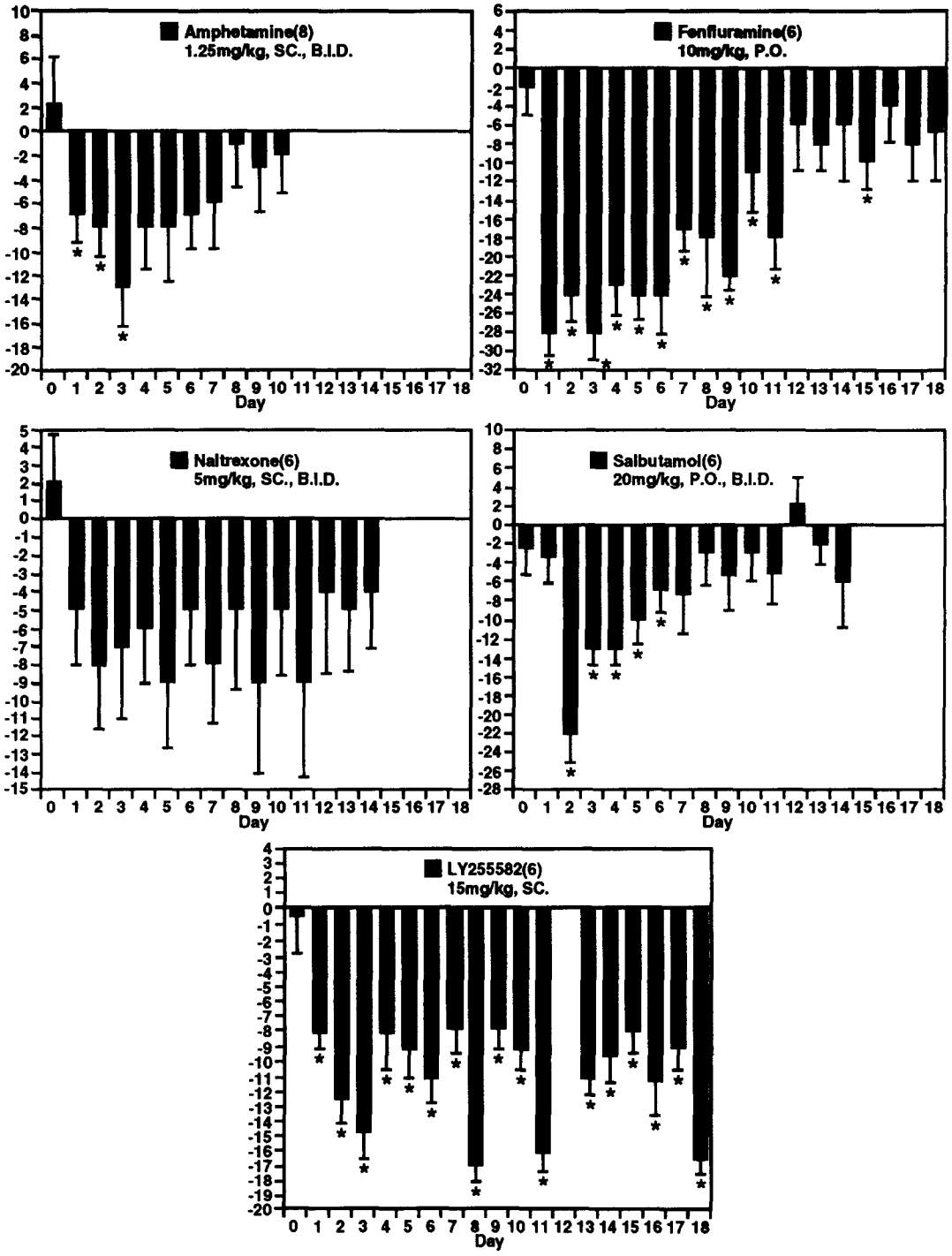

FIG. 6. The effect of several compounds in this study on food intake of obese Zucker rats. The bar graph in each instance represents the mean change ± SEM in food intake due to the administration of that drug from that of the control group on that day. The number in parentheses indicates the total number of obese rats in each experimental group. The * indicates a change that is statistically significant ($p > 0.05$). The dose and route of administration are indicated, and the frequency of administration was one dose daily except where B.I.D. is indicated, meaning twice daily dosage. Data for day 12 in LY255582 studies was not available due to a balance malfunction.

for 41 days. Figure 2 shows the effect on food consumption and body weight gain. For the first 14 days of treatment, fenfluramine significantly decreased food consumption. From day 15 onward, there was no significant suppression of appetite. Over the first 4 days of this study there was a weight loss that was sustained for an additional 5 days. At that time, weight gain began to increase, paralleling that of the control group. The initial period of weight loss plus the period during which the loss was maintained resulted in shifting the body weight gain curve of the treated obese rats to the right of the control group.

The third substance tested was naltrexone. Naltrexone was administered chronically to fed obese Zucker rats twice daily at 0800 and 1600 at 5 mg/kg subcutaneously for 16 days followed by 14 days at 10 mg/kg. Figure 3 shows the effect of naltrexone on food consumption and body weight gain. Although the mean appetite of the treated group was less than the control group, at no time was this effect statistically significant. Body weight gain for the first 5 days was decreased and returned to predrug level by day 7. From that time on, the treated group gained weight at the same rate as the control group. On day 17 of the study, the dose of naltrexone was doubled to 10 mg/kg. There was no significant change in food consumption or body weight gain. More careful examination of the data indicates that the appetite of female obese rats was suppressed more than that of male obese rats, contributing the large SEM for the treated group.

Salbutamol has been shown to be an anoretic agent when administered acutely by the intraperitoneal route to meal-fed normal Sprague–Dawley rats (2). We administered salbutamol chronically at an oral dose of 20 mg/kg twice daily (0800 and 1600) for 29 days. Figure 4 shows the effect of salbutamol on food consumption and body weight gain of fed obese Zucker rats. A significant decrease in food consumption occurred only for the first 6 days of treatment. A loss in body weight occurred during this 6-day period followed by 8 days during which the weight gain paralleled that of the control group. After day 12, body weight gain of the treated group changed drastically, such that over the period from day 12 to day 29, the control obese rats gained 38 g and the salbutamol-treated group gained only 5 g, a period over which there was no anoretic effect. Thus, the anoretic effect of salbutamol in chronically treated obese rats is of short duration and the subsequent effect on body weight, which occurred after 12 days of treatment, is related to some mechanism other than to appetite suppression.

It has been shown previously (20) that LY255582, administered subcutaneously at a dose of 0.31 mg/kg to meal-fed obese Zucker rats for a period of 69 days, reduced food consumption and body weight gain significantly. We compared the effect of LY255582 to that of amphetamine, fenfluramine, naltrexone, and salbutamol. LY255582 was administered subcutaneously to fed obese Zucker rats once daily (0800) at 15 mg/kg for 30 days. The results are shown in Fig. 5. LY255582 significantly reduced food consumption and body weight gain of the obese rats at all times during the entire treatment period. The mean food consumption of the LY255582-treated group was always significantly less than the controls. Body weight gain of the LY255582-treated group was always significantly less than the controls.

DISCUSSION

The purpose of this study was to compare the effect of amphetamine, fenfluramine, naltrexone, salbutamol, and LY255582 on food consumption of obese Zucker rats, to determine whether any decrease in food intake translated to any effect on body weight and to determine the duration of these effects. It was not an aim to determine relative efficacies of the five substances and this cannot be read into this data since two routes of administration (subcutaneous and oral) were used and insufficient dose ranging studies were done.

Each of the compounds studied has been found to affect the food consumption of laboratory animals. Amphetamine is believed to function by activating central noradrenergic and dopaminergic systems (4,9). Fenfluramine acts through central serotonergic systems (5). Salbutamol, with respect to anorexia, acts as a β_2-adrenergic agonist (3,6). Both naltrexone (12,17) and LY255582 (21) are considered to be opioid antagonists and have been shown to suppress appetite. Figure 6 was drawn, based on data already shown in Figs. 1–5, to make it easier to illustrate some of the conclusions. In Fig. 6, we have shown the actual change in food consumption on a day-to-day basis from that day's control level as a bar graph when the various drugs were administered.

The effect of amphetamine (Fig. 6) on food intake of the fed obese rat lasted 3–5 days. It was accompanied by a short-lived decrease in body weight gain. When the dose was increased to 2.5 mg/kg, effects on both parameters lasted for only 2–3 days (Fig. 1).

The effect of fenfluramine (Fig. 6) shows a significantly decreased food intake for about 11 days. Body weight gain was decreased below the pretreatment level and was maintained for about 10 days (Fig. 2).

Naltrexone at 5 mg/kg (Fig. 6) caused a modest decrease in food intake, which was not at any time statistically significant. Body weight gain was reduced only for the first 5 days of treatment (Fig. 3). Increasing the dose to 10 mg/kg caused no change in either parameter.

Salbutamol produced a significant decrease in food intake on the second day of treatment, which gradually lessened, so that by day 7 there was no statistically significant change (Fig. 6). Body weight gain was reduced below the initial level for several days followed by weight gain paralleling that of the controls until day 18. At that time, body weight gain ceased to change and maintained a plateau until the end of the study at day 29. Thus, in the obese rat, the anoretic effect of salbutamol at an oral dose of 20 mg/kg is of short duration. This kind of a change in body weight gain in these obese rats is reminescent of the response of these rats to thermogenic phenethanolamines LY79771 (18) and LY104119 (19). In these salbutamol-treated obese rats, the rectal temperature, as well as the total body oxygen consumption, increased significantly on day 17 when compared to the controls and remained so for the rest of the study (data not shown). The protein content and tissue oxygen consumption of intrascapular brown adipose tissue from the salbutamol-treated obese rats when compared to that of the control animals was also increased (data not shown), like that previously seen for LY104119 (19).

Finally, Fig. 6 shows that LY255582 decreased food intake significantly from the first day of treatment. This lasted for the duration of the study (Fig. 5). This was accompanied by a decrease in body weight gain that became more evident as the study progressed. This effect on food intake with LY255582 is clearly different from the other compounds studied. This suggests that the obese Zucker rat did not develop tolerance to the effects of LY255582 administered chronically, in contrast to amphetamine, fenfluramine, naltrexone, and salbutamol. These data obtained in the fed obese Zucker rat agree

with that previously found (21) when LY255582 was administered for 69 days to meal-fed obese Zucker rats.

Whether the results obtained in these studies can be truly ascribed to the development of a tolerance to the particular drug can be debated. We have attempted to correlate the effect of the various agents on food intake with effect on body weight gain and have shown that LY255582, unlike amphetamine, fenfluramine, naltrexone, and salbutamol, continues to reduce food intake of the obese rat and to cause decreased body weight gain for as long as LY255582 was administered. This does not indicate with surety that similar responses will be seen in man, but at least it suggests a preclinical difference in chronic aspects for LY255582 compared to other prototypical appetite suppressants.

It has been shown that LY255582, administered intravenously once daily, decreased food intake and body weight gain of normal Sprague–Dawley rats over a treatment period of 7 days (11). It was concluded that LY255582 was a potent and long-acting appetite suppressant. The present studies with obese rats are in agreement with the conclusion that LY255582 is a long-acting appetite suppressant and both studies agree that this effect on food intake results in decreased body weight gain.

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