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# Effects of preexposure to dexfenfluramine, phentermine, dexfenfluramine–phentermine, or fluoxetine on sibutramine-induced hypophagia in the adult rat☆

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**Abstract**

The antiobesity drug sibutramine suppresses food intake via inhibition of reuptake of both norepinephrine (NE) and serotonin (5-HT) into brain terminals. The present study examined whether preexposure to other antiobesity drugs (fluoxetine [FLUOX], phentermine [PHEN], and dexfenfluramine [DEX]) that alter noradrenergic and/or serotonergic activity in brain induces tolerance or sensitization to the subsequent hypophagic action of sibutramine. Accordingly, adult male rats were treated (administered orally once per day for 21 days) with DEX (0, 1, or 3 mg/kg) and/or PHEN (0, 5, or 10 mg/kg), alone and in combination, or with the selective 5-HT reuptake inhibitor FLUOX (0, 15, or 30 mg/kg). Daily administration of PHEN persistently reduced food intake and body weight whereas tolerance developed to the hypophagic action of DEX or of FLUOX within the first week of daily administration. Moreover, low doses of DEX (1 mg/kg) and PHEN (5 mg/kg) interacted in a supra-additive manner to inhibit food intake and water intake and decrease body weight over the 21-day exposure period. After a recovery period of 9 days, a series of food intake trials were conducted to assess the hypophagic action of sibutramine (0, 1, 3, and 9 mg/kg po). Preexposure to PHEN (5 or 10 mg/kg), DEX (3 mg/kg), or FLUOX (30 mg/kg) resulted in a significant attenuation of the hypophagia induced by sibutramine over an 8-h, but not a 2-h, testing period. The pattern of cross-tolerance noted in this study is consistent with the observation that sibutramine inhibits eating via an interaction with noradrenergic and serotonergic mechanisms. Whether PHEN and DEX preexposure in humans alters subsequent sibutramine effectiveness is unknown.


*Keywords:* Noradrenergic; Serotonergic; Drug tolerance; Drug sensitization; Obesity

## 1. Introduction

The antiobesity drug sibutramine is known to induce dose-dependent suppression of body weight in obese patients (Bray, 1999; Bray et al., 1999; Lean, 1997; McNeely and Goa, 1998). Preclinical studies in rats demonstrate that sibutramine produces a dose-dependent inhibition of food intake (Jackson et al., 1997a; Mitchell et al., 1998; Strack et al., 2002) and stimulation of thermogenesis (Connoley et al., 1999; Liu et al., 2002). The antiobesity action of sibutramine in humans mostly results from reduced caloric intake. Whereas sibutramine has variable actions on thermogenesis in humans (Danforth, 1999; Hansen et al., 1999), Chapelot et al. (2000) reported that a single morning dose (15 mg) of sibutramine reduced actual intake of fat, carbohydrate, and protein and reduced hunger ratings in lean human subjects.

The hypophagic action of sibutramine is related to an inhibitory action on reuptake of norepinephrine (NE) (Balcioglu and Wurtman, 2000) and serotonin (5-HT) into neuron terminals in brain (Buckett et al., 1988; Gundlah et al., 1997; Heal et al., 1998a,b; Luscombe et al., 1989). Significant attenuation of the hypophagic action of sibutramine has been reported in rats pretreated with prazosin ($\alpha_1$-adrenergic antagonist), metergoline (nonselective 5-HT antagonist), ritanserin (5-$HT_{2A/2C}$ antagonist), or SB200646 or SB206553 (5-$HT_{2B/2C}$ antagonists) (Grignaschi et al., 1999; Jackson et al., 1997b; Mitchell et al., 1998). These observations indicate that $\alpha_1$-adrenergic and 5-$HT_{2B/2C}$

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receptors play prominent roles in the inhibitory action of sibutramine on food intake.

The clinical use of sibutramine as an antiobesity agent in the United States closely followed the withdrawal of fenfluramine and dexfenfluramine (DEX) from the market in September of 1997 (Anonymous, 1997). Fenfluramine and its active enantiomer DEX have important neuropharmacological actions on the release of 5-HT in brain and the uptake of 5-HT into presynaptic neurons, and the fenfluramines may exert direct actions on postsynaptic serotonergic receptors (Balcioglu and Wurtman, 1998; Curzon et al., 1997; Garratini et al., 1975; Gobbi et al., 1992). Moreover, the antiobesity agent phentermine (PHEN) was commonly coadministered with one of the fenfluramines (thus forming the ''FEN–PHEN'' cocktail) to reduce body weight (Anonymous, 1997). The interactions of PHEN with the NE systems of brain include the blockade of NE reuptake and the release of NE (Lancashire et al., 1998; Rothman et al., 2001). PHEN alone can enhance extracellular 5-HT levels in rat brain (Balcioglu and Wurtman, 1998; Prow et al., 2001; Tao et al., 2002). The observation that the fenfluramines and PHEN interact with the NE and 5-HT systems, which are the very systems by which sibutramine reduces food intake, raises the experimental question as to whether preexposure to DEX, PHEN, or to the combination of DEX and PHEN (DEX–PHEN) alters the subsequent hypophagic action of sibutramine. Thus, the intent of the present study was to examine whether repeated administration (once per day for 21 days) of DEX (0, 1, or 3 mg/kg po) and/or PHEN (0, 5, or 10 mg/kg po), alone and in combination, alters the subsequent hypophagic response of adult rats to sibutramine (0, 1, 3, and 9 mg/kg po). In addition, the present experiment examined whether preexposure to the selective 5-HT reuptake inhibitor fluoxetine (FLUOX: 0, 15, or 30 mg/kg) altered the subsequent hypophagic action of sibutramine.

## 2. Methods

### 2.1. Subjects and housing

The subjects were 120 male Sprague–Dawley (Harlan, Houston, TX) outbred rats weighing 300–350 g at the start of the study. The rats were maintained under a reverse-day/night schedule (lights off at 0900 h). Each rat was housed in a standard polycarbonate cage outfitted with a wire floor positioned over a cardboard pad (used to collect food spillage). Food (see Diet) and tap water were freely available throughout the dark phase (0900–1700 h) of each day of the study. Animal procedures were accomplished under a 40-W red light.

### 2.2. Drugs

Sibutramine HCl was provided by Abbott Laboratories. DEX HCl was obtained from Research Biochemicals (Natick, MA) while FLUOX HCl and PHEN HCl were obtained from Sigma (St. Louis, MO). Sibutramine, PHEN, and DEX were dissolved in sterile deionized water, while FLUOX was dissolved in partially acidified (glacial acetic acid) sterile deionized water (final solution pH = 4.0). All drug doses were calculated as the salt and were administered orally in a volume of 2 ml/kg using metal gavage feeding needles.

### 2.3. Diet

A palatable wet-mash diet (1.89 kcal/g) consisted of 250 ml sweetened condensed milk (Albertson's, Boise, ID), 1000 ml ground rat chow (Teklad), and 850 ml tap water. The mash diet was prepared fresh each morning and was presented in the home cage in a Pyrex custard dish. The mash diet does not spoil over an 8-h period, is readily consumed by rats, and is rarely spilled during consumption (Cooper, 1987).

### 2.4. Procedure

The rats were maintained in the colony room for 7 days prior to the start of the study to adapt them to colony procedures (handling, weighing, etc.). At 0900 h on each of seven baseline days, the rats were weighed (nearest 1.0 g) and placed in a clean cage. Each cage contained a glass custard dish (containing a weighed amount of the wet-mash diet) as well as a drinking tube containing tap water. At 1100 h, the diet plus dish was weighed to the nearest 0.1 g, as was the drinking tube (plus water). The 2-h intake values were calculated as the difference between 0900- and 1100-h values for each rat. The diet dish and the drinking tube were returned to the cage until 1700 h, at which point the 6-h food and water intake values were each recorded to the nearest 0.1 g. The 6-h values were added to the 2-h values to yield total 8-h food and water consumption values. During the 16-h period between intake trials, the rats were given access to water, but not food.

Twelve groups of rats ($n = 10$ rats/group) were formed based on comparable 2- and 8-h food intakes during the last 3 days of the baseline period. During the subsequent 21-day preexposure period, each rat received an oral infusion (2 ml/kg) of vehicle (deionized water), PHEN (5 or 10 mg/kg), DEX (1 or 3 mg/kg), DEX in combination with PHEN (1–5, 1–10, 3–5, or 3–10 mg/kg), or FLUOX (0, 15, or 30 mg/kg) at 0900 h each day. Body weights and food and water consumption values (2 and total 8 h) after drug or vehicle infusion were measured as during the baseline procedures. During the 16-h period between successive daily tests, the rats were given access to water, but not food.

Daily drug or vehicle infusions ceased at the end of the 21-day preexposure period. During the ensuing 9-day recovery period (no vehicle infusions), the rats were given

daily access to the wet-mash diet (and tap water) as during the baseline period. Body weights were recorded daily during the recovery period.

At the end of the recovery period, the rats underwent a series of 10 ingestive trials. On Days 1, 4, 7, and 10, each rat received an infusion of one of the sibutramine doses (0, 1, 3, and 9 mg/kg po). A random order of dose administration was used for each rat. Food and water consumption were measured as described for the preexposure period. The four sibutramine test trials were each separated by two non-injection trials.

### 2.5. Design and data analyses

The primary design of this experiment was a $3 \times 3$ factorial with between-group factors of PHEN (0, 5, or 10 mg/kg) and DEX treatment (0, 1, or 3 mg/kg). A secondary design considered the impact of FLUOX (0, 15, or 30 mg/kg) dose on sibutramine hypophagia. Separate analyses of variance (ANOVA) were computed using SYSTAT (Version 8.0, SPSS, Chicago, IL) for body weight, food intakes (2 and 8 h) and for water intakes (2 and 8 h) during the 21-day pretreatment phase.

Inspection of the individual treatment groups revealed that the high-dose 10 mg/kg PHEN condition (i.e., 0–10-mg/kg DEX–PHEN group) and the high dose 3 mg/kg DEX treatment condition (i.e., 3–0-mg/kg DEX–PHEN group) produced marked reductions of food intake during the 2-h period, which may represent a floor boundary that would preclude analysis of the interactions of DEX with PHEN on food intake. An additional analysis involving residual calculations was computed using the low-dose DEX (1 mg/kg) and PHEN (5 mg/kg) groups. To estimate variations in additivity (which may indicate drug–drug synergism), a series of difference calculations were made in which the effects of the individual drug groups (i.e., 1–0 and 0–5 mg/kg DEX–PHEN) were subtracted from the effects of the combined drugs (1–5 mg/kg DEX–PHEN group) relative to the 0–0-mg/kg DEX–PHEN control group. In these effect-additive analyses, positive values represent supra-additive effects, wherein the individual treatment group effects do not completely account for the combined actions of these drugs, while negative values represent an instance where the combined drug effect is less than the sum of the individual drug effects. The expected value of the residual measure, given complete additivity of drug effects, would be zero.

Recovery of body weight and food intake after termination of drug preexposure was examined using food intake and body weight on Day 29, the day before the first sibutramine trial. Analyses of the effects of drug pretreatment on sibutramine-induced hypophagia were conducted in separate ANOVAs with sibutramine dose as a within-group factor. Comparisons among group means were made using Tukey contrasts ($P < .05$).

## 3. Results

### 3.1. Baseline analyses

There were no differences among the groups with regard to the 3-day baseline measures of food intake, water intake, or body weight prior to the start of the 21-day preexposure period (data not presented).

### 3.2. Changes in ingestion and body weight produced by administration of DEX, PHEN, and the DEX–PHEN combinations on Days 1–21

#### 3.2.1. Food intake

The impact of DEX and of PHEN, given alone or in combination, on 2-h food intake for all groups is depicted in Fig. 1. ANOVA of these data revealed significant effects of DEX treatment [$F(2,83) = 31.23$, $P = .01$] and of PHEN treatment [$F(2,83) = 137.7$, $P = .01$], as well as a significant interaction among DEX and PHEN treatments [$F(4,83) = 6.6$, $P = .01$]. Moreover, there was a significant effect of days of exposure [$F(20,1660) = 3.19$, $P = .01$], as well as a significant interaction between PHEN treatment and days of exposure [$F(40,1660) = 1.9$, $P = .01$].

Fig. 2 (top panel) depicts the treatment groups and residual analyses for 2-h food intake for rats treated with vehicle, 5 mg/kg PHEN, 1 mg/kg DEX, or 1–5 mg/kg DEX–PHEN combination. Treatment with 5 mg/kg PHEN produced a consistent inhibition of eating during the 2-h test interval across the 21-day treatment period and there was no evidence of tolerance in this group. In contrast, 1 mg/kg DEX produced a suppression of eating during the first 4 days of treatment, and by Day 5, the intakes of this group during the first 2 h after injection had risen to the level of the vehicle control group (0–0-mg/kg DEX–PHEN group). On days thereafter, the food intakes of the DEX group was at or slightly below the intake values of the vehicle control group. The 1–5-mg/kg DEX–PHEN combination group exhibited a substantially larger suppression of eating that persisted across the 21-day treatment period. Eighteen of the 21 daily residual values computed were positive and these values summed to a composite of 66.0. The residual analyses confirm that the combination of a low DEX dose (1 mg/kg) with a low PHEN dose (5 mg/kg) exerted a greater hypophagic effect than that produced by the individual drug treatments.

Fig. 2 (bottom panel) depicts food intakes over the 8-h test interval for rats treated with vehicle, 5 mg/kg PHEN, 1 mg/kg DEX, or 1–5 mg/kg DEX–PHEN combination. As can be seen, administration of 5 mg/kg PHEN did not exert a reliable effect on eating during the 8 h test interval, whereas 1 mg/kg DEX dose inhibited eating during the first 2 days of treatment and not thereafter. As was the case for the 2-h test interval, the 1–5-mg/kg DEX–PHEN combination group exhibited a greater suppression of eating, but this effect waned across the 21 day treatment period. The

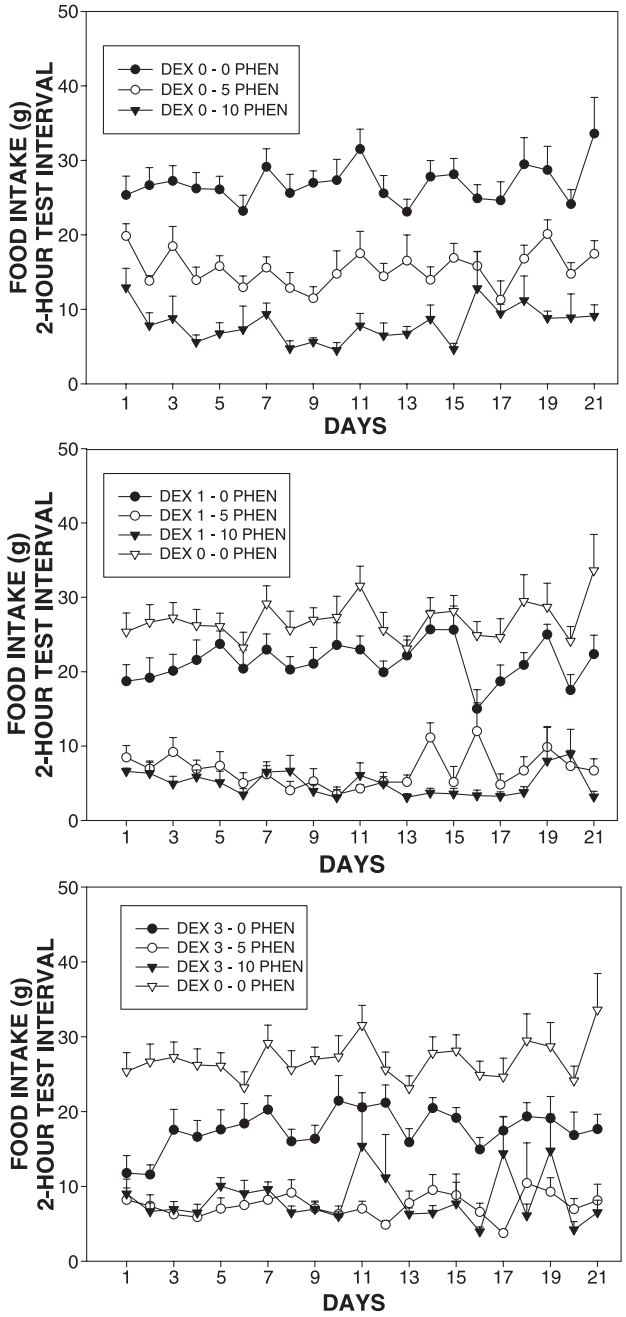


Fig. 1. Mean group daily 2-h food intake values (g) during drug treatment Days 1–21 for rats treated once daily with 0 (top panel), 1 (middle panel), or 3 mg/kg DEX (lower panel) alone or in combination with 0, 5, or 10 mg/kg PHEN. The lines above each symbol represent the S.E.M. Rats in the 0–0-mg/kg DEX–PHEN group consumed 26.9 ± 1.6 g/2 h.

gradual tolerance to the supra-additive effect of these doses is evident in the daily residual analyses, wherein the residual values are positive during the first 7 days of treatment, but are a mixture of positive and negative values during the last 14 days of the treatment period.

Fig. 3 depicts the impact of DEX and of PHEN on food intake over an 8-h interval after drug administration. To facilitate comparison of the groups, the data from the 0–0-mg/kg DEX–PHEN control group is repeated in these panels. ANOVA of 8-h test interval food intake data revealed significant main effects of DEX exposure [$F(2,83) = 13.3$, $P = .01$] and of PHEN exposure [$F(2,83) = 4.5$, $P = .027$]. There was no significant interaction, however, between PHEN and DEX exposure [$F(4,83) = 1.8$, $P = .105$]. There was a significant interaction between the factor of days and every other factor and interaction term in this analysis ($P$ = at least .013). Thus, tolerance developed to the hypophagic action of DEX–PHEN.

Food intake recorded during the 8-h test interval increased to control levels during the 9-day period separating the end of the drug pretreatment phase and the start of the sibutramine trials. ANOVA of the food intakes recorded during an 8-h test interval on the day prior to the start of the sibutramine trial revealed no significant between-group differences ($P$'s = at least .252).

### 3.2.2. Water intakes

Fig. 4 depicts the changes in water intake recorded during a 2-h test interval after treatment with DEX and

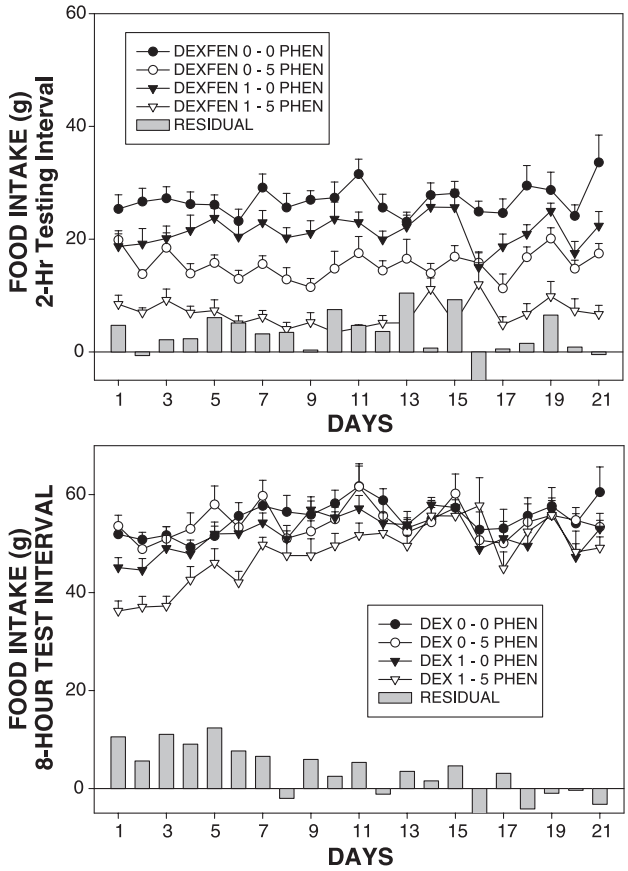


Fig. 2. Mean group daily 2- (top panel) and 8-h food intake values (bottom panel) for the 1–5-mg/kg DEX–PHEN combination group relative to the 0–0-, 1–0-, and 0–5-mg/kg DEX–PHEN groups. The inset bars represent the mean difference between the 0–0- and 1–5-mg/kg DEX–PHEN groups, less the differences of the 0–5- (relative to the 0–0-mg/kg DEX–PHEN group) and the 1–0-mg/kg DEX–PHEN groups (relative to the 0–0-mg/kg DEX–PHEN group).

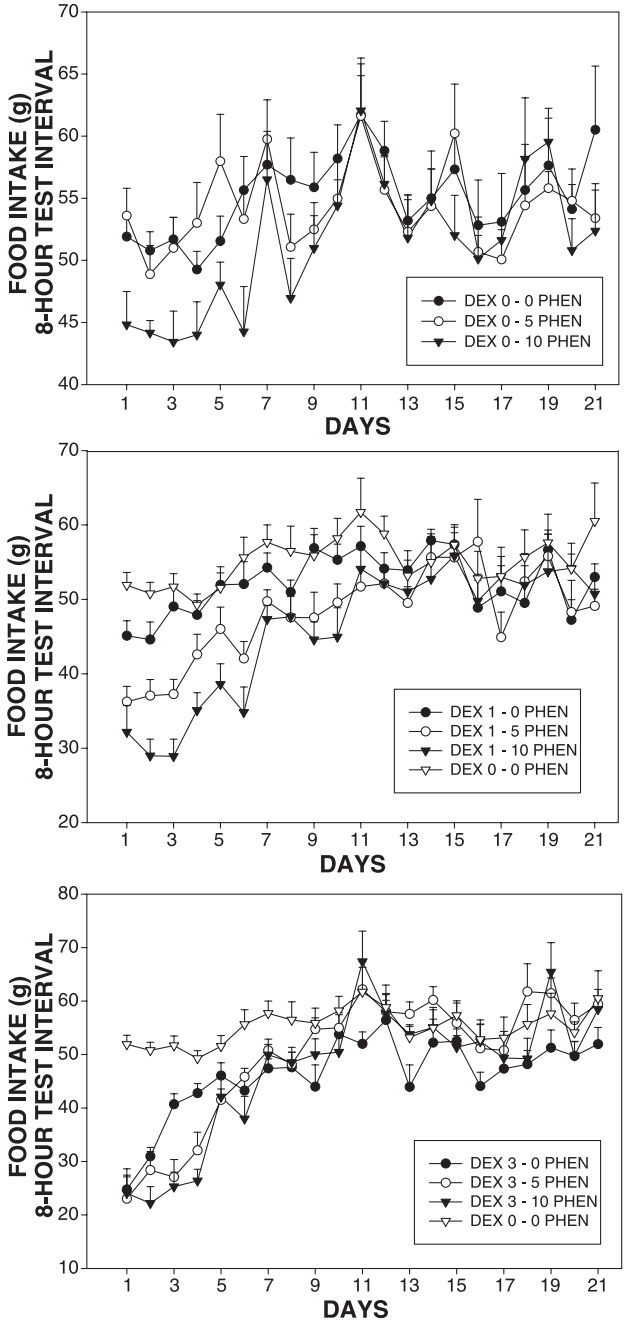


Fig. 3. Mean group daily 8-h food intake values (g) during drug treatment Days 1–21 for rats treated once daily with 0 (top panel), 1 (middle panel), or 3 mg/kg DEX (lower panel) alone or in combination with 0, 5, or 10 mg/kg PHEN. The lines above each symbol represent the S.E.M. The 0–0-mg/kg DEX–PHEN data are repeated in each panel to facilitate data inspection.

with PHEN. ANOVA of these data indicated a significant effect of DEX treatment [$F(2,82)=6.9$, $P<.01$] and of PHEN treatment [$F(2,83)=73.5$, $P=.01$]. Moreover, these analyses indicated a significant interaction between DEX and PHEN treatment [$F(4,82)=7.32$, $P=.01$]. There was a significant effect of days of exposure [$F(20,1640)=12.09$, $P=.01$], but no significant interaction between days of exposure and the other terms of this analysis.

Fig. 5 depicts the changes in water intake over a 8-h test interval after treatment with DEX and PHEN. ANOVA of these data indicated significant effects of DEX treatment [$F(2,83)=27.3$, $P=.01$] and of PHEN treatment [$F(2,83)=134.5$, $P=.01$], as well a significant interaction between DEX and PHEN treatments [$F(4,83)=5.8$, $P=.01$]. In this analysis, there was a significant effect of

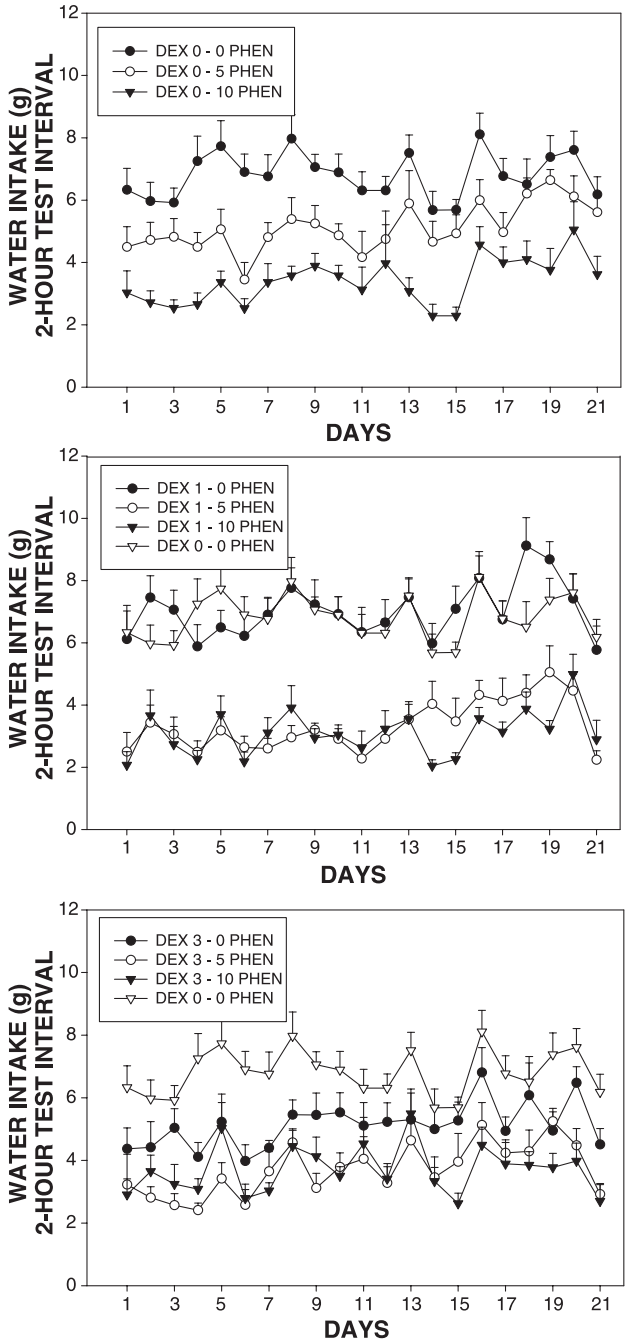


Fig. 4. Mean group daily 2-h water intake values (g) during drug treatment Days 1–21 for rats treated once daily with 0 (top panel), 1 (middle panel), or 3 mg/kg DEX (lower panel) alone or in combination with 0, 5, or 10 mg/kg PHEN. The lines above each symbol represent the S.E.M. The 0–0-mg/kg DEX–PHEN data are repeated in each panel to facilitate data inspection. Rats in the 0–0-mg/kg DEX–PHEN group consumed 55.2 ± 1.2 g/8 h.

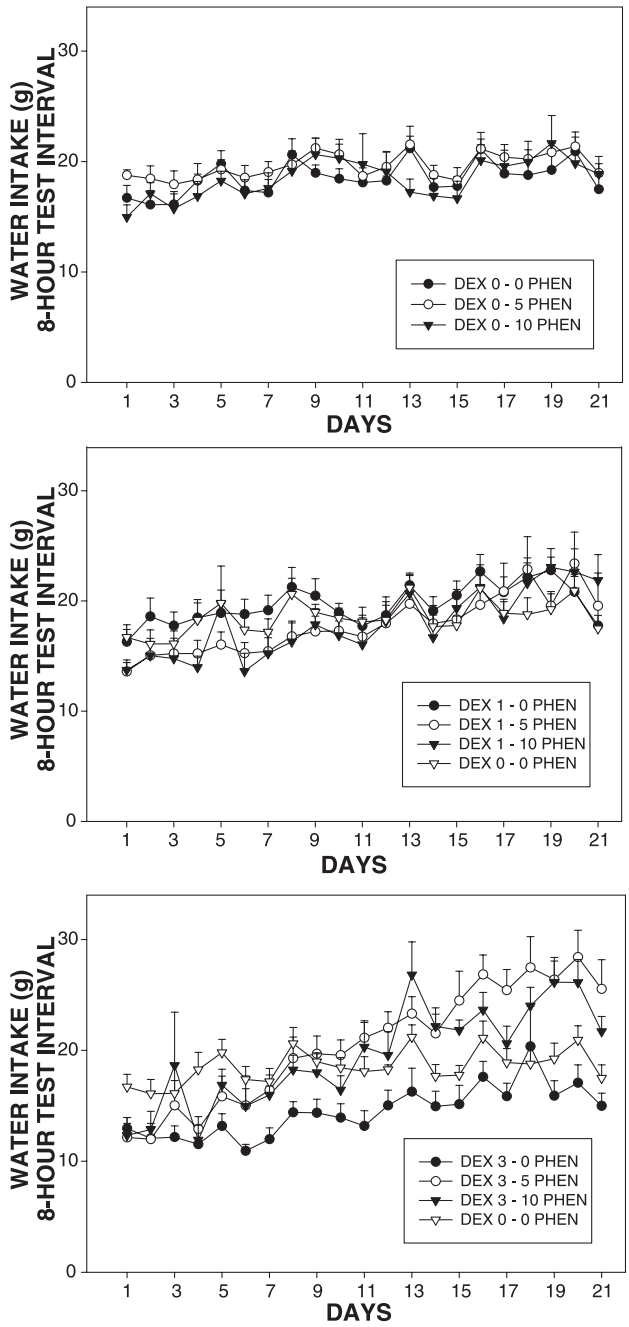


Fig. 5. Mean group daily 8-h water intake values (g) during drug treatment Days 1–21 for rats treated once daily with 0 (top panel), 1 (middle panel), or 3 mg/kg DEX (lower panel) alone or in combination with 0, 5, or 10 mg/kg PHEN. The lines above each symbol represent the S.E.M. The 0–0-mg/kg DEX–PHEN data are repeated in each panel to facilitate data inspection.

days of exposure [$F(20,1660) = 12.09$, $P = .01$] and significant interactions between days of exposure and PHEN treatment [$F(40,1660) = 1.92$, $P = .01$]. Remarkably, rats treated with 3 mg/kg DEX in combination with either 5 or 10 mg/kg PHEN consumed more water during the 8-h test interval than did rats treated with 3 mg/kg DEX only.

### 3.2.3. Body weight

The baseline body weights of the rats (approximately 340 g) were comparable prior to drug administration. Control rats (0–0-mg/kg DEX–PHEN group) gained approximately 70 g of body weight during the 21-day period. ANOVA of the changes in body weight during drug administration revealed a significant main effect of day of exposure [$F(20,1660) = 317.9$, $P = .01$]. Administra-

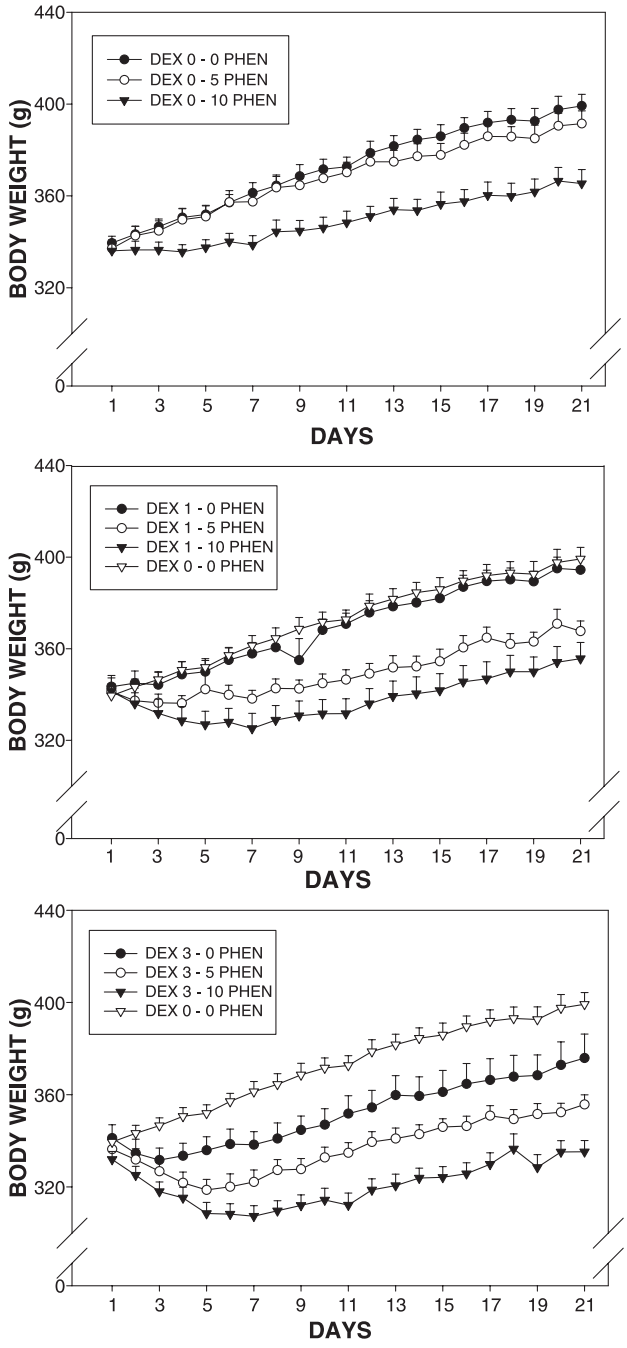


Fig. 6. Mean group daily body weight values (g) during drug treatment Days 1–21 for rats treated once daily with 0 (top panel), 1 (middle panel), or 3 mg/kg DEX (lower panel) alone or in combination with 0, 5, or 10 mg/kg PHEN. The lines above each symbol represent the S.E.M. The 0–0-mg/kg DEX–PHEN data are repeated in each panel to facilitate data inspection.

tion of DEX and of PHEN produced significant reductions in body weight gain during the 21-day period (see Fig. 6). ANOVA of these changes in body weight revealed significant effects of DEX administration [$F(2,83)=22.3$, $P=.01$] and of PHEN administration [$F(2,83)=25.2$, $P=.01$]. There was no significant interaction between DEX and PHEN administration [$F(4,83)=0.645$, $P<.632$], nor was there a significant interaction between the factors of days and DEX and PHEN administration [$F(80,1660)=1.132$, $P<.204$].

As was the case for food intake and water intake analyses above, a secondary analysis of the body weight data was computed using the low-dose DEX (1 mg/kg) and PHEN (5 mg/kg) groups. The individual treatment groups as well as the residual analyses are depicted in Fig. 7. As can be clearly seen, the 1-mg/kg DEX and the 5-mg/kg PHEN groups were similar in weight gain to the vehicle control (0–0 mg/kg DEX–PHEN) group. In contrast, there was a significant suppression of weight gain during the 21-day period evident in the group receiving the combination of 1–5 mg/kg DEX–PHEN. Each of the 21 daily residual values were positive; thus, confirming that the combination of these lower doses of DEX and PHEN produced a greater inhibition of weight gain than could be accounted for by either treatment alone.

Body weights increased for all groups during the 9-day period separating the end of the drug pretreatment phase and the start of the sibutramine trials (data not presented). ANOVA of body weight values on the day prior to the sibutramine trials, however, revealed significant between group differences in body weight in the PHEN [$F(2,83)=10.8$, $P=.01$] and DEX groups [$F(2,83)=3.2$, $P=.047$]. There was no significant interaction among these factors ($P=.92$). Because significant between-group differences in body weight were evident at the start of the sibutramine trials, subsequent analyses of food intake during the sibutramine trials were computed using food intake per unit body weight (g/kg).

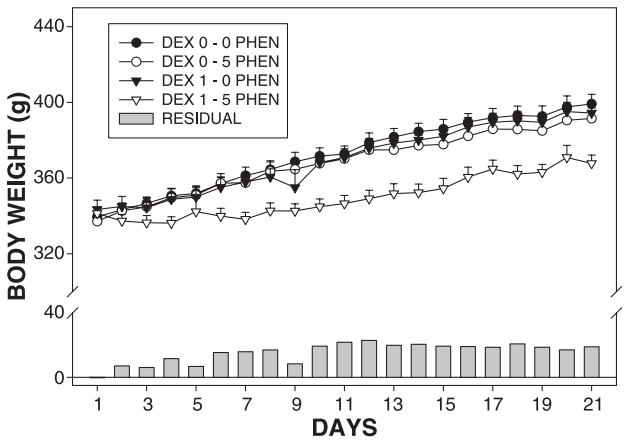


Fig. 7. Mean group body weight values (g) for the 1–5-mg/kg DEX–PHEN combination group relative to the 0–0-, 1–0-, and 0–5-mg/kg DEX–PHEN groups. The inset bars represent the mean difference between the 0–0- and 1–5-mg/kg DEX–PHEN groups, less the differences of the 0–5- (relative to 0–0-mg/kg DEX–PHEN group) and 1–0-mg/kg DEX–PHEN group (relative to the 0–0-mg/kg DEX–PHEN group).

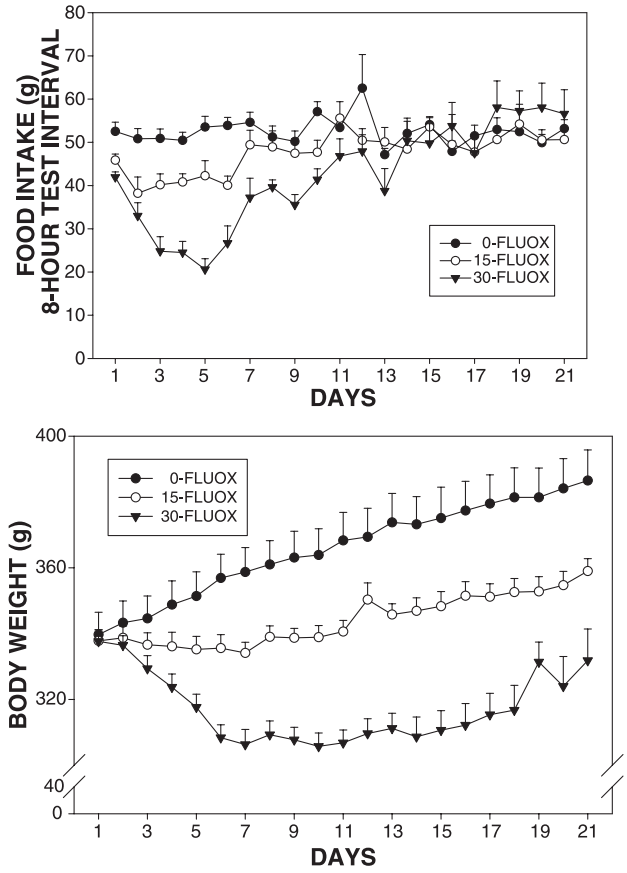


Fig. 8. Mean group 8-h food intake values (g) (top panel) or mean group body weight (g) (bottom panel) during drug treatment Days 1–21 for rats treated once daily with 0, 15, or 30 mg/kg FLUOX.

### 3.3. Changes in ingestion and body weight produced by administration of FLUOX on Days 1–21

#### 3.3.1. Food intake

Intakes of the mash diet during an 8-h interval over Days 1–21 in rats treated with 0 mg/kg FLUOX were similar to those of rats treated with 0–0 mg/kg DEX–PHEN (see top panel of Fig. 8). FLUOX produced significant reductions in food intake during an 8-h test interval after drug administration. For rats treated with 30 mg/kg FLUOX, food intakes reached a nadir at Day 5 and then gradually returned to levels comparable to and slightly above that of control rats (Day 21, ns) ANOVA of these data revealed a significant main effect of FLUOX treatment [$F(2,28)=5.8$, $P=.01$], a significant main effect of day of exposure [$F(20,560)=10.8$, $P=.01$], and a significant interaction between FLUOX treatment and day of exposure [$F(40,560)=4.9$, $P=.01$]. The interaction between FLUOX and day resulted from the large separation in food intake values between the FLUOX groups during the first 10 days of the study and the minimal

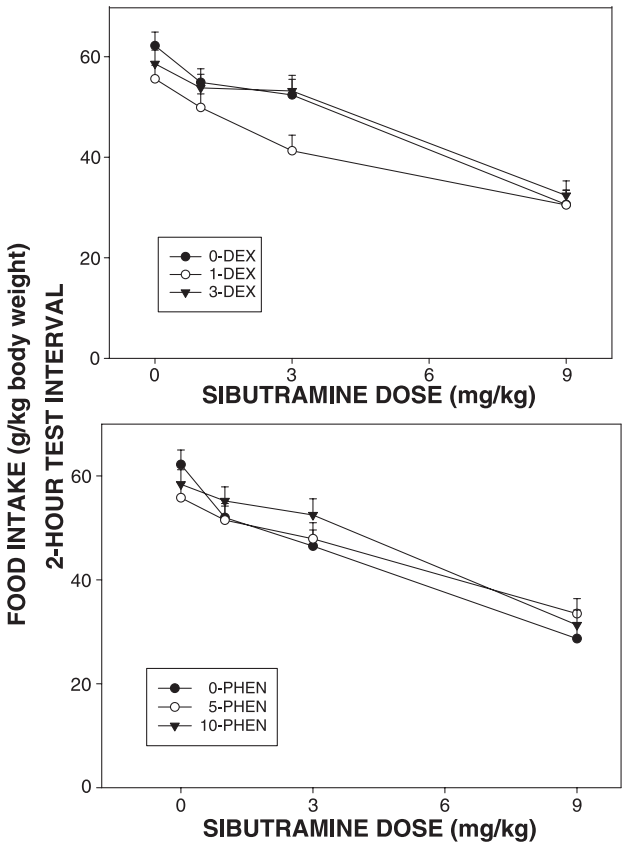


Fig. 9. Mean group food intake values (g/kg body weight) during a 2-h interval after administration of 0, 1, 3, or 9 mg/kg sibutramine for rats pretreated (on Days 1–21) with 0, 1, or 3 mg/kg DEX (top panel) or with 0, 5, or 10 mg/kg PHEN (bottom panel). The DEX values are collapsed across PHEN groups and the PHEN values are collapsed across the DEX groups.

difference in food intake between the groups during the last 5 days of FLUOX administration. At the end of the 8-day recovery period (i.e., the day prior to the sibutramine trials), the mean group food intakes of the 15- and 30-mg/kg FLUOX groups (59.5 and 63.5, respectively) were slightly higher than that of the 0-mg/kg FLUOX vehicle (57.1 g), but these differences were not significant ($P \geq .107$).

### 3.3.2. Body weight

Rats treated with 0 mg/kg FLUOX gained approximately 70 g in weight over the 21-day pretreatment period, a weight gain similar to that of rats treated with the 0–0-mg/kg DEX–PHEN control condition (see bottom panel of Fig. 8). In contrast to the effects of FLUOX on food intake, chronic administration of FLUOX, at doses of 15 and 30 mg/kg produced marked and persistent group separations in body weight over the 21-day period. ANOVA of the changes in body weight during Days 1–21 revealed significant main effects of FLUOX treatment [$F(2,28) = 20.7$, $P = .01$] and day [$F(25,60) = 21.4$, $P = .01$], as well as a significant interaction between the factors of FLUOX treatment and day [$F(45,60) = 12.9$, $P = .001$]. The separation of the FLUOX groups in body weight diminished over the 9-day recovery period such that on Day 29 (the day prior to sibutramine trials), the differences between FLUOX groups in body weight were not statistically significant ($P \geq .534$).

## 3.4. Changes in sibutramine-induced hypophagia produced by prior exposure to DEX, PHEN, and the DEX–PHEN combination

### 3.4.1. Food intake

Fig. 9 presents changes in food intake during the first 2 h after sibutramine administration for rats previously treated with DEX, PHEN, or combinations of DEX and PHEN. ANOVA of these changes in 2-h food intake values revealed a significant main effect of sibutramine dose on food intake [$F(3,249) = 61.9$, $P = .01$]. Subsequent Tukey contrasts revealed significant suppression of food intake by sibutramine (3 and 9 mg/kg) relative to vehicle ($P < .05$). Although there was a significant main effect of DEX preexposure on food intake [$F(2,83) = 3.3$, $P = .04$], subsequent Tukey contrasts comparing DEX group means (0 versus 1 or 3 mg/kg) were not significant ($P$'s>.05). There was no significant effect of PHEN preexposure nor were

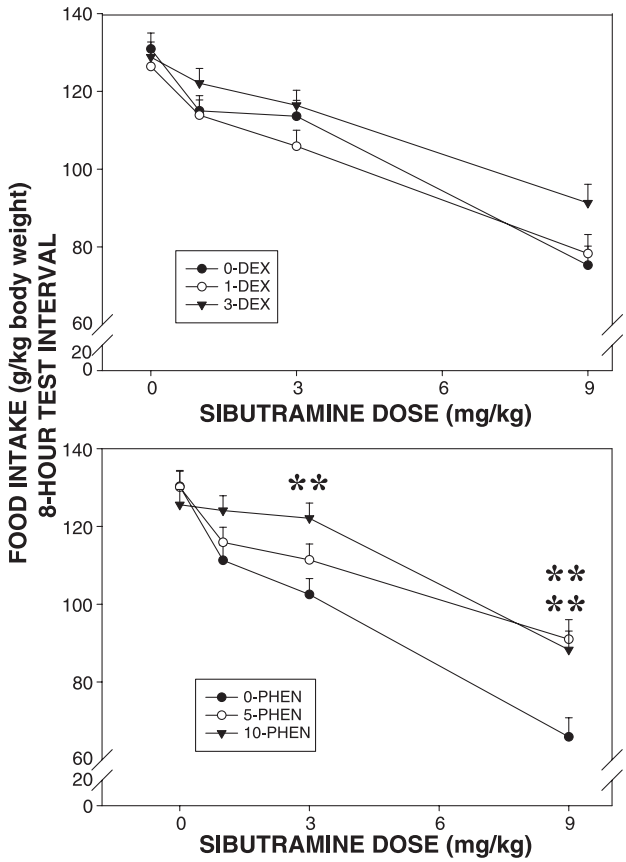


Fig. 10. Mean group food intake values (g/kg body weight) during a 8-h interval after administration of 0, 1, 3, or 9 mg/kg sibutramine for rats pretreated (on Days 1–21) with 0, 1, or 3 mg/kg DEX (top panel) or with 0, 5, or 10 mg/kg PHEN (bottom panel). A single star above a symbol represents a significant difference ($P < .05$) between that group and the relevant control group at a single concentration of sibutramine (** $P < .01$).

there significant two- or three-way interactions in the 2-h food intake analyses ($P$'s>.05).

ANOVA of the food intakes recorded during the 8-h test interval after sibutramine treatment (see Fig. 10) revealed a significant main effect of sibutramine dose [$F(3,249) = 71.3$, $P$=.01], DEX preexposure [$F(2,83) = 3.6$, $P$=.033], and PHEN preexposure [$F(2,83) = 8.0$, $P$=.01]. The only significant two-way interaction was that between sibutramine and PHEN [$F(6,249) = 3.3$, $P$=.01]. Subsequent evaluation of the DEX effect on sibutramine hypophagia revealed a significant difference ($P < .05$) between the DEX doses (3 mg/kg versus 1 mg/kg), whereas no other contrasts were statistically significant.

Subsequent contrasts were used to evaluate the significant interaction between sibutramine dose and PHEN preexposure. At a 0-mg/kg dose of sibutramine, no contrast between the PHEN doses (0, 5, or 10 mg/kg) was significant. The contrasts between the 0- and 10-mg/kg PHEN doses were significant at the 3-and 9-mg/kg sibutramine dose (but not at a dose of 1 mg/kg sibutramine). Moreover, the contrast between 0 and 5 mg/kg PHEN was significant at the 9-mg/kg dose of sibutramine. These analyses indicate that PHEN attenuated the hypophagic action of sibutramine and these effects were greatest for the high dose of PHEN (10 mg/kg) as well as the high dose of sibutramine (9 mg/kg).

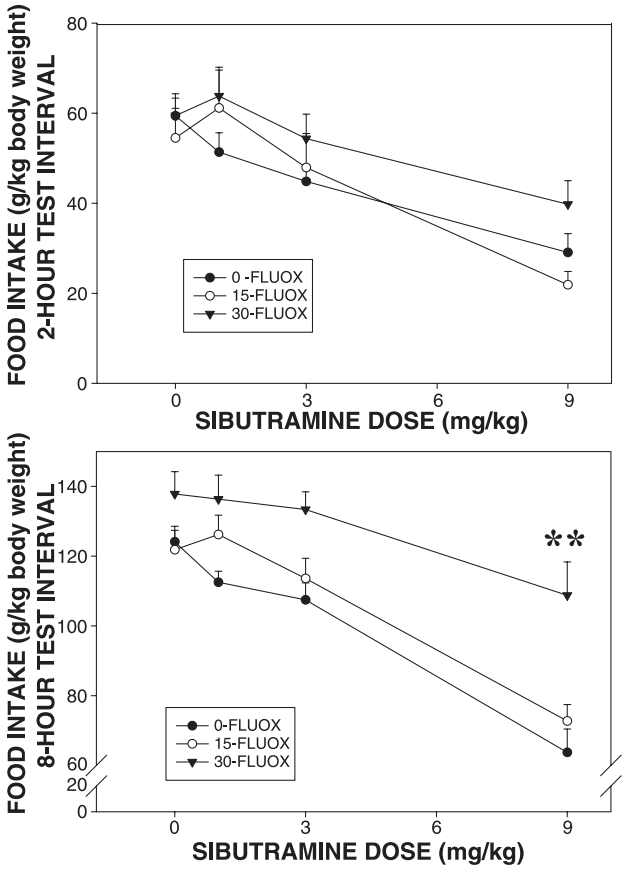


Fig. 11. Mean group food intake values (g/kg body weight) during a 2-(top panel) or 8-h interval (bottom panel) after administration of 0, 1, 3, or 9 mg/kg sibutramine for rats pretreated (on Days 1–21) with 0, 15, or 30 mg/kg FLUOX. Tukey contrasts indicated a significant difference in 8-h food intake (collapsed across sibutramine dose) between 0 and 30 mg/kg FLUOX groups ($P < .01$).

### 3.5. Changes in sibutramine-induced hypophagia produced by prior exposure to FLUOX

FLUOX pretreatment during Days 1–21 did not alter the hypophagic action of sibutramine during the first 2 h after sibutramine administration (see top panel of Fig. 11). ANOVA of the 2-h food intake data revealed a significant main effect of sibutramine dose [$F(2,84) = 29.4$, $P$=.01], but no significant effect of FLUOX exposure or of the interaction between FLUOX exposure and sibutramine dose ($P$'s>.172). In contrast, there was a significant attenuation of the hypophagic action of sibutramine produced by FLUOX preexposure over the 8-h test interval (see bottom panel of Fig. 11). ANOVA of these food intake data revealed a significant main effect of FLUOX exposure [$F(2,84) = 17.7$, $P$=.01], but no significant interaction between FLUOX exposure and sibutramine dose ($P$>.122). Subsequent Tukey contrasts revealed that the 30-mg/kg FLUOX dose produced significant ($P < .01$) attenuation of the hypophagic action of sibutramine relative to the 0- and 15-mg/kg FLUOX doses (but the 0- and 15-mg/kg FLUOX doses were not significantly different).

## 4. Discussion

Repeated daily administration of PHEN, DEX, and FLUOX produced significant suppressions of eating in rats during the first week of drug exposure, but these effects tended to decrease with continued drug treatment. The hypophagic action of DEX and of FLUOX over the 2- and 8-h test periods after drug administration waned within the first week of treatment, whereas the hypophagic action of PHEN during a 2-h test period did not. In contrast, there was a persistent effect of these drugs on body weight evident over the 21-day drug exposure period. Each group of rats gained weight during the 9-day period separating the termination of drug on Day 21 and the start of the sibutramine tests. On the day before sibutramine testing, there were no differences in food intake between the groups, although there remained significant differences in body weight among the PHEN and DEX groups.

Moreover, the present study noted that a combination of PHEN (5 mg/kg) and DEX (1 mg/kg) produced a greater suppression of food intake, water intake, and body weight than what would be expected from a summation of their individual effects. Higher doses of these compounds given in combination (i.e., in 1–10-, 3–5-, or 3–10-mg/kg DEX–PHEN groups) exerted effects on feeding and body weight that were either additive or less than additive. The former findings provide partial support for the notion that DEX and

PHEN may act in a synergistic manner (Wellman and Maher, 1999) on eating and on body weight in the rat, thus confirming earlier studies (Roth and Rowland, 1998, 1999). It should be noted that these effects were evident only for lower doses of these drugs and the synergistic effect on food intake and body weight did not extend to higher doses of these drugs in combination. The neuropharmacological basis of this effect may be related to changes in 5-HT (Tao et al., 2002; Wellman and Maher, 1999), but such changes in hypothalamic 5-HT were not the focus of this study.

In the present study, significant dose-dependent suppressions of eating were evident during the first 2 h after sibutramine administration. Preexposure to DEX, PHEN, or FLUOX did not significantly attenuate the hypophagic action of sibutramine during the first 2 h after sibutramine administration. In contrast, during the 8-h testing interval, rats preexposed to PHEN at either dose exhibited a significant attenuation of the feeding suppressive action of sibutramine. In contrast, only the highest doses of DEX (3 mg/kg but not 1 mg/kg) and FLUOX (30 mg/kg, but not 15 mg/kg) produced an attenuation of the hypophagic action of sibutramine and these effects were only noted for the 9 mg/kg sibutramine dose.

PHEN exerts important actions on brain noradrenergic systems (Rothman et al., 2001), but also exerts important actions on brain dopamine and 5-HT systems (Rothman et al., 2001; Tao et al., 2002). A common action of PHEN and sibutramine relates to the action of these drugs on brain noradrenergic systems and a consequent activation of brain $\alpha_1$-noradrenergic receptors that inhibit eating (Rothman et al., 2001; Wellman, 2000; Wellman et al., 1993). Indeed, the capacity of PHEN and sibutramine to inhibit eating are attenuated by prior administration of the $\alpha_1$-noradrenergic receptor antagonist prazosin (Jackson et al., 1997b). One explanation of the impact of PHEN on sibutramine hypophagia would invoke the notion that PHEN preexposure produced tolerance to the hypophagic effects of PHEN, which then resulted in cross-tolerance to sibutramine. However, in the present study, PHEN doses of 5 or 10 mg/kg produced dose-dependent hypophagia during the 2- and 8-h test intervals, and these responses did not diminish across the 21-day PHEN treatment period. The present study indicates that preexposure to high doses of PHEN that reduces food intake and produces persistent reductions in body weight are associated with a reduced hypophagic response to certain doses of sibutramine. The present study does not identify the mechanism that underlies this diminished hypophagia to sibutramine in rats preexposed to PHEN.

As expected and consistent with earlier studies (Rowland et al., 2000), daily administration of DEX and of FLUOX resulted in tolerance to their hypophagic actions within the first week of exposure, which persisted through the 21-day exposure period. Yet, only the highest DEX and FLUOX doses produced an attenuation of sibutramine hypophagia, and this occurred only for the 9-mg/kg sibutramine dose, a dose that is considered to be several fold above that required to reduce eating in rats by 50% (Jackson et al., 1997a). One explanation for these data is that these drug treatments may deplete brain 5-HT, thus compromising the subsequent hypophagic response to sibutramine that is thought to reflect a joint action of sibutramine on noradrenergic and serotonergic systems. While it is known that DEX depletes brain 5-HT (McCann et al., 1998), no such depletion is evident for PHEN (Halladay et al., 1998; Lew et al., 1997; McCann et al., 1998) or for FLUOX (Kalia et al., 2000). In the latter study, FLUOX administered at a dose of 114 mg/kg in rats (twice per day for 4 days) did not alter 5-HT levels within rat prefrontal cortex. Similarly, Gobbi et al. (1997) reported no effect of chronic FLUOX exposure (15 mg/kg, twice per day for days; 7 day washout period) in rats on 5-HT uptake (cortex, hippocampus), 5-HT transporter binding (cortex, hippocampus, raphe, ventral tegmental area), presynaptic 5-$HT1_b$ autoreceptor function, or ligand binding to 5-$HT_3$ receptors (cortex) or 5-$HT_4$ receptors (substantia nigra). Finally, Heal et al. (1998a,b) examined the impact of FLUOX (10 mg/kg) or DEX (10 mg/kg) given once per day for 14 days on 5-$HT_{2A}$ receptor number in rat frontal cortex. DEX reduced 5-$HT_{2A}$ receptor number by 61%, whereas FLUOX was without effect. These studies suggest that a depletion of brain 5-HT by these drugs is unlikely to account for the pattern of findings in the present study.

The attenuation of sibutramine hypophagia noted in the present preexposure study is unlikely to reflect changes in basal food intakes per se given that the rats were allowed a 9-day recovery period following termination of drug exposure. On the day before the start of sibutramine testing, there were no significant between-group differences in food intake. Moreover, these effects are similarly unlikely to reflect differences in body weight per se. The food intake data of the sibutramine study were analyzed as food intake per kilogram body weight to equate the groups for any differences in body weight immediately before the sibutramine tests. Moreover, the results from rats treated with 3 mg/kg DEX indicated a robust attenuation of sibutramine hypophagia during the 8-h test interval. Yet, this group exhibited a small difference in body weight relative to the control group (0–0 mg/kg DEX–PHEN) on the day before sibutramine testing.

The observation that sibutramine hypophagia was attenuated over a longer 8-h test interval, but not a shorter 2-h test interval, suggests that this effect may be related to an alteration of the metabolism or elimination of sibutramine. Sibutramine is demethylated within the liver to form the active metabolites 1 and 2 (Cheetham et al., 1993; Van Gaal et al., 1998), which are in turn hydroxylated and eventually excreted via the kidney (Cheetham et al., 1993; Van Gaal et al., 1998).[1] Preexposure to PHEN and to high doses of FLUOX or DEX may enhance liver enzyme activity such that sibutramine is more rapidly cleared from blood. This

[1] Dr. David Heal, personal communication, October 17, 2000.

effect would be evident over longer time intervals and would be expected to exert the largest effect on relatively high doses of sibutramine. Plasma levels of sibutramine and of the two sibutramine metabolites were not assessed in the present study; thus, a final determination of this explanation will require assessment of the kinetics of sibutramine and its metabolites in rats preexposed to DEX, PHEN, and FLUOX.

It is uncertain as to the extent to which the present findings are of direct relevance with regard to clinical use of sibutramine as an antiobesity drug in humans. The doses of PHEN, DEX, and FLUOX used in the present animal study exceed the daily clinical doses used in humans. The PHEN doses (5 and 10 mg/kg) used in the present study produced a significant attenuation of sibutramine hypophagia in rats, whereas human clinical doses of PHEN are in the range of 0.2–0.5 mg/kg/day. For DEX, the human clinical doses are less than 1 mg/kg/day, a dose level that is less than the 3-mg/kg DEX dose noted in the present study to attenuate sibutramine hypophagia in rats. Finally, attenuated sibutramine hypophagia was evident in the present study at for rats treated with FLUOX at 30 mg/kg/day, a dose level substantially higher than the human clinical FLUOX dose of less than 1 mg/kg/day (assuming a 60-mg/day dose and a 70-kg human).

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