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Long-Term Administration of Dexfenfluramine to Genetically Obese (ob/ob) and Lean Mice: Body Weight and Brain Serotonin Changes

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ROWLAND, N. E. *Long-term administration of dexfenfluramine to genetically obese (ob/ob) and lean mice: Body weight and brain serotonin changes.* PHARMACOL BIOCHEM BEHAV **49**(2) 287–294, 1994.—The weight-reducing and brain 5-HT-depleting properties of dexfenfluramine (DFEN) or dexnorfenfluramine (DNOR) were measured in genetically obese (ob/ob) and lean mice. These agents were infused for 14 days via osmotic minipumps to mice fed either a low fat or a moderate fat diet. Weight loss was observed in only the obese mice, with DNOR more potent than DFEN. At the end of 14 days, neither agent caused a consistent change in either plasma glucose or corticosterone concentrations, although some effects of diet and differences between batches of mice were apparent. The levels of brain 5-HT, or of paroxetine binding that correlates with 5-HT level, were reduced by 24 mg DFEN/kg/day, a decline that persisted for at least 14 days after the end of treatment. Plasma and brain concentrations of DFEN and DNOR were measured on the last day of pump function. DNOR accounted for about 30% of the total drug + metabolite content, a ratio comparable to that in human plasma. Brain concentrations exceeded plasma by 10-fold at 6 mg DFEN/kg/day and by 17-fold at 24 mg DFEN/kg/day. The levels were higher in mice fed the moderate-fat compared with the low-fat diet. Depletions in brain 5-HT parameters were found only in the high-dose groups, and at brain total levels above about 20 μM.

Anorectic agent Brain and plasma concentrations Steady state Dexfenfluramine Corticosterone Tolerance Dietary fat content

DEXFENFLURAMINE (DFEN) is a potent weight-reducing agent in clinical use in many countries (13,18). A large body of evidence indicates that an increase in synaptic levels of serotonin (5-HT) in brain underlies, at least in part, the reduced appetite and food intake caused by DFEN (5,23). Its principal cellular actions are inhibition of 5-HT uptake and stimulation of 5-HT release, but the specific 5-HT projections involved in the anorectic effect remain largely unknown (16).

It has been known for many years that high doses of DFEN or its active metabolite, dexnorfenfluramine (DNOR), cause depletions of brain 5-HT in several animal species. The extent and duration of depletion are both dose-related and species-dependent (e.g., 24,27–29). Most of the neurochemical work has been performed in rats, a species that rapidly dealkylates acutely administered DFEN to DNOR such that plasma and brain levels of DNOR exceed those of DFEN within 2 h (9). Because some of the presynaptic actions of DFEN and DNOR differ (6), it is possible that these agents may also differ in their 5-HT-depleting potency or action (15). This might be clinically significant because humans, unlike rats, are able to deaminate DFEN and DNOR efficiently to inactive polar metabolites that are excreted in urine. As a result, plasma levels of DFEN always exceed those of DNOR (9,17).

Mice have a drug-to-metabolite ratio similar to that in humans, and unlike rats. This is because, relative to rats, mice dealkylate DFEN and deaminate DNOR slowly, resulting in high plasma levels of both DFEN and DNOR, and a correspondingly high urinary excretion of these compounds (8,17).

Acute injection of either DFEN or racemic fenfluramine produces only small and transient depletions of brain 5-HT in mice compared with much larger and longer-lasting effects in rats (26,28). The present study will attempt to discriminate between three possible explanations for this species difference.

The first possibility is that the species difference relates to the longer plasma half-life for DFEN, and shorter for DNOR, in mice compared with rats. To overcome the problem inherent of widely fluctuating drug levels using injection paradigms, we administered drugs by osmotic minipump; this chronic mode of administration also mimics the much longer half-life of DFEN in humans compared with rodents.

The second possibility is that DNOR (or another metabolite), and not DFEN, is responsible for 5-HT depletions. Thus,

mice would be protected from 5-HT depletion because they form relatively less DNOR. To assess this, in Experiment 1 we compared infusions of DFEN and DNOR; because DNOR has about twice the anorectic potency and twice the half-life of DFEN, DNOR was used at one half the dose of DFEN.

The third possibility is that for a given tissue exposure to either DFEN or DNOR (or another metabolite), 5-HT neurons in mice are simply less susceptible to long-term 5-HT-depleting effects. This would have important implications for the underlying mechanisms. To assess this, plasma and brain concentrations of DFEN and DNOR were measured in Experiment 2, and the resultant effects on brain 5-HT parameters were compared with rat data.

We also measured the effects of these regimens on body weight and plasma corticosterone and glucose. Previous short-term and long-term studies in normal and obese mice have found that very high doses of fenfluramine (relative to effective doses in rats) are needed to produce modest anorexia or weight loss (1,3,11,12,19,20,26). These doses are more effective in obese than in lean mice, possibly because the half-life of DFEN is longer (4). Because differences in dietary fat content may affect plasma binding and elimination of the agents, we have compared low-fat and moderate-fat diets. The latter approximates the fat content of human diets in postindustrial societies.

Long-term use of fenfluramine has been reported to decrease plasma glucose in hyperglycemic, insulin-resistant ob/ob mice (20), and so we measured plasma glucose as an index of this metabolic action. In the short term, DFEN stimulates hypothalamic release of corticotropin releasing hormone; thus, plasma corticosterone level may be an indication of the functional status of a subset of brain 5-HT synapses in chronic treatment. The decline in DFEN-stimulation of corticosterone after chronic DFEN may reflect feedback regulation or adaptation to the agent (2). In our study this was assessed for the first time in mice. Two experiments were performed: in the first, administration of DFEN and DNOR were compared. In the second, high and low doses of DFEN were compared. In each experiment, groups fed either low- or moderate-fat diets were included.

METHODS

Animals, Housing, and Diet

Female obese (ob/ob) and lean (ob/- and ?/-) C57BL/6J mice, 4–6 weeks of age, were purchased from Jackson Labs (Bar Harbor, ME). Sixty-four mice were used in Experiment 1 and 320 mice were used in Experiment 2. They were housed individually in stainless-steel cages (18 × 12 × 13 cm) in a vivarium with lights on from 0600 to 1800 h. Half of the mice from each phenotype were fed either a low-fat diet (RC; Purina Rodent Chow 5001; approximately 10% of calories are from fat, 25% protein, and 65% carbohydrate) or a moderate-fat diet (MC; Purina Mouse Chow 5008; approximately 25% calories are from fat, 25% protein, and 50% carbohydrate), both presented as pellets in a hopper. The designated food, as well as tap water, were available at all times. Mice were assigned randomly to those groups and were fed the designated diet for about 4 weeks before the experimental phase.

General Procedure

Mice of each phenotype and diet condition were then assigned at random to treatment groups in Experiments 1 and 2. The treatment phase lasted 14 days. After sedation with methoxyflurane, the mice were implanted subcutaneously, via an incision in the scapular region, with osmotic minipumps (Alzet 2002; 0.5 μl/h × 14 days). The incision was closed with a wound clip. The pumps were filled with either DFEN or DNOR at a concentration to deliver the desired dose in milligrams (of the HCl salts) per kilogram per day. Control mice were sedated, but received no implant. All mice were weighed immediately after surgery and were returned to their home cages. They were weighed every 2 days thereafter during the treatment phase.

Either in the middle of day 14, before nominal expiration of the pumps, or at designated times after the end of the treatment, mice were decapitated and two hematocrit tubes of blood were collected and centrifuged. Plasma was extruded and one sample assayed for glucose (YSI analyzer); another was assayed for corticosterone (^{125}I-radioimmunoassay; Ventrex). In Experiment 2, trunk blood was also collected for measurement of DFEN and DNOR. The brain was removed for assay of DFEN and DNOR, and various 5-HT parameters. All samples were obtained between 0900 and 1300 h (first half of the light phase) to minimize time-of-day effects.

Measurements on Brain Tissue

5-HT was measured by high-performance liquid chromatography with electrochemical detection. Samples of cerebral cortex and hypothalamus were homogenized immediately in cold 0.2 M $HClO_4$ containing EDTA (10 μM) and an internal standard (homovanillyl alcohol, 1 μM) and centrifuged, and the supernatant was assayed for serotonin (5-HT) concentration. Peak areas were integrated automatically and normalized, and tissue concentrations of 5-HT were calculated (cf. 22).

An additional sample of cortex was frozen and subsequently assayed for 5-HT uptake carrier density using paroxetine binding (14). Total binding was determined in triplicate Tris homogenate of cortex incubated with a saturating concentration (10 nM) of ^{3}H-paroxetine (DuPont) for 2 h at room temperature. Fluoxetine (1000 nM) was added to additional tubes to determine nonspecific binding. Tissue-bound label was counted by liquid scintillation spectrometry after vacuum filtration (GF/B Whatman discs) and washing. Total binding (mean total minus mean nonspecific) was expressed relative to protein content of the homogenate as determined by the BCA (Pierce Labs) method.

DFEN and DNOR Assays

Plasma samples and the left half of the brain (Experiment 2) were immediately frozen at −70°C and were later shipped in solid CO_2 to Dr. D. B. Campbell (Servier, UK) for assay of DFEN and DNOR by gas chromatography (21).

Experiment 1: DFEN and DNOR Compared

Five mice from each phenotype and diet group were implanted with a minipump filled with either DFEN dissolved in water at a concentration to deliver 6 mg/kg/day (dose expressed as HCl salt) or with DNOR to deliver 3 mg/kg/day. Another six mice from each condition served as controls and received no pump (in pilot work, we found no difference between saline pump and no implant controls). All mice were killed on day 14 for determination of plasma corticosterone and glucose and brain 5-HT concentration and paroxetine binding.

Experiment 2: High and Low Doses of DFEN Compared

Because the drug effects in Experiment 1 were small, a higher dose of DFEN was tested and the persistence of effects was examined up to 2 weeks after the end of the treatment. This large experimental design necessitated that the study be performed in three stages (starting March, May, and December 1991). Approximately equal numbers of mice from each of the treatment groups were used in each stage. The data from each stage were comparable, and were combined for statistical analysis. A total of 160 each of ob/ob and lean mice were fed either RC or MC. There were three treatment groups: 1) 14-day minipump infusion of DFEN (6 mg/kg per day), 2) 14-day infusion of DFEN (24 mg/kg per day), and 3) uninfused controls.

Mice were sacrificed on either day 14 (last day of infusion), day 16, or day 28 (2 or 14 days after infusion). Controls were not expected to (nor did they) differ across days, and their data were combined to form overall control means. There were six to eight mice per group (two phenotypes, two diets, three treatments, and three time points). Body weight changes were measured every 2 days as before. In mice killed on day 14, plasma corticosterone and glucose were measured and plasma and brain samples frozen for DFEN and DNOR assay. The right frontal cortex was used to measure paroxetine binding to the 5-HT uptake site in all mice (days 14, 16, and 28).

Statistics were performed by analysis of variance (ANOVA)

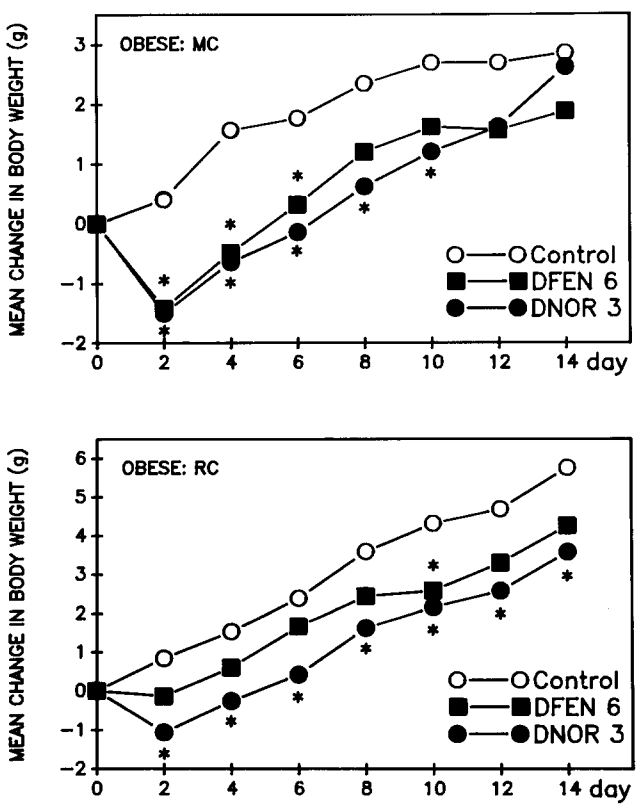

FIG. 1. Mean change in body weight of ob/ob mice (N = 5–6) fed either low-fat rat chow (RC) or moderate-fat mouse chow (MC) and infused with either nothing (control), DFEN (6 mg/kg per day), or DNOR (3 mg/kg per day) via 14-day osmotic minipump. *$p < 0.05$, differs from control. The SE of the means (not shown) ranges from 0.2–0.7.

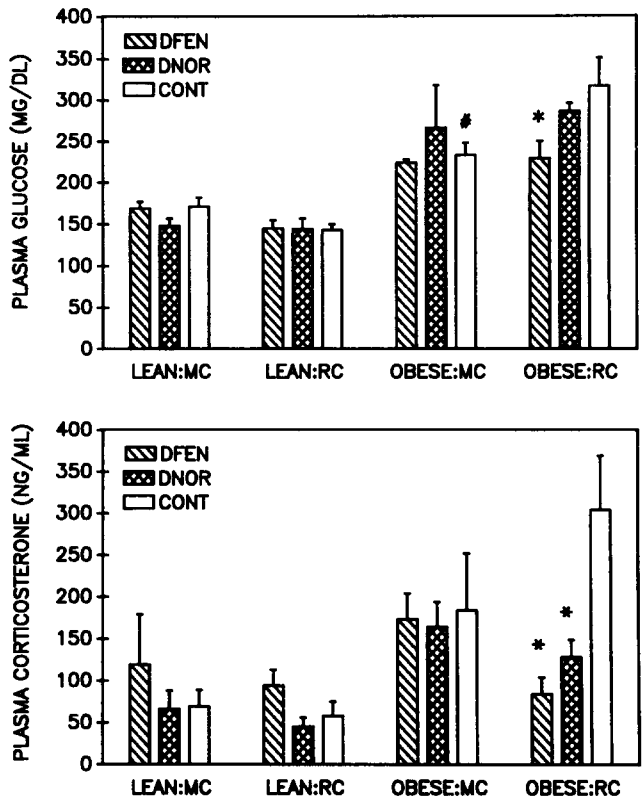

FIG. 2. Mean (±SE) plasma glucose and corticosterone in lean and ob/ob mice (N = 5–6) after 14 days' treatment, as indicated in Fig. 1. *$p < 0.05$, differs from control. #$p < 0.05$, MC differs from RC condition.

(SAS PC version) and post hoc (Duncan) tests with significance level set at $p < 0.05$.

RESULTS

Experiment 1: DFEN and DNOR Compared

Body weight (Fig. 1). Ob/ob mice weighed 2–3 times more than lean mice; those fed the MC diet were about 20% heavier than the RC-fed mice of the same phenotype; and all gained weight during the study. The mean weights at the start of the experimental phase (i.e., after 4 weeks of adaptation to the diets) were 59.4 (obese MC), 49.0 (obese RC differed significantly from obese MC), 26.1 (lean MC), and 23.4 (lean RC) g. Overall ANOVA of body weight during the experimental phase revealed main effects (each $p < 0.01$) of phenotype, diet, and days. The increase in body weight from day of pump implantation (day 0) was significantly attenuated by drug ($p < 0.001$) in both the RC and MC ob/ob groups. The increase in body weight was significantly lower ($p < 0.05$) in the DNOR-infused ob/ob mice for most of the treatment period in both RC and MC groups than in their respective controls. DFEN had a less marked effect on body weight than DNOR, and their change in weight differed from controls only through day 6 in the MC-fed and through day 10 in the RC-fed obese groups. At no time did DNOR and DFEN groups differ from each other. Thus, DNOR was at least as effective as twice the dose of DFEN. In contrast to these effects in obese mice, there were no significant effects of either DFEN or

TABLE 1

BRAIN 5-HT CONCENTRATION AND PAROXETINE BINDING (EXPERIMENT 1)

	Control	DFEN (6)	DNOR (3)
Hypothalamic 5-HT (nmol/g)			
Obese	4.55 ± 0.69	6.09 ± 0.87	6.00 ± 0.88
Lean	1.93 ± 0.55	2.39 ± 0.40	1.89 ± 0.47
Cortical 5-HT (nmol/g)			
Obese	3.70 ± 0.34	4.10 ± 0.29	3.16 ± 0.51
Lean	1.16 ± 0.55	1.57 ± 0.18	1.53 ± 0.45
Cortical paroxetine binding (cpm/mg protein)			
Obese	12.4 ± 0.8	9.7 ± 1.0	13.0 ± 0.8
Lean	11.8 ± 0.6	12.4 ± 0.9	13.0 ± 0.4

Shown are the means ± SE for eight to 12 samples (low- and moderate-fat diets combined). Tissues were collected on day 14 of treatment with either DFEN (6 mg/kg/day) or DNOR (3 mg/kg/day), or no drug (control).

DNOR administration on body weight change in lean mice compared with untreated controls.

Plasma glucose (Fig. 2A). As expected, ob/ob mice were hyperglycemic, and this was more pronounced with RC. In the RC-fed obese group, DFEN significantly reduced glucose level, but no other drug-related effects were detected.

Plasma corticosterone (Fig. 2B). As expected, ob/ob mice had high daytime titers of corticosterone compared with lean mice, and this was more pronounced in the RC group. Treatment with both DFEN and DNOR significantly reduced the elevated corticosterone in RC-fed, but not in the corresponding MC-fed ob/ob mice. There were no effects of either agent in lean mice compared with controls.

Brain 5-HT measures (Table 1). 5-HT levels were higher in hypothalamus and cortex of ob/ob compared with lean mice ($p < 0.001$). Because there was no consistent effect of diet, the RC and MC data were combined. There were no significant overall effects (each $p < 0.2$) of either DFEN or DNOR on 5-HT concentration or paroxetine binding.

Experiment 2: High and Low Doses of DFEN Compared

Body weight (Fig. 3). Absolute body weights were similar to Experiment 1, with mean initial values 53.5 (obese MC), 49.0 (obese RC), 23.6 (lean MC), and 21.2 (lean RC) g. In the uninfused control animals, the change in body weight during the 14-day treatment period was comparable in RC and MC

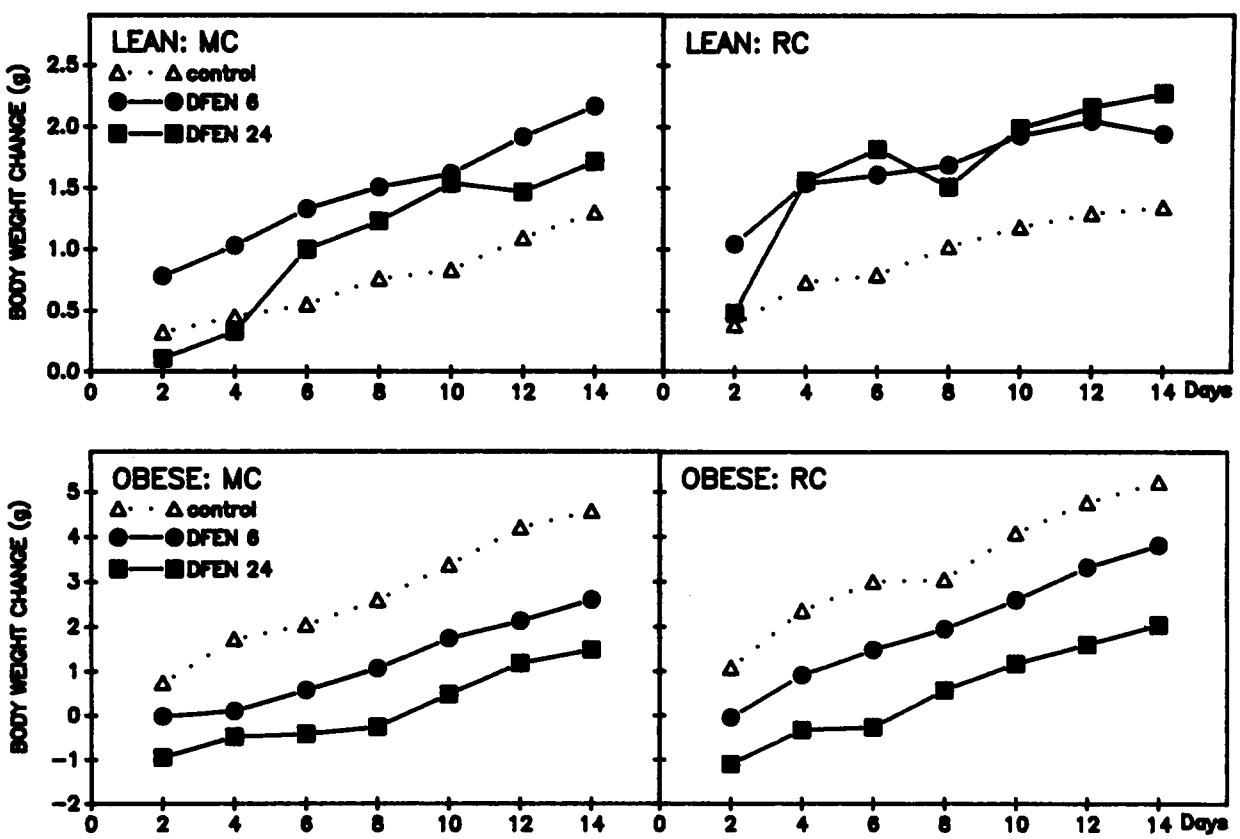

FIG. 3. Mean change ($N = 6$) in body weight of lean and ob/ob mice fed either moderate-fat (MC) or low-fat (RC) diets and infused for 14 days with either nothing (cont), or 6 or 24 mg DFEN/kg/day. (Note the different ordinate scales for lean and obese groups). DFEN reduced body weight at all times in the ob/ob group.

TABLE 2

PLASMA GLUCOSE AND CORTICOSTERONE (EXPERIMENT 2)

	Control	DFEN (6)	DFEN (24)
Plasma glucose (mg/dl)			
Obese, MC	302 ± 47	512 ± 78	371 ± 74
Obese, RC	257 ± 16	316 ± 49	318 ± 43
Lean, MC	205 ± 14	198 ± 11	181 ± 9
Lean, RC	202 ± 23	187 ± 10	191 ± 16
Plasma corticosterone (ng/ml)			
Obese, MC	445 ± 112	235 ± 37	229 ± 27
Obese, RC	266 ± 42	168 ± 68	330 ± 68
Lean, MC	62 ± 20	75 ± 21	90 ± 40
Lean, RC	62 ± 14	58 ± 9	211 ± 83*

Shown are means ± SE for numbers of five to seven on day 14 of treatment with either 6 or 24 mg DFEN/kg/day. MC, moderate-fat diet; RC, low-fat diet. *$p < 0.05$ compared with corresponding control group.

groups, but was significantly (2–3 times) greater in mice of the obese phenotype.

In ob/ob mice, DFEN produced a sustained dose-related decline in weight gain relative to controls. Thus, in both RC and MC groups, the weight change after both low- and high-dose DFEN was lower than in controls ($p < 0.05$, one-way ANOVA + Duncan tests). The weight change was reliably lower in mice treated with the high compared with the low dose of DFEN. Most of the weight change relative to controls occurred early in the treatment period.

In lean mice, DFEN significantly and unexpectedly increased body weight change at all times. This was similar across diet and DFEN dosages and could be a consequence of the pump implantation, in part because of the rapid occurrence of the effect. If this "nonspecific" increase also occurred in the ob/ob groups, it would partly mask the effects of DFEN, and drug-related weight loss would possibly be larger than indicated in Fig. 3. The reason this effect was not observed in Experiment 1 is not clear.

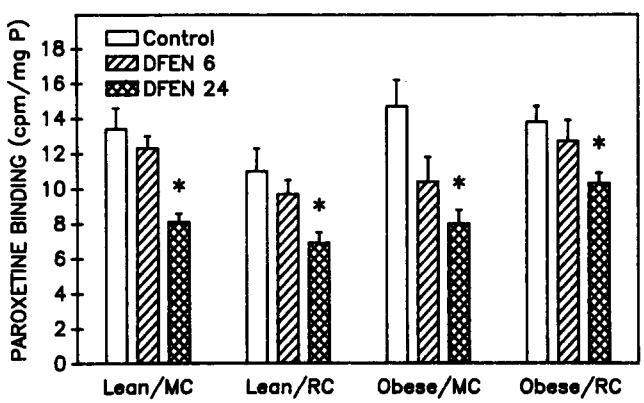

FIG. 4. Mean (±SE) paroxetine binding (in counts per minute per milligram of protein) in cerebral cortex of mice treated for 14 days with DFEN, as indicated in Fig. 3 (N = 12–18). The data have been combined for mice sacrificed on day 14 of treatment, and 2 and 14 days afterward. *$p < 0.05$ compared with uninfused control.

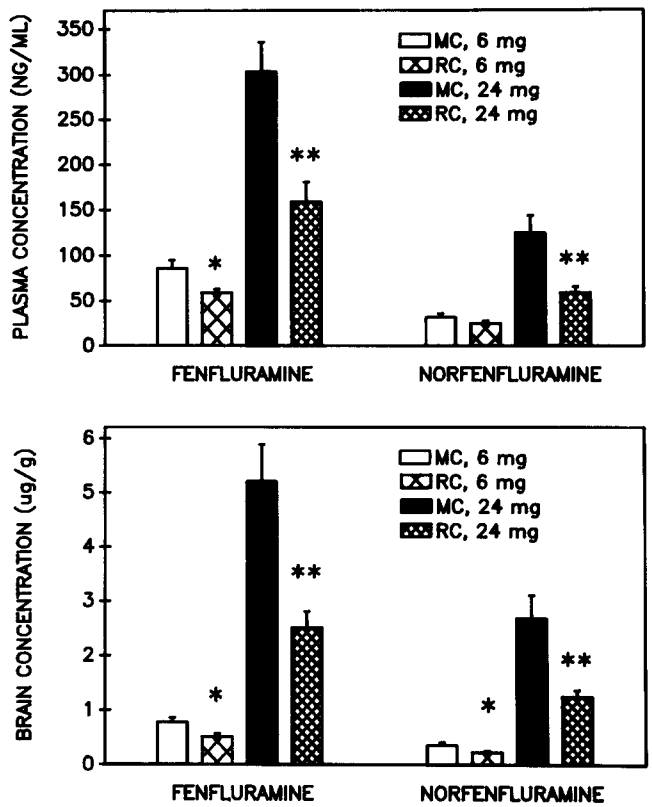

FIG. 5. Plasma and brain concentrations of DFEN and DNOR in lean and ob/ob mice (combined) on day 14 of the various treatment groups, as described in Fig. 3. *$p < 0.05$. **$p < 0.01$, RC differs from MC condition. Shown are means ± SE for groups of 12–14.

Plasma glucose and corticosterone (Table 2). On day 14, plasma glucose was unaffected by either diet or DFEN in either phenotype. Lean mice had higher, marginally hyperglycemic plasma glucose levels than in Experiment 1. Ob/ob mice had more variable plasma glucose, especially with the MC diet when some values were extremely high. In contrast to Experiment 1, plasma corticosterone was higher in the ob/ob MC than the RC control group on day 14. The low dose of DFEN reduced corticosterone in ob/ob mice, but this effect was not significant. Plasma corticosterone in lean mice was elevated above control in only the high-dose DFEN RC group. Plasma values on days 16 and 28 after the pumps expired were similar to respective control values (data not shown).

Paroxetine binding (Fig. 4). Mean paroxetine binding was reduced by 30–40% in mice treated with the high dose of DFEN ($p < 0.001$). The low dose of DFEN reduced binding significantly in only the ob/ob MC group. The data were similar at each sacrifice day (main effect of sacrifice day [$F(2, 190) = 0.9$]) and the data for all 3 days have been combined in Fig. 4. Paroxetine binding was significantly higher in obese compared with lean phenotypes ($p < 0.05$, ANOVA main effect).

Plasma and brain DFEN and DNOR levels (Figs. 5 and 6). The plasma concentration of DNOR was slightly less than half that of DFEN in all groups (Fig. 5), yielding a DNOR/(DFEN + DNOR) ratio of 0.3 (Fig. 6). The plasma concentrations of DFEN and DNOR did not differ significantly between lean and ob/ob groups, and so have been combined in Figs. 5 and

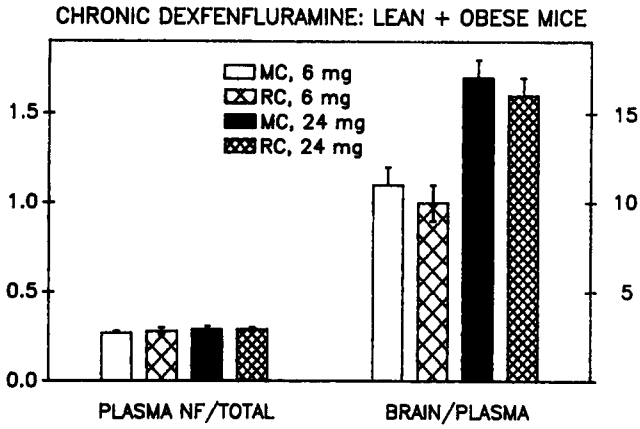

FIG. 6. Left panel: ratio of DNOR/total (DFEN + DNOR) active drug in lean combined with ob/ob mice on day 14 after infusion with either 6 or 24 mg DFEN/kg/day. Right panel: corresponding ratio of levels in brain compared with plasma. Shown are means ± SE for N = 12–14.

6. As expected, plasma levels of both compounds were higher in mice infused with 24 vs. 6 mg DFEN/kg/day, with the ratio near 4 : 1, indicating that metabolic clearance was not markedly different between the two doses. There was a consistent diet effect ($p < 0.01$), with plasma levels 50–100% higher in mice fed the MC diet compared with the RC diet.

The brain concentrations of DFEN and DNOR reflected all of these trends in the plasma concentrations (Fig. 5). The brain concentration (in nanograms per gram) always exceeded the plasma concentration (in nanograms per milliliter), but the ratio was dose-dependent. At the 6 mg DFEN/kg/day dose, the brain concentration averaged 10 times plasma levels, whereas at the 24-mg DFEN/kg/day dose, the ratio was 17 times (Fig. 6).

There was about a threefold range in plasma and brain levels of the agents in individuals within a treatment group. Nonetheless, on day 14, there were significant negative correlations within treated mice between cortical paroxetine binding and brain levels of DFEN ($r[45] = -0.37$, $p < 0.02$), DNOR ($r[45] = -0.44$, $p < 0.01$), and DFEN plus DNOR ($r[45] = -0.40$, $p < 0.01$).

DISCUSSION

These studies were designed to investigate three explanations for the reported insensitivity of mice to the anorectic and 5-HT-depleting effects of DFEN. The first hypothesis, relating to the relatively short half-life of DFEN in mice (4 h), was not tested explicitly because all of our studies used minipump infusions.

Body Weight, Glucose, and Corticosterone

The weight losses that we observed with 6 mg DFEN/kg/day were similar to those with 3 mg DNOR/kg/day and were greater than those reported in ob/ob mice given daily short-term injections of up to 40 mg racemic fenfluramine/kg for 14 days (20). Thus, as is also evident in rats (22), smaller doses of DFEN given by slow delivery to obese mice are as effective as higher doses given quickly. Because the half-life of DNOR is about 2 times that of DFEN in mice (9), the molar potencies of these molecules may not be markedly different in this model.

In Experiment 2, the higher dose of DFEN produced, in obese mice, body weight losses of a few percent comparable to those produced by lower doses in rats (10): weight loss of a few percent was sustained only in obese animals. However, as will be discussed subsequently, the tissue levels of drug at a given dose are lower in mice than rats, so the anorectic and weight loss effect does not seem to differ between species once kinetic differences are taken into consideration.

In Experiment 1, DFEN and DNOR lowered plasma glucose in the obese-hyperglycemic mice, as has previously been reported (20). However, this was not confirmed in Experiment 2. Potential changes in either the physiology of the mice or the diet compositions may have occurred between our two experiments. As expected, in Experiment 1 the ob/ob mice fed the RC diet were hyperglycemic relative to those fed the MC (lower carbohydrate) diet. The reverse occurred in Experiment 2, in which MC-fed ob/ob mice had the highest blood glucose levels. The most hyperglycemic mice developed perigenital irritation and fur loss, presumably as a result of glycosuria. To control this, all mice were given an antibiotic (Tribrissin) in the drinking water and paper bedding (Nestlets) in the cage. None of the RC mice in Experiment 2 developed these lesions.

Plasma corticosterone levels paralleled the glucose measures. In the short-term, DFEN administration causes large (e.g., 10-fold) increases from basal in plasma corticosterone in both rats and mice, and it is obvious from Fig. 2 and Table 2 that such stimulation of the pituitary-adrenal axis showed adaptation or tolerance during the 14-day administration. Partial tolerance was observed in rats given repeated injections of racemic fenfluramine (2), and after 28-day minipump infusion of DFEN when corticosterone was only twofold that of untreated rats (10).

Brain 5-HT Parameters

The second hypothesis advanced in the introduction to this report, that the absence of a 5-HT-depleting effect of DFEN in mice exists because they do not produce large amounts of the putative depleting molecule, DNOR, is not sustained by the results of Experiment 1. The half-life of DNOR is about twice that of DFEN, and so the steady-state levels of DFEN achieved with 6 mg DFEN/kg/day should have been comparable to those of DNOR with 3 mg DNOR/kg/day. Neither agent had significant depleting effects on 5-HT parameters, suggesting that DNOR does not have a markedly greater potency than DFEN. In relation to 5-HT depletion, a similar conclusion was reached in a recent study in rats, in which DFEN/DNOR ratios were manipulated (7).

The first hypothesis, that short tissue exposures after brief injections of DFEN or DNOR are responsible for resistance to long-term 5-HT depletion in mice compared with rats, is not proven by Experiment 2. DFEN (24 mg/kg/day) reduced paroxetine binding, an effect that persisted unchanged for at least 2 weeks after the end of treatment. This contrasts with the rapid (48 h) recovery of 5-HT from a comparable initial depletion after a brief dose of 20 mg/kg (26). Steranka and Sanders-Bush (28) also reported rapid recovery of brain 5-HT levels in mice after racemic fenfluramine administration. These authors also found comparably rapid recovery of 5-HT after short-term injection but not after minipump infusion of *p*-chloroamphetamine, an agent sharing some structural and 5-HT-ergic properties of DFEN (27). This suggests that, independent of short-term depletion, there is a critical duration, as

well as a threshold concentration, of exposure of brain to these agents for long-term 5-HT depletion.

The third hypothesis, that for a given tissue exposure to either DFEN or DNOR, 5-HT neurons in mice are less susceptible to long-term 5-HT-depleting effects than in rats is not sustained by the results of Experiment 2. The combined plasma concentrations of DFEN + DNOR averaged near 0.4 and 1.7 nmol/ml in the mice of either phenotype treated with 6 and 24 mg DFEN/kg per day, respectively. This compares with a corresponding concentration of about 1 nmol/ml in rats infused with 3 mg DFEN/kg per day (26). It can thus be estimated that 4–5 times the daily dosage of DFEN is needed to achieve comparable steady-state plasma levels of DFEN + DNOR in mice compared with rats. This estimate is consistent with known relations between body mass (0.75 exponent) and drug clearance, shown by the greater fractional excretion of DFEN by mice compared with rats (17), and with a previous estimate from short-term studies (28). On these and other (4) grounds, we had expected to find higher levels of DFEN and DNOR in obese compared with lean mice, but this was not the case. This suggests that clearance must have been faster in the obese mice.

Previous studies on pharmacokinetics of brief doses of DFEN showed that mice metabolize DFEN to DNOR relatively slowly, and so may be a better model of the human condition than rats (9). Experiment 2 extends this observation to the long-term condition. The ratio of DFEN to total (DFEN + DNOR) was about 0.7 in all groups and in both brain and plasma. These data contrast with a corresponding brain DFEN : total ratio in rats of 0.29–0.37 after lengthy administration of DFEN (3 mg/kg per day by minipump for 4 weeks) (26). Brain concentrations were 10 and 17 times plasma concentrations in mice given the low and high dosages, respectively. These brain–plasma ratios are intermediate between the ratio 4–5 in rats given 3 mg DFEN/kg per day by minipump (26) and 31–41 in rats given brief injections of 2.5–5 mg DFEN or DNOR/kg (7). Both plasma and brain levels of both DFEN and DNOR were higher in mice fed the MC diet. The functional half-life of DFEN may be increased as a result of lipid binding; it would be interesting to know whether dietary fat likewise affects plasma levels of DFEN in humans.

Paroxetine binding to the cerebral cortical 5-HT uptake carrier varies in rough parallel to 5-HT content (e.g., 25,29). Binding was reduced by the high dose of DFEN (Fig. 5), which may be related to the brain levels of DFEN + DNOR. Thus, MC-fed mice that had the highest plasma and brain levels of the agents had the lowest paroxetine binding. Brain levels (on day 14) of DFEN + DNOR in excess of 5 μg/g (20 μM) seem necessary to produce reliable declines in paroxetine binding. Comparable total brain levels (>5 μM) are needed to produce 5-HT depletion in rats (26). We show that when declines in paroxetine binding occur in mice, they persist for at least 14 days. We do not know the critical duration of treatment for this long-term effect.

In conclusion, mice may be better than rats as models of long-term exposure to DFEN because the biotransformation of DFEN to DNOR is slow in both mice and humans. However, because the half-life for elimination of DFEN is rapid in mice compared with humans, higher doses and long-term administration were needed to make this a suitable model. The doses of DFEN used in this study produced plasma levels of DFEN and DNOR that exceeded by 10–40-fold those in humans receiving a clinical dose (0.5 mg/kg per day). The brain–plasma ratio is a nonlinear function of dose and plasma level, and thus the brain concentrations in mice may be up to 100 times those expected clinically. After accounting for kinetic differences, we conclude there is no evidence for differences in brain effects of DFEN or DNOR in mice compared with rats. If these data are extrapolated to humans, it can be estimated that brain levels of DFEN expected at clinical doses of DFEN should be 10–100 times lower than those needed to produce depletions of brain 5-HT in healthy humans.

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