ELSEVIER

Hormonal Responses to d- and d,l-Fenfluramine in Healthy Human Subjects

Emil F. Coccaro, M.D., Richard J. Kavoussi, M.D., Thomas B. Cooper, M.A., and Richard L. Hauger, M.D.

Two different doses of d-fenfluramine HCl and d,l-fenfluramine HCl (0.5 mg/kg and 1.0 mg/kg) were administered to 11 healthy male volunteers to compare the neuroendocrine responses to these two forms of fenfluramine in human subjects. Prolactin (PRL) responses to d- and d,l-fenfluramine were significantly greater than those to placebo and were equivalent at both dose levels. Adrenocortiatrophic-releasing hormone (ACTH) and cortisol (CORT) responses to d-fenfluramine at both dose levels were also significantly greater than those to placebo. In contrast, the higher dose of d,l-fenfluramine was associated only with a significant CORT response in comparison to placebo. PRL responses to d-fenfluramine were higher than the PRL response to d,l-fenfluramine at either dose level. The PRL response to d-fenfluramine at 0.5 mg/kg was very highly correlated with the PRL responses to d,l-fenfluramine at 1.0 mg/kg ($r = 0.97$, $n = 10$). Homovanillic acid (HVA) were not altered by either d, or d,l-fenfluramine at either dose in a subsample of subjects ($n = 4$). ACTH/CORT responses to d- and d,l-fenfluramine were modestly intercorrelated. These data suggest that the PRL response evoked by d-fenfluramine is quantitatively very similar to that evoked by d,l-fenfluramine. ***[Neuropsychopharmacology 15:595–607, 1996]***

KEY WORDS: *Serotonin; Fenfluramine; Prolactin; ACTH; Cortisol*

The hormonal response to the 5-HT releasing agent/uptake inhibitor fenfluramine has been used as an indicator of central 5-HT system function in humans for more than a decade (Coccaro and Kavoussi 1994). Most studies have been conducted with the racemic formulation that contains approximately equal quantities of fenfluramine's d- and l-isomer. Recently, animal studies suggested that the d- and l-isomers have somewhat different properties and potencies in the serotonergic and catecholaminergic neurotransmitter systems. Specifically, the d-isomer is more potent at 5-HT release and uptake inhibition than the l-isomer (Invernizzi et al. 1986). In addition, the l-isomer has been shown to have clear effects on the central dopaminergic system as evidenced by its ability to increase brain levels of homovanillic acid (HVA), a major metabolite of dopamine (Crunelli et al. 1980). Although the specific mechanism of l-fenfluramine's effect on brain HVA is not clear, some investigators suggest that l-fenfluramine exerts a neurolepticlike action on dopamine receptors (Garattini et al. 1988). Such an action would appear to compromise fenfluramine's ability to assess central 5-HT system function, particularly where the prolactin (PRL) response to fenfluramine is used as the primary outcome variable (van Praag et al. 1987), because dopamine exerts a powerful inhibitory influence on the pituitary lactotrogh (Ben-Jonathan et al. 1989). Accordingly, PRL responses to fenfluramine could be due to both the enhancement in serotonergic function and the inhibi-

From the Department of Psychiatry (EFC, RJK), Clinical Neuroscience Research Unit, Medical College of Pennsylvania, Philadelphia, Pennsylvania; the Department of Psychiatry (TBC), Analytical Psychopharmacology Laboratory, College of Physicians and Surgeons, Columbia University, New York, New York; and, Department of Psychiatry (RLH), Neuroscience Research Laboratory, University of California, San Diego, California.

Address correspondence to: Emil F. Coccaro, M.D., Clinical Neuroscience Research Unit, Department of Psychiatry, Medical College of Pennsylvania and Hahnemann University, 3200 Henry Avenue, Philadelphia, PA 19129.

Received September 12, 1995; accepted May 15, 1996.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/96/$15.00
PII S0893-133X(96)00133-X

tion of the tuberoinfundibular dopamine system that directly regulates PRL secretion.

At least one study in human subjects suggests that l-fenfluramine (as part of the d,l-formulation) is associated with an inhibition of the tuberoinfundibular dopamine system. Mitchell and Smythe (1991) reported that 60 mg of d,l-fenfluramine was associated with a robust increase in plasma levels of HVA. This increase in plasma HVA was temporally correlated with the PRL response to d,l-fenfluramine and was linearly correlated with the maximal PRL response to d,l-fenfluramine challenge. These data were interpreted as evidence that the PRL response to d,l-fenfluramine represented at best a mixed signal reflecting central serotonergic and tuberoinfundibular dopaminergic system function.

In this study, we sought to systematically compare the hormonal responses to d- and d,l-fenfluramine HCl at two dose levels (0.5 mg/kg and 1.0 mg/kg) compared to each other and to placebo. In addition, we examined the plasma HVA response to both formulations of fenfluramine at both dose levels to replicate the work previously published by Mitchell and Smythe (1991).

METHODS

Subjects

Subjects were 11 medically healthy male volunteers recruited by advertisement. Subjects were medically evaluated by history, physical examination, standard laboratory studies (hematology, chemistries, thyroid function, etc.) and ECG. Six subjects had no history of any DSM-III-R Axis I or Axis II disorder. Four had an Axis II personality disorder (Antisocial PD; $n = 2$; Passive-Aggressive PD; $n = 2$; PD-Not Otherwise Specified; $n = 2$; Narcissistic PD; $n = 1$); of these four subjects three had current Axis I disorders (i.e., Social Simple Phobia; Depressive Disorder Not-Otherwise Specified; Adjustment Disorder with depressed mood), and two had a past Axis I disorder (alcoholism; drug abuse). A final subject had no personality disorder but had a past history of an Axis I disorder only (Depressive Disorder-Not Otherwise Specified). The mean age of the sample was 31.7 ± 7.4 years (range, 23–42 years; median, 30 years). Nine subjects completed a placebo challenge and two sets of d- and d,l-fenfluramine challenges. One subject did not complete a placebo challenge, and one other subject did not undergo the d,l-fenfluramine challenge at the 1.0-mg/kg dose.

Fenfluramine Challenges

Subjects were no less than 2 weeks' medication free. No subjects had a history of psychotropic treatment. Subjects were instructed to follow a low-monoamine diet for 3 days prior to each test session. All subjects came to the Clinical Neuroscience Research Unit Procedures Suite at approximately 8:00 A.M. after an overnight fast. An IV was inserted in a forearm vein and kept open by NS TKO. Basal blood samples for PRL were obtained at 9:45 A.M. and at 9:55 A.M. d-Fenfluramine, d,l-fenfluramine, or placebo was given at 10:00 A.M. The doses were 0.0 mg/kg (placebo) 0.5 mg/kg, and 1.0 mg/kg. The order of the placebo or 0.5 mg/kg doses was randomized, but no subject in this study was given the 1.0-mg/kg dose until they had received the 0.5 mg/kg dose. Post-fenfluramine blood samples were obtained every 30 minutes for a total of 5 hours (3:00 P.M.). Samples for plasma HVA were obtained in four (4) subjects at 9:45 A.M., and 9:55 A.M. (basal levels) and every 60 minutes thereafter for 5 hours (3:00 P.M.). Prolactin (Diagnostic Products Corporation, Los Angeles, CA), adrenocorticotrophic-releasing hormone (ACTH); Nichols Institute Diagnostics, San Juan Capistrano, CA), and CORT (Diagnostic Products Corporation; Los Angeles, CA) were assayed by RIA. Intraassay CV's for the PRL kit range from 4.4% (at 9 ng/ml) to 2.8% at (43 ng/ml); for the ACTH kit, it ranged from 3% (at 35 pg/ml) to 3.2% (at 366 pg/ml); for the CORT kit, it ranged from 3.5% (at 7.7 μg/dl) to 4.5% (at 32.5 μg/dl). Interassay CVs for the PRL kit range from 8.6% (at 7 ng/ml) to 7.6% at (45 ng/ml); from 3.0% (at 35 pg/ml) to 3.2% (at 366 pg/ml); for the ACTH kit and from 7.8% (at 36 pg/ml) to 6.8% (at 358 pg/ml) for the CORT kit range. Plasma HVA was assayed by GC/MS (Hunneman 1983), with an intra–interassay CV of 6% and 8%, respectively. Plasma levels of fenfluramine and norfenfluramine were determined by gas chromatography with electrochemical detection (Krebs et al. 1984), with an intra- and interassay CV lower than 7%. Challenges with d- or d,l-fenfluramine were separated by no less than 5 days. The mean interval between d-fenfluramine challenges was 15.5 ± 6.7 days; the mean interval between d,l-fenfluramine challenges was 11.4 ± 3.8 days; and the mean interval between full sets of d- and d,l-fenfluramine challenges = 27.7 ± 20.4 days). Six subjects underwent the d-fenfluramine challenge sets first, and five underwent the d,l-fenfluramine challenge sets first.

Statistical Analysis

No significant differences in any hormonal or plasma fenfluramine (FEN) level variable were noted between the six healthy volunteers (i.e., no Axis I or II disorder) and the five psychiatric (i.e., positive history of Axis I and/or II disorder) volunteers; accordingly, data from these two groups were combined for further analysis. A multifactorial repeated-measures analysis of variance (RM-ANOVA) with condition (placebo, d-FEN 0.5 mg/kg, d-FEN 1.0 mg/kg, d,l-FEN 0.5 mg/kg, and d,l-FEN 1.0 mg/kg), time, and sequence (d-FEN first vs. d,l-FEN first) was used first to analyze PRL, ACTH, CORT, and

HVA levels over time. A second multifactorial RM-ANOVA with (0.5 mg/kg vs. 1.0-mg/kg doses), isomeric form (d-FEN vs. d,l-FEN), time, and sequence (d-FEN first vs. d,l-FEN first) was performed next. Subsequent multifactorial RM-ANOVAs were then performed to determine the presence or absence of differences between the various conditions. For analyses involving peak delta PRL(FEN), ACTH(FEN), CORT(FEN) values, hormonal levels at −15 and −5 minutes were averaged to determine a single baseline value to use with postfenfluramine hormonal levels to calculate a peak delta hormonal value ("peak post-FEN value" minus "averaged baseline value"). Area under the curve (AUC) values were estimated by the trapezoid rule. Correlations were performed by Pearson correlation. All the alpha probability values reported are two-tailed.

RESULTS

Plasma Fenfluramine Concentrations at the Two Dose Levels

As expected, plasma fenfluramine and norfenfluramine (FEN + NorFEN) concentrations over time were significantly higher in subjects undergoing the 1.0-mg/kg, compared to 0.5-mg/kg, protocol (Figure 1). Multifactorial RM-ANOVA revealed a significant main effect for dose [$F(1,8) = 120.87$, $p < .001$], time [$F(2,16) = 240.94$, $p < .001$], and for the dose by time interaction [$F(2,16) = 54.46$, $p < .001$]. There was no significant effect as a function of isomer [d vs. d,l:$F(1,8) = 0.01$, $p = .92$] and no significant isomer by dose [$F(1,8) = 0.82$, $p = .392$], isomer by time [$F(2,16) = 0.78$, $p = .476$], or isomer by dose by time [$F(2,16) = 0.45$, $p = .643$] interaction. Sequence was not a significant factor by itself [$F(1,8) = 0.60$, $p = .459$], although there was a trend for a sequence by isomer by dose by time interaction [$F(2,16) = 3.29$, $p = .063$] whereby subjects who underwent d-FEN challenges after d,l-FEN challenges had slightly higher total FEN/Nor-FEN plasma levels over time than those who underwent d-FEN challenges first.

There were no significant differences in peak total FEN/Nor-FEN plasma levels between d- and d,l-, at the 0.5-mg/kg dose (e.g., peak total d-FEN/Nor-FEN = 37.5 ± 7.6 ng/ml; peak total d,l-FEN/Nor-FEN = 41.8 ± 5.7 ng/ml; p = ns) or the 1.0-mg/kg dose (e.g., peak total d-FEN/Nor-FEN = 83.5 ± 17.6 ng/ml; peak total d,l-FEN/Nor-FEN = 81.3 ± 16.2 ng/ml; p = ns). Peak total FEN/Nor-FEN plasma concentrations at the 0.5-mg/kg dose level for d- and d,l-FEN were highly correlated ($r = 0.75$, $n = 11$, $p < .01$); the corresponding correlation for d- and d,l-FEN at the 1.0-mg/kg dose level was not statistically significant ($r = 0.32$, $n = 10$, p = ns).

Prolactin Response to d- and d,l-Fenfluramine

PRL responses over time for the placebo condition and for the two doses of each fenfluramine isomer are displayed in Figure 2. Multifactorial RM-ANOVA revealed a significant main effect for condition [$F(4,28) = 11.68$, $p < .001$], time [$F(11,77) = 13.34$, $p < .001$], and for the condition by time interaction [$F(44,308) = 5.49$, $p < .001$]. There was no effect for sequence by itself [$F(1,7) = 0.46$, $p = .518$] or for the sequence by condition by time interaction [$F(44,308) = 1.25$, $p = .141$]. Subsequent RM-ANOVA analyses revealed a significant ef-

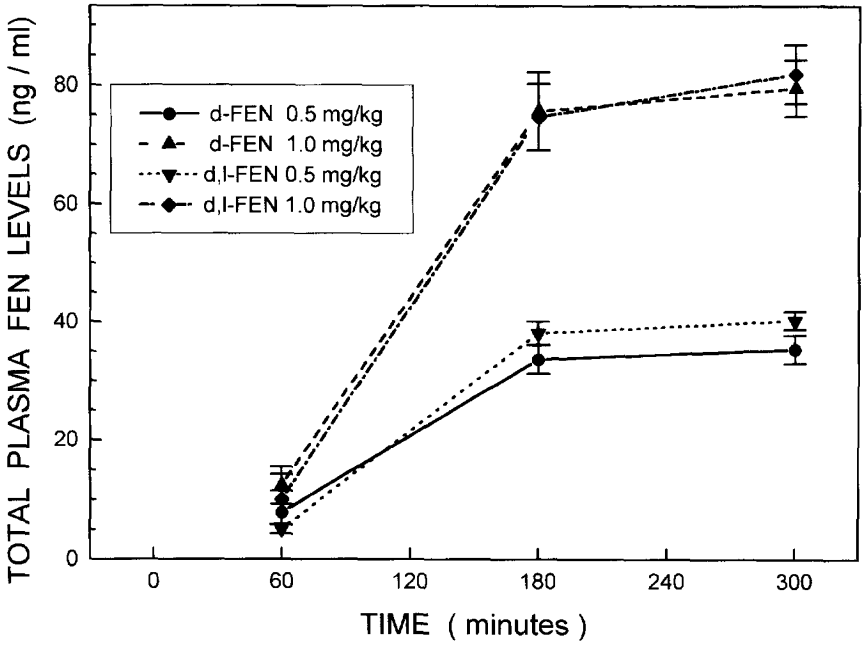

Figure 1. Mean (±SEM) plasma total (FEN + Nor-FEN) d-FEN and d,l-FEN levels over time in the 11 healthy human subjects.

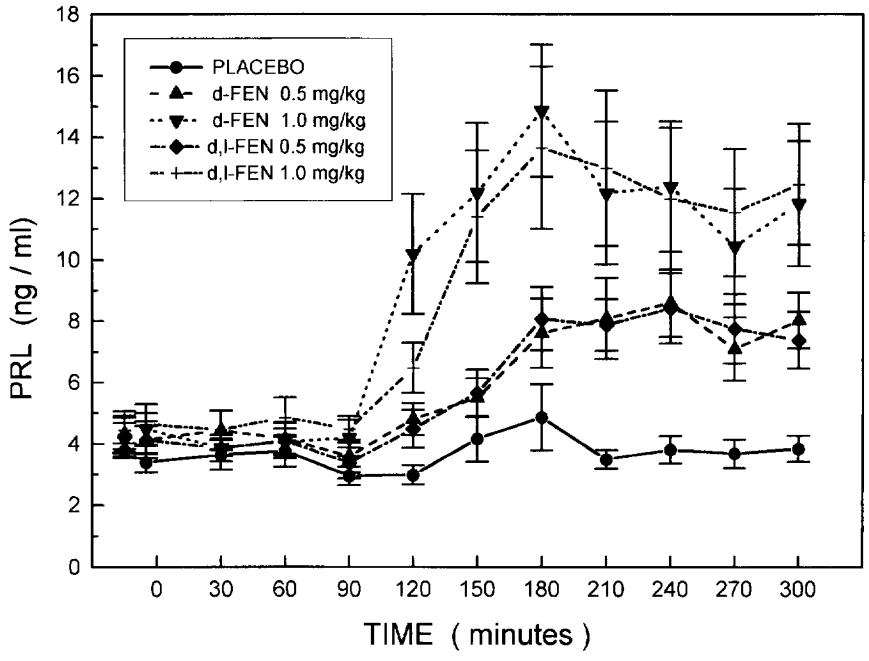

Figure 2. Mean (±SEM) plasma prolactin responses over time to placebo, d,l-fenfluramine, and d-fenfluramine in the 11 healthy human subjects.

fect of condition, time, and dose by time interaction for both doses of d-, and d,l-FEN compared with placebo, indicating that both doses of d-, or d,l-FEN increased PRL(FEN) levels over time more than placebo. There were no significant effects for sequence alone or for the interaction of sequence by condition by time for any of these comparisons. Among the drug conditions, specifically, there was a significant effect for dose [$F(1,8) = 13.83$, $p = .006$], time [$F(11,88) = 14.54$, $p < .001$], and for the dose by time interaction [$F(11,88) = 8.47$, $p < .001$]. There was no effect for isomer [$F(1,8) = 0.08$, $p = .785$], isomer by time [$F(1.33) = 1.33$, $p = .220$], or an isomer by dose by time interaction [$F(11,88) = 1.09$, $p = .376$]. There was no effect for sequence [$F(1,8) = 0.31$, $p = .593$] either or for its interaction with dose by isomer by time [$F(11,88) = 0.56$, $p = .855$]. This indicates that d-FEN elicited PRL(FEN) responses equivalent to those of d,l-FEN at both dose levels.

Mean peak delta prolactive responses to d- and d,l-fenfluramine and placebo are displayed in Figure 3. These data yield similar results as PRL(FEN) over time data. An RM-ANOVA revealed a significant main effect for condition [$F(4,28) = 18.56$, $p < .001$] but not for sequence [$F(1,7) = 1.01$, $p = .349$] or for the condition by sequence interaction [$F(4,28) = 4.28$, $p = .129$]. This was accounted for by higher PRL(FEN) responses for both d-FEN and d,l-FEN at both doses than for placebo. Among the drug conditions specifically peak delta PRL(FEN) data revealed a significant effect for dose [$F(1,8) = 30.22$, $p < .001$] but no effect for isomer [$F(1,8) = 0.00$, $p = .995$], for sequence [$F(1,8) = 1.89$, $p = .206$], or for the dose by isomer [$F(1,8) = 0.42$, $p = .534$] or the dose by isomer by sequence interaction [$F(1,8) = 0.02$, $p = .893$]. Peak delta PRL(FEN) responses for both the d-FEN 0.5-mg/kg and 1.0-mg/kg dose were highly correlated with their respective AUC PRL(d-FEN) responses (0.5 mg/kg: $r = .99$, $n = 11$, $p < .001$; 1.0 mg/kg: $r = .95$, $n = 11$, $p < .001$). Peak delta PRL[FEN] responses for both the d,l-FEN 0.5-mg/kg and 1.0-mg/kg dose were also highly correlated with their respective AUC PRL(d,l-FEN) responses (0.5 mg/kg: $r = .88$, $n = 11$, $p < .001$; 1.0 mg/kg: .95, $n = 10$, $p < .001$). Accordingly, analyses involving AUC PRL(FEN) values yielded similar results as those involving peak delta values (Figure 3).

ACTH Response to d- and d,l-Fenfluramine

ACTH responses over time for the placebo condition and for the two doses of each fenfluramine isomer are displayed in Figure 4. Multifactorial RM-ANOVA revealed a significant main effect for condition [$F(4,28) = 7.18$, $p < .001$], time [$F(11,77) = 7.50$, $p < .001$] and for the condition by time interaction [$F(44,308) = 3.35$, $p < .001$]. There was no effect for sequence by itself [$F(1,7) = 0.07$, $p = .793$] or for an interaction between sequence by condition by time [$F(44,308) = 0.72$, $p = .911$]. Subsequent RM-ANOVA analyses revealed a significant effect of condition, time, and condition by time interaction for d-FEN at both doses compared with placebo [e.g., condition by time: 0.5 mg/kg: $F(11,88) = 4.43$, $p < .001$; 1.0 mg/kg: $F(11,88) = 5.49$, $p < .001$]. For d,l-FEN, ACTH(FEN) responses over time were not significantly different from those to placebo for the 0.5-mg/kg dose, but they were significantly different for the d,l-FEN 1.0-

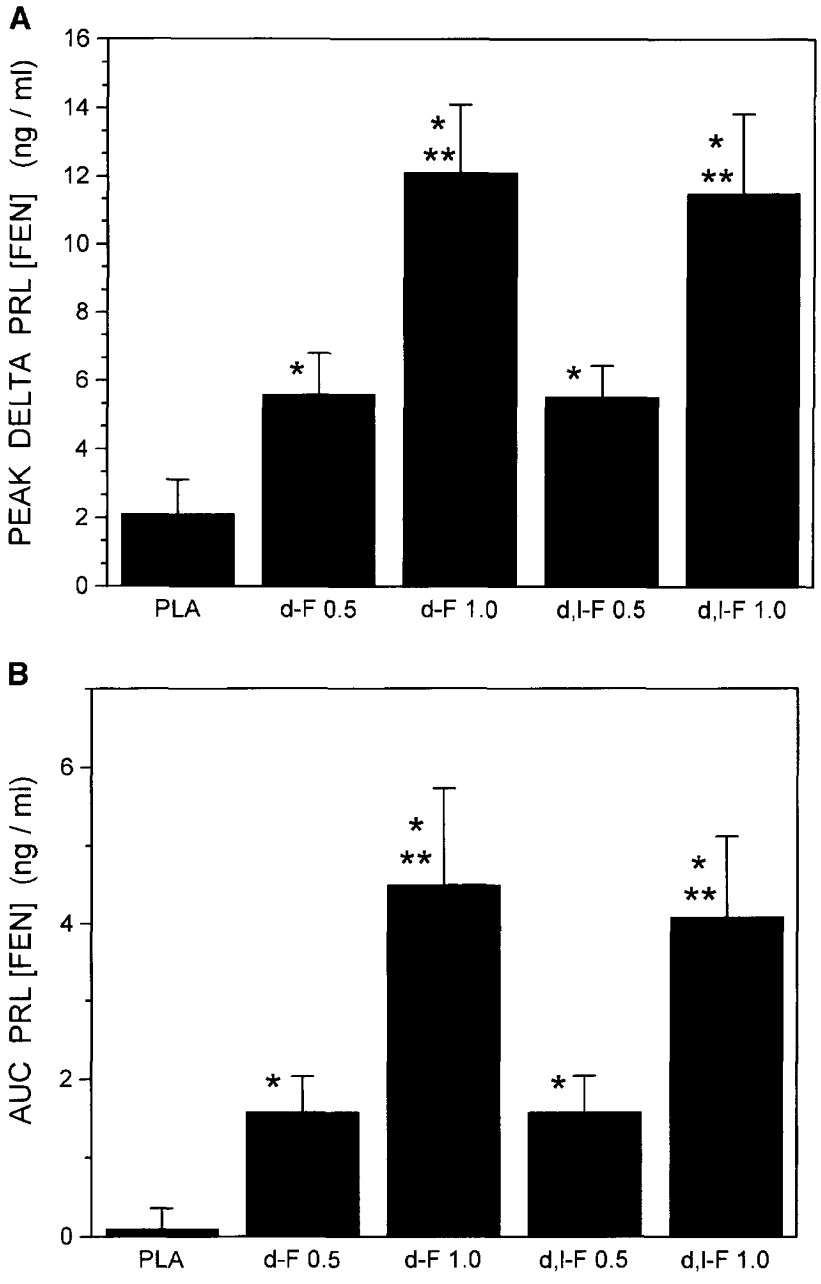

Figure 3. Mean (±SEM) peak delta (**A**) and AUC (**B**) prolactin responses over time to placebo, d,l-fenfluramine, and d-fenfluramine in the 11 healthy human subjects. *, response greater for drug condition ($p < .05$) than for placebo condition; **, response for 1.0-mg/kg dose greater ($p < .05$) than for 0.5-mg/kg dose.

mg/kg dose at least for the condition by time interaction [$F(11,77) = 2.57$, $p = .008$]. There were no significant effects for sequence alone or for the interaction of sequence by condition by time for any of these comparisons. Among the drug conditions, specifically, there was a significant effect for dose [$F(1,8) = 6.22$, $p = .037$], time [$F(11,88) = 8.96$, $p < .001$], and for the dose by time [$F(11,88) = 3.51$, $p < .001$] interaction. In contrast to PRL(FEN) responses, there was a significant effect for isomer [$F(1,8) = 11.71$, $p = .009$], isomer by time [$F(11,88) = 5.70$, $p < .001$], but not for the isomer by dose by time interaction [$F(11,88) = 1.12$, $p = .358$]. This was due to significant ACTH(FEN) response to d-FEN at both doses with a nonsignificant ACTH(FEN) response to d,l-FEN at the 0.5-mg/kg dose. There was no effect for sequence [$F(1,8) = 0.52$, $p = .493$] or for its interaction with dose by isomer by time [$F(11,88) = 0.37$, $p = .965$].

Mean peak delta ACTH responses to d- and d,l-fenfluramine and placebo are displayed in Figure 5. These data yield similar results as the ACTH(FEN) over time data. An RM-ANOVA revealed a significant main effect for condition [$F(4,28) = 7.77$, $p < .001$] but not for sequence [$F(1,7) = 0.35$, $p = .571$] or the condition by sequence interaction [$F(4,28) = 0.11$, $p = .979$]. This was accounted for by higher peak delta ACTH(FEN) re-

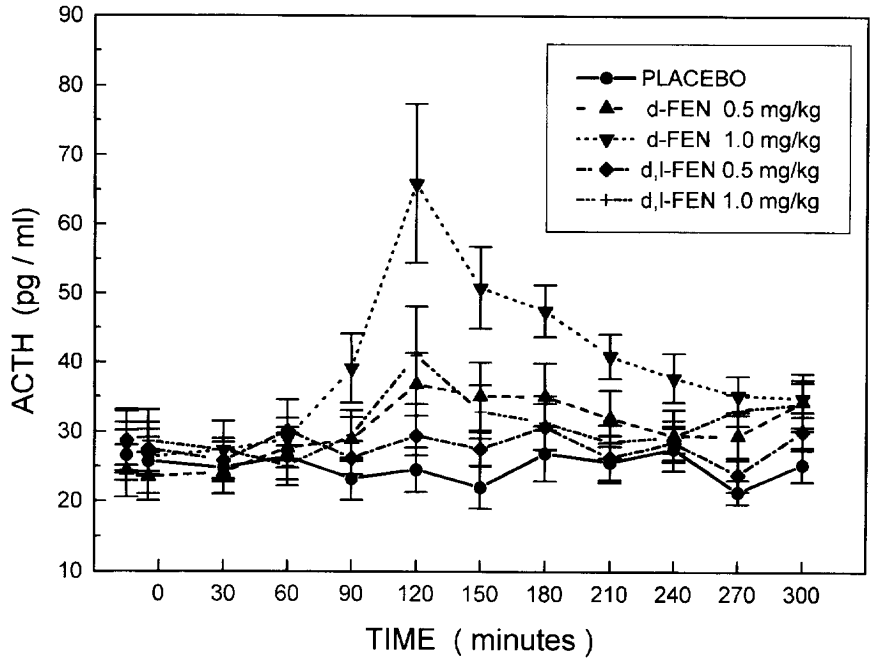

Figure 4. Mean (±SEM) plasma ACTH responses over time to placebo, d,l-fenfluramine, and d-fenfluramine in the 11 healthy human subjects.

sponses for the 1.0-mg/kg dose level for both d-FEN [$F(1,8) = 14.17, p = .006$] and d,l-FEN [$F(1,7) = 6.77, p = .035$] than for placebo; peak delta ACTH(FEN) responses at the 0.5-mg/kg dose level for either d-, or d,l-FEN were not significantly different from placebo responses. Among the drug conditions, specifically peak delta ACTH(FEN), data revealed a significant effect for dose [$F(1,8) = 18.02, p = .003$] with only a trend for an effect of isomer [$F(1,8) = 4.38, p = .070$] or for a dose by isomer interaction [$F(1,8) = 3.45, p = .082$]. These findings were largely accounted for by the higher peak delta ACTH(FEN) responses for a d-FEN 1.0-mg/kg dose than for the other drug conditions. Peak delta ACTH(FEN) responses for the 0.5-mg/kg dose for both d-FEN and d,l-FEN were correlated with their respective AUC ACTH(d-FEN) responses (d-FEN: $r = .73, n = 11, p = .011$; d,dl-FEN: $r = .70, n = 11, p = .016$). Unlike PRL(FEN) responses, there were no significant correlations between peak delta ACTH(FEN) and AUC ACTH(FEN) values for either isomeric form for the 1.0-mg/kg dose (d-FEN: $r = .48, n = 11, p = .139$, d,l-FEN: $r = .22, n = 10, p = .538$). Analyses of AUC ACTH(FEN) responses revealed a significant main effect for condition [$F(4,28) = 6.33, p = .001$] with no significant effect for sequence [$F(1,7) = 1.47$] or condition by sequence interaction [$F(4,28) = 0.48, p = .748$]. This was due to larger AUC ACTH(FEN) responses to d-FEN at both 0.5 mg/kg [$F(1,8) = 11.72, p = .009$] and 1.0 mg/kg [$F(1,8) = 23.40, p < .001$], but not d,l-FEN at either dose. Among the drug conditions, specifically, there was a trend for an effect of dose [$F(1,8) = 5.22, p = .052$], a significant effect for isomer [$F(1,8) = 12.98, p = .007$], but not for a dose by isomer interaction [$F(1,8) = 0.58, p = .469$]. This was due to larger AUC ACTH(FEN) responses to d-FEN at both dose levels (Figure 5).

Cortisol Response to d- and d,l-Fenfluramine

Cortisol responses over time for the placebo condition and for the two doses of each fenfluramine isomer are displayed in Figure 6. A multifactorial RM-ANOVA revealed a significant main effect for condition [$F(4,28) = 9.63, p < .001$], time [$F(11,77) = 5.63, p < .001$], and for the condition by time interaction [$F(44,308) = 4.40, p < .001$]. There was no effect for sequence by itself [$F(1,7) = 2.32, p = .171$] or for an interaction of sequence by condition by time [$F(44,308) = 1.29, p = .111$]. Subsequent RM-ANOVA analyses revealed a significant or near significant effect of dose and time for all conditions compared with placebo and for the dose by time interaction for d-FEN at both the 0.5-mg/kg [$F(11,88) = 2.91, p = .003$] and 1.0-mg/kg [$F(11,88) = 6.29, p < .001$] dose levels and for d,l-FEN only at the 1.0-mg/kg dose level [$F(11,77) = 3.28, p = .001$]. There were no significant effects for sequence alone or for the interaction of sequence by condition by time for any of these comparisons. Among the drug conditions, specifically, there was a significant effect for dose [$F(1,8) = 26.99, p < .001$], time [$F(11,88) = 6.32, p < .001$], and for dose by time [$F(11,88) = 5.99, p < .001$] and significant, or near significant, effects for isomer [$F(1,8) = 3.57, p = .095$], isomer by time [$F(11,88) = 2.15, p = .024$], or isomer by dose by time [$F(11,88) = 2.27, p = .017$]. This was due to significantly greater CORT(FEN) responses for d-FEN

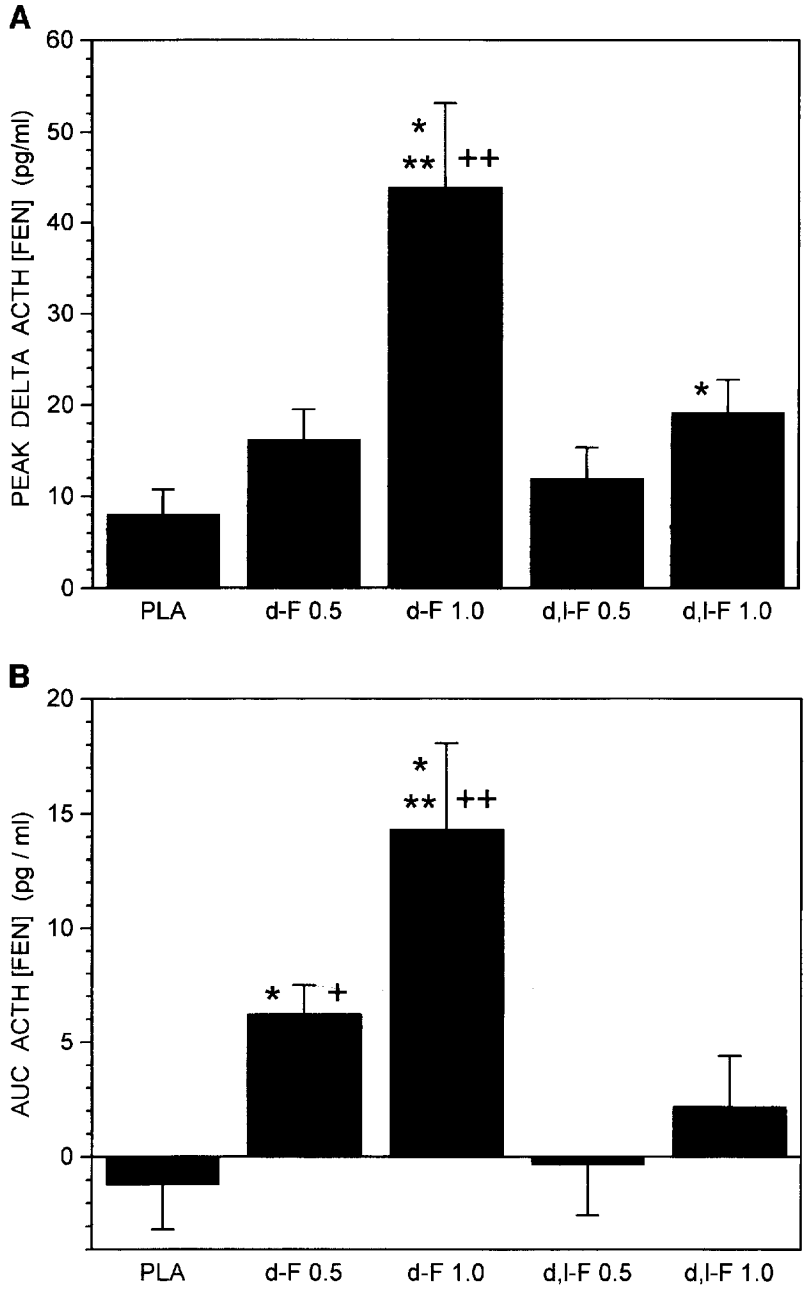

Figure 5. Mean (±SEM) peak delta (**A**) AUC (**B**) ACTH responses over time to placebo, d,l-fenfluramine, and d-fenfluramine in the 11 healthy human subjects. *, Response for the drug condition greater ($p < .05$) than for the placebo condition; **, Response for the 1.0-mg/kg dose greater ($p = .051$) than for the 0.5-mg/kg dose; +, Response for d-FEN greater ($p < .05$) than for d,l-FEN at a 0.5-mg/kg dose; ++, Response for d-FEN greater ($p = .054$) than for d,l-FEN at a 1.0-mg/kg dose.

(both doses) and d,l-FEN at the 1.0-mg/kg dose only. There was no effect for sequence itself or for its interaction with dose, isomer, or time individually, but there was a significant sequence by dose by isomer by time interaction [$F(11,88) = 2.49$, $p = .009$]. This appeared to be due to a somewhat higher CORT(FEN) response over time to d-FEN 0.5 mg/kg in subjects who underwent d-FEN studies after undergoing d,l-FEN studies first.

Mean peak delta corticol responses to d- and d,l-fenfluramine and placebo are displayed in Figure 7. These data yield similar results as the CORT(FEN) over time data. An RM-ANOVA revealed a significant main effect for condition [$F(4,28) = 11.38$, $p < .001$] but not for sequence [$F(1,8) = 0.14$, $p = .720$] or the condition by sequence interaction [$F(4,28) = 0.66$, $p = .628$]. This was accounted for by higher peak delta CORT(FEN) responses for both d-FEN [$F(1,8) = 21.91$, $p = .002$] and d,l-FEN [$F(1,7) = 7.41$, $p = .030$] at the 1.0-mg/kg dose than for placebo; peak delta CORT(FEN) responses at the 0.5-mg/kg dose level, for either d- or d,l-FEN, were not significantly different from placebo responses. Among the drug conditions, specifically, peak delta CORT(FEN) data revealed a significant effect for dose [$F(1,8) = 10.40$, $p < .012$], a significant effect for isomer [$F(1,8) = 47.92$, $p < .001$], but not for sequence [$F(1,8) = 0.03$, $p = .869$],

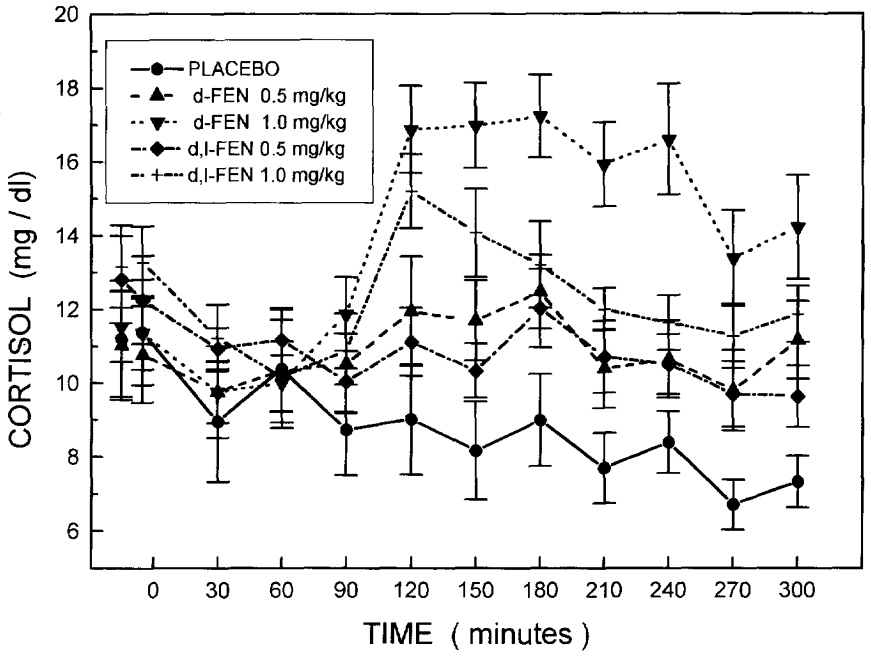

Figure 6. Mean (±SEM) plasma cortisol responses over time to placebo, d,l-fenfluramine, and d-fenfluramine in the 11 healthy human subjects.

the dose by isomer interaction [$F(1,8) = 1.77$, $p = .220$], the isomer by sequence interaction [$F(1,8) = 2.88$, $p = .128$], or the dose by isomer by sequence interactions [$F(1,8) = 2.00$, $p = .195$]. Overall, peak delta CORT(FEN) responses resembled those of peak delta ACTH(FEN) responses. d-FEN produced significantly greater peak delta CORT(FEN) responses than d,l-FEN at both doses, and only the 1.0-mg/kg dose of d- or d,l-FEN elicited peak delta CORT[FEN] responses significantly greater than for placebo. Peak delta CORT(FEN) responses for both the d-FEN 0.5-mg/kg and 1.0-mg/kg dose were highly correlated with their respective AUC CORT(d-FEN) responses (0.5 mg/kg: $r = .99$, $n = 11$, $p < .001$; 1.0 mg/kg: $r = .95$, $n = 11$, $p < .001$). Similarly, peak delta CORT(FEN) responses for both the d,l-FEN 0.5-mg/kg and 1.0-mg/kg dose were highly correlated with their respective AUC CORT(d,l-FEN) responses (0.5 mg/kg: $r = .88$, $n = 11$, $p < .001$; 1.0 mg/kg: .95, $n = 10$, $p < .001$). Accordingly, analyses involving AUC CORT(FEN) values yielded similar results as those involving peak delta values (Figure 7).

Plasma HVA Response to d- and d,l-Fenfluramine

Plasma HVA levels before and after fenfluramine/placebo challenge over time for placebo, 0.5 mg/kg; and 1.0 mg/kg in four of the subjects are displayed in Figure 8. A multifactorial RM-ANOVA revealed a main effect for time [$F(6,12) = 6.16$, $p = .004$) But no significant effect for condition [$F(4,8) = 0.66$, $p = .635$], sequence [$F(1,2) = 8.60$, $p = .099$], or for the condition by time [$F(24,48) = 1.27$, $p = .238$] or for the condition by time by sequence [$F(24,48) = 0.53$, $p = .954$) interactions.

Correlations between Body Weight, Total Fenfluramine Levels, and Hormonal Responses to d- and d,l-Fenfluramine

There were no significant correlations between body weight and peak delta (PRL(FEN), ACTH(FEN), or CORT(FEN) responses to or between body weight and total FEN/Nor-FEN plasma levels for either dose of d-FEN or d,l-FEN. There was no significant correlation either between total FEN/Nor-FEN plasma levels and peak delta PRL(FEN), ACTH(FEN), or CORT(FEN) responses to either dose of d-FEN or d,l-FEN.

Correlations between Hormonal Responses to d- and d,l-Fenfluramine

Peak delta PRL responses to d- and d,l-fenfluramine were positively correlated at both the 0.5-mg/kg ($r = .57$, $n = 11$, $p < .07$) and the 1.0-mg/kg ($r = .77$, $n = 10$, $p < .01$) dose. The difference in the magnitude of these correlations at the two doses may have been due to an increased intrasubject variability in PRL(FEN) response associated with a prolonged interval between challenges in one subject in whom d- and d,l-fenfluramine challenges at the 0.5-mg/kg dose level were separated more than 3 months (i.e., performed during different seasons of the year). The correlation among the remaining subjects at the 0.5-mg/kg dose level was similar to that observed at the 1.0-mg/kg dose level ($r = .69$, $n = 10$, $p < .05$). The correlation between the peak delta prolactin response to d-fenfluramine at the 0.5-mg/kg dose level (the dose of d-fenfluramine most often used) and

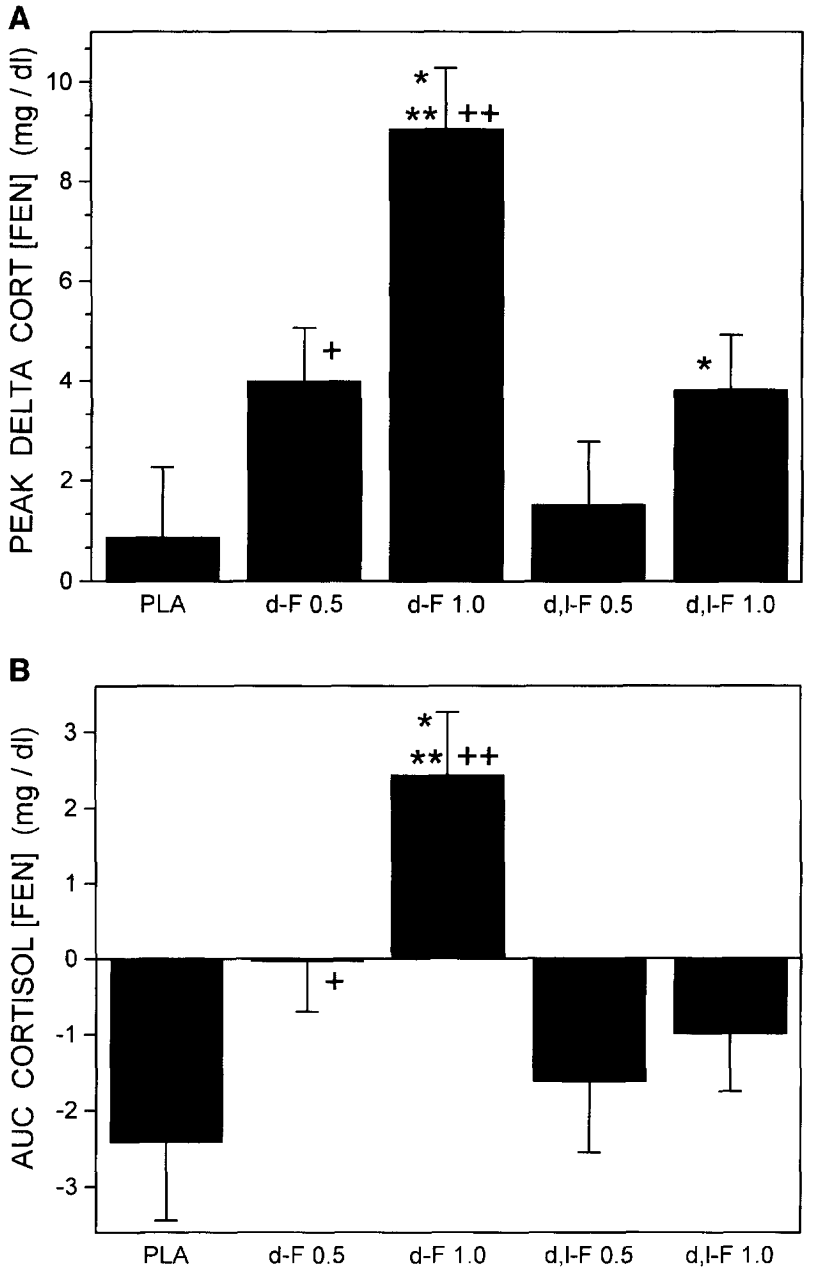

Figure 7. Mean (±SEM) peak delta (**A**) and AUC (**B**) cortisol responses over time to placebo, d,l-fenfluramine, and d-fenfluramine in the 11 healthy human subjects. *, Response for the drug condition greater ($p < .05$) than for the placebo condition; **, Response for the 1.0-mg/kg dose greater ($p < .05$) than for the 0.5-mg/kg dose; +, Response for d-FEN greater ($p < .05$) than for d,l-FEN at 0.5-mg/kg dose; ++, Response for d-FEN greater ($p < .05$) than for d,l-FEN at a 1.0-mg/kg dose.

to that of d,l-fenfluramine at the 1.0-mg/kg dose level (the dose of d,l-fenfluramine most often used), however, was extremely high: $r = .97$, $n = 10$, $p < .001$ (Figure 9).

Peak data ACTH and CORT responses to d- and d,l-fenfluramine were poorly correlated in general. The most relevant correlations for peak delta ACTH(FEN) were as follows: $r = .52$ ($p = .104$ for the 0.5-mg/kg dose); $r = -.02$ (p = NS for the 1.0-mg/kg dose); $r = .14$ (p = NS for the 0.5-mg/kg d-FEN/1.0-mg/kg d,l-FEN correlation). The most relevant correlations for peak delta CORT(FEN) were as follows: $r = 0.16$ (p = NS for the 0.5-mg/kg dose); $r = .43$ ($p = .189$ for the 1.0-mg/kg dose); $r = -.42$ (p = NS for the 0.5-mg/kg d-FEN/1.0-mg/kg d,l-FEN correlation). The lack of intercorrelation between d- and d,l-FEN, with respect to ACTH or CORT at either dose, is probably due to the fact that d,l-fenfluramine was not associated with a significant ACTH response at either dose and had a significant CORT response to FEN only at the 1.0-mg/kg dose.

DISCUSSION

These data demonstrate that prolactin responses to equiweighted doses of d- and d,l-fenfluramine are essentially identical even when accounting for any potential ordering effect of receiving d-FEN before or after

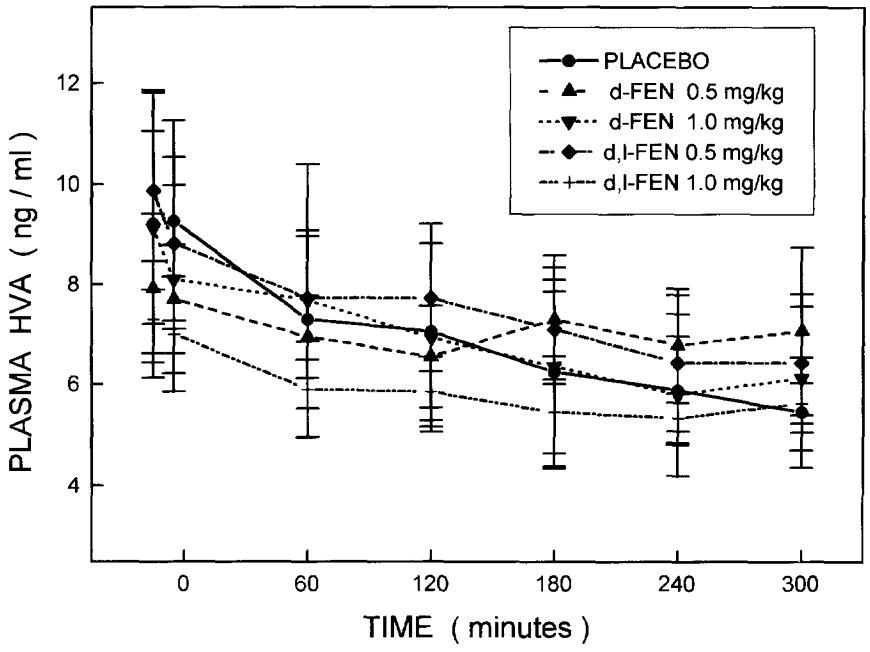

Figure 8. Mean (±SEM) plasma homovanillic acid (HVA) responses over time to placebo, d,l-fenfluramine, and d-fenfluramine in four healthy human subjects.

d,l-FEN. First, the time plots of these responses for two different doses were indistinguishable. Second, the mean peak delta PRL(FEN) values for two different doses were indistiguishable and intercorrelated. Of greatest interest, the correlation between the PRL(FEN) responses to d-FEN at 0.5 mg/kg and d,l-FEN at 1.0 mg/kg nearly reached unity. The significance of the latter observation is that there should be good agreement between data obtained with d-FEN at its usual dose of 0.5 mg/kg (O'Keane and Dinan 1991; O'Keane et al. 1992; Gorard et al. 1993) and data obtained with d,l-FEN at the dose close to that used in clinical studies (i.e., 60 mg: 0.8 to 1.0 mg/kg depending on body weight of the subject). Because PRL(d,l-FEN) responses at 1.0 mg/kg were quantitatively higher than those of PRL(d-FEN) at 0.5 mg/kg, data obtained with the d,l-FEN at 1.0 mg/kg (or 60 mg) cannot simply be combined with data obtained with d-FEN at 0.5 mg/kg. However, it is unlikely that differences in PRL(FEN) response data obtained with d-FEN at 0.5 mg/kg and d,l-FEN at 60 mg can be ascribed to putative pharmacological differences between d-FEN and d,l-FEN.

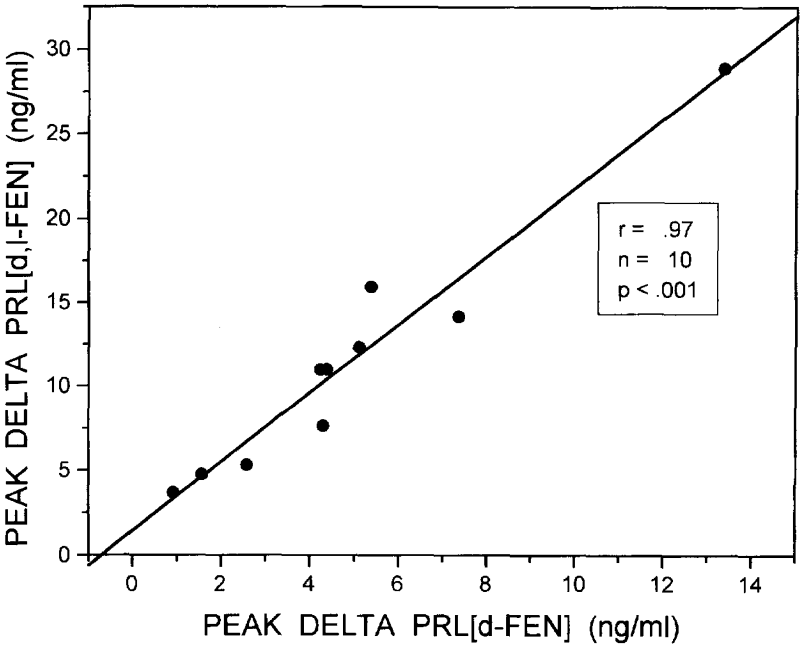

Figure 9. Correlation between peak delta prolactin response to d-fenfluramine at 0.5 mg/kg with peak delta prolactin response to d,l-fenfluramine at 1.0 mg/kg in 10 healthy human subjects.

These data raise the question of why d-fenfluramine challenge should be dosed at 0.5 mg/kg (or 30 mg) rather than at 1.0 mg/kg (or 60 mg), as is d,l-fenfluramine. As d- and d,l- forms of fenfluramine yield essentially PRL(FEN) responses, it would appear that d-FEN should be dosed in the same manner as d,l-FEN. However, in other studies with d-FEN challenge, we found that the behavioral response to d-FEN at 1.0 mg/kg Po was intolerable when the subject had not been previously exposed to d-FEN 0.5 mg/kg, as they had in this study. Three out of three d-FEN-naive subjects given a d-FEN dose of 1.0 mg/kg experienced adverse behavioral reactions, each commencing 90 minutes after ingestion of d-FEN. The reactions were as follows: subject #1, panic attack and dysphoria; subject #2, dysphoria, irritability, paranoid ideation; subject #3, dysphoria. None of these reactions were reported or observed in these subjects when a repeat of d-FEN dose of 0.5 mg/kg was given at a later date (4–12 weeks later). Moreover, no significant adverse behavioral reactions were observed in any of the subjects in the present study where the lower dose of d-FEN preceded the higher dose.

Despite the fact that the d-stereoisomer comprises no more than 50% of the d,l-FEN administered, it appears that the l-isomer is equipotent in its ability to evoke a PRL response. In vitro data suggest that the l-isomer is less potent than the d-isomer as a 5-HT releaser and as a 5-HT uptake inhibitor (Garattini et al. 1987), but it is important to note that these differences are of less than an order of magnitude and thus are not relevant in vivo. On the other hand, it is possible that a modest, but important, contribution of dopamine receptor antagonism, which would act to increase PRL levels at the level of the pituitary, could also be present and lead to the appearance of equipotency on PRL responses for the d- and l-forms of fenfluramine.

The possibility of an important in vivo contribution of dopaminergic antagonism of l-fenfluramine to the PRL response to d,l-FEN is also suggested by data from Mitchell and Smythe (1991) that demonstrated robust increases in plasma levels of HVA, covarying with PRL responses, in healthy volunteers undergoing d,l-FEN challenge (60 mg Po). In contrast to those data, we did not find a robust plasma HVA response to d- or d,l-FEN at either dose. Although our subsample with plasma HVA data was small, there was little evidence of an increase or decrease in plasma HVA levels to fenfluramine in any of the four subjects at either dose of d- or d,l-FEN. Moreover, one other study of the effect of d,l-FEN challenge on plasma HVA response in healthy subjects, reports no change in plasma HVA levels (Hollander et al. 1992). Our own plasma HVA data in a larger sample with an additional eight subjects undergoing d-FEN challenge at the 0.5-mg/kg dose does not demonstrate any of d-FEN effect on plasma HVA levels in comparison to placebo either (Coccaro et al. unpublished data). Although this seems to argue against an important role for dopaminergic antagonism in the PRL response to d,l-FEN, it is possible that plasma HVA may not be sufficiently sensitive an index to detect small, but important, effects of dopaminergic antagonism at the level of the pituitary. In the rat, for example, the increase in brain levels of total and free HVA after treatment with a dopamine antagonist (e.g., haloperidol) ranges from 50% (total HVA) to 90% (free HVA) greater than the corresponding increase seen in the plasma (Kendler et al. 1981). Thus, plasma HVA data may only be able to rule in, but not rule out, a role of dopaminergic antagonism in the case of the PRL response to d,l-FEN. On the other hand, the high correlation between PRL responses to d- and d,l-FEN challenge would seem to suggest that if there is a contribution of dopaminergic antagonism to the PRL response to l-fenfluramine, it is very small.

In fact, only indirect evidence is available to rule out (or in) the in vivo role of dopaminergic antagonism for the l-isomer component of d,l-FEN. Recently, we reported the absence of a correlation between the PRL response to d,l-FEN and to TRH in personality-disordered subjects (Coccaro et al. 1994). Because the PRL response to TRH appears to be mediated through dopaminergic pathways (Ghigo et al 1985), these data suggested that dopaminergic tone at the level of the pituitary was not involved in the PRL response to d,l-FEN. Hence, even if the putative antidopaminergic activity of l-fenfluramine is not significantly associated with PRL release, it is possible that even a small contribution of dopaminergic antagonism may be important in other ways in regions of the brain not related to the PRL response to fenfluramine. Thus, these data should not be interpreted to mean that d- and d,l-fenfluramine are equivalent in every respect. For example, d-fenfluramine is more potent than d,l-fenfluramine as an anorectic agent by a factor of 2 (Silverstone et al. 1987). Accordingly, weight-reduction doses of d-fenfluramine are half of those of d,l-fenfluramine.

Finally, the differential effects of d- and d,l-fenfluramine on the ACTH and CORT responses merit some comment. In this study, we did not observe as clear a dose–response relationship for ACTH(FEN) or CORT(FEN) as we did for PRL(FEN). This may be due to the variability in the ACTH and CORT response to d-FEN, particularly at lower doses. Gorard et al. (1993) recently reported that neither the ACTH or CORT response to d-FEN challenge at 0.5 mg/kg was significantly greater than that for placebo in healthy volunteers. In the present study we found a significant effect of the d-FEN (but not d,l-FEN) 0.5-mg/kg dose for the ACTH(FEN) and CORT(FEN) over time data (though not for the peak delta data). This is probably because the average (i.e., 0.5-mg/kg) d-FEN dose our subjects received was at least 20% higher than the uniform 30-mg dose received

in Gorard et al. (1993). It is of note that neither ACTH or CORT concentrations were significantly increased by the 0.5-mg/kg dose of d,l-FEN. Because ACTH and CORT responses were elicited by the 1.0-mg d,l-FEN dose, it appears that racemic fenfluramine can affect these hormones provided a sufficient dose is administered. This points to differences between d- and d,l-FEN with respect to its effects on PRL and ACTH)FEN or CORT(FEN) responses. Based on these data, there does not appear to be a similarity for ACTH(FEN) or CORT(FEN) responses with respect to d- and d,l-fenfluramine. Accordingly, parallels between ACTH(FEN) or CORT(FEN), in contrast to PRL(FEN), data with d,l- and d-fenfluramine may not be appropriate.

Differences in the PRL(FEN) and ACTH(FEN) or CORT(FEN) responses to d- and d,l-FEN may be due to different 5-HT subtype receptors and/or neuronal pathways that underlie these responses (Van de Kar et al. 1985). Unfortunately, there are limited data examining potential differences between the hormonal responses in this regard. PRL responses to fenfluramine can be completely blocked by 5-HT$_{-2a/2c}$ (Goodall et al. 1993; Coccaro et al. in press), but not by 5-HT$_{-1a}$ (Park and Cowan 1995) antagonists. Data related to 5-HT$_{-3}$ receptors in humans have not been published in this regard, although animal studies suggest that PRL responses can be elicited by stimulation of 5-HT$_{-1a}$, 5-HT$_{-2}$; and 5-HT$_{-3}$ receptors (Jorgenson et al. 1992). Much less is known about the 5-HT receptor subtypes underlying ACTH or CORT responses to FEN, although animal studies suggest that ACTH(FEN) or CORT(FEN) responses can be elicited by stimulation of either 5-HT$_{-1a}$ or 5-HT$_{-2}$ receptors (Koenig et al. 1987). Of greater importance, however, is animal data that suggest that CORT, but not ACTH, responses to 5-HT releasing agents such as fenfluramine are due to both a central and a peripheral component (Van de Kar et al. 1985). If true, the value of CORT(FEN) responses as a central index of 5-HT function may be less than that of ACTH(FEN) responses.

In conclusion, these data demonstrate that the PRL, but not the ACTH/CORT response to d- and d,l-fenfluramine are remarkably similar. It is likely that observations made using the PRL response to d,l-fenfluramine will closely parallel those made with the PRL response to d-fenfluramine; observations made with ACTH/CORT with d,l-fenfluramine may not necessarily parallel those made with d-fenfluramine. These data also suggest that there may be little, if any, contribution of dopaminergic antagonism from l-fenfluramine in the PRL response to d,l-fenfluramine, but a small role of dopaminergic antagonism in this regard cannot be ruled out entirely. Moreover, these data do not rule out a role for dopaminergic antagonism of l-fenfluramine in regard to other possible effects of d,l-fenfluramine on other physiological or behavioral effects to stimulation with d,l-fenfluramine.

REFERENCES

Ben-Jonathan N, Abbogast LA, Hyde JF (1989): Neuroendocrine regulation of prolactin release. Prog Neurobiol 33:399–447

Coccaro EF, Kavoussi RJ (1994): The neuropsychopharmacologic challenge in biological psychiatry. Clin Chem 40: 319–327

Coccaro EF, Klar HM, Siever LJ (1994): Prolactin responsiveness to fenfluramine challenge does not correlate with prolactin responsiveness to TRH challenge in psychiatric patient. Biol Psychiatr 36:344–346

Coccaro EF, Kavoussi RJ, Oakes M, Cooper TB, Hauger RL (1996): Amesergide completely blocks the prolactin response to d-fenfluramine in healthy male subjects. Psychopharmacology 126:24–30

Crunelli V, Bernasconi S, Samanin R (1980): Effects of d- and l-fenfluramine on striatal homovanillic acid concetrations in rats after pharmacological manipulation of brain serotonin. Pharmacol Res Commun 12:215–223

Garattini S, Mennini T, Samanin R (1987): From fenfluramine racemate to d-fenfluramine: Specificity and potency of the effects on the serotonergic system and food intake. Ann NY Acad Sci 499:156–165

Garattini S, Bizzi A, Caccia S, Mennini T, Samanin R (1988): Progress in assessing the role of serotonin in the control of food intake. Clin Neuropharmacol 11(supp1):S8–S32

Ghigo E, Goffi S, Malinatti GM, Camanni F, Massara F (1985): Prolactin and TRH responses to both domperidone and TRH in normal and hyperprolactinemic women after dopaminergic synthesis blockade. Clin Endocrinol 23: 155–160

Goodall EM, Cowan PJ, Franklin M, Silverstone T (1993): Ritanserin attenuates anorectic, endocrine and thermal responses to d-fenfluramine in human volunteers. Psychopharmacology 112:461–466

Gorard DA, Taylor TM, Medback SH, Perry LA, Libby GW, Farthing MJG (1993): Plasma prolactin, adrenocorticotrophic hormone and cortisol after administration of d-fenfluramine or placebo to healthy subjects. Int Clin Psychopharmacol 8:123–128

Hollander E, Stein DJ, Saoud JB, DeCaria CM, Cooper TB, Islam MN, Liebowitz MR, Stanley M (1992): Effects of fenfluramine on plasma homovanillic acid in healthy subjects. J Neural Trans 90:81–84

Hunneman DH (1983): Mass fragmentographic determination of homovanillic acid and 4-hydroxy-3methoxy mandelic acids in 50 μl plasma. Clin Chem Acta 135: 169–174

Invernizzi R, Berettera C, Garattini S, Samanin R, (1986): D- and L-isomers of fenfluramine differ markedly in their interaction with brain serotonin and catecholamines in the rat. Eur J Pharmacol 120:9–15

Jorgenson H, Knigge U, Warberg J (1992): Involvement of 5-HT$_{-1}$, 5-HT$_{-2}$, and 5-HT$_{-3}$ receptors in the mediation of the prolactin response to serotonin and 5-hydroxytryptophan. Neuroendocrinology 55:336–343

Kendler KS, Heninger GH, Roth RH (1981): Brain contribution to the haloperidol-induced increase in plasma homovanillic acid. Eur J Pharmacol 71:321–326

Koenig JI, Gudelsky GA, Meltzer HY (1987): Stimulation of corticosterone and beta-endorphin secretion in the rat by selective 5-HT receptor subtype activation. Eur J Pharmacol 137:1–8

Krebs HA, Chens LK, Wright GJ (1984): Determination of fenfluramine and norfenfluramine in plasma using a nitrogen-sensitive detector. J Chromatography 310:412–417

Mitchell PB, Smythe GA (1991): Endocrine and amine responses to d,l-fenfluramine in normal subjects. Psychiatr Res 39:141–153

O'Keane V, Dinan TG (1991): Prolactin and cortisol responses to d-fenfluramine in major depression: Evidence for diminished responsivity of central serotonergic function. Am J Psychiatr 148:1009–1015

O'Keane V, Moloney E, O'Neill H, O'Connor A, Smith C, Dinan TG (1992): Blunted prolactin responses to d-fenfluramine in sociopathy: Evidence for subsensitivity of central serotonergic function. Br J Psychiatr 160:643–646

Park SBG, Cowan PJ (1995): Effect of pindolol on the prolactin response to d-fenfluramine. Psychopharmacology 118:471–474

Silverstone T, Smith G, Richards R (1987): A comparative evaluation of dextrofenfluramine and d,l-fenfluramine on hunger, food intake, psychomotor function, and side effects in normal human subjects. In Bender AE, Brookes LJ, (eds), Body Weight Control: The Physiology, Clinical Treatment and Prevention of Obesity. Edinburgh, Churchill-Livingstone

Van de Kar LD, Karteszi M, Bethea CL, Ganong WF (1985): Serotonergic stimulation of prolactin and corticosterone secretion is mediated by different pathways from the mediobasal hypothalamus. Neuroendocrinology 41: 380–384

van Praag HM, Lemus C, Kahn R (1987): Hormonal probes of central serotonergic activity: Do they really exist? Biol Psychiatry 22:86–98