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Administration of Dexfenfluramine in Pregnant Rats: Effect on Brain Serotonin Parameters in Offspring

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ROWLAND, N. E. AND R. M. ROBERTSON. *Administration of dexfenfluramine in pregnant rats: Effect on brain serotonin parameters in offspring.* PHARMACOL BIOCHEM BEHAV **42**(4) 855–858, 1992.—Dexfenfluramine (DFEN) was infused SC at doses of either 6 or 12 mg/kg/day during the last week of pregnancy in rats. Compared with untreated controls, weight gain of dams was attenuated by DFEN without effect on the number or birth weight of offspring. Brain serotonin (5-HT) concentration and/or paroxetine binding to the 5-HT uptake carrier was reduced by 20% on the day after birth in one study but not in two other studies. No decreases in brain 5-HT parameters were observed on or after the sixth postnatal day. In contrast, mothers sustained large depletions of brain 5-HT when measured at least 3 weeks after giving birth. These data indicate that fetal brains either are protected from or recover rapidly from the 5-HT-depleting actions of high-dosage regimens of DFEN in rats.

Dexfenfluramine Serotonin Pregnancy Neurotoxicity Infant rats

THE *d*(+)-enatiomer of fenfluramine, dexfenfluramine (DFEN), is an anorectic agent useful in the treatment of severe obesity (5) and as an experimental tool to study the role of brain serotonin (5-HT) processes in feeding behavior (11). There has been some discussion concerning the safety of DFEN: It has long been known that administration of relatively high dosages of DFEN (and other phenethylamines) to rats produces long-lasting depletions of brain 5-HT content and of the 5-HT uptake carrier and at least transient decreases in the number and intensity of 5-HT-immunoreactive axons in cerebral cortex [e.g., (2,4,7,8,14,16)]. The significance of these results is currently under investigation in several laboratories.

In rats, it is agreed that once a dose of DFEN is reached that causes initial 5-HT depletion that depletion lasts a few weeks. In contrast, hamsters given a large dose of fenfluramine that causes substantial initial depletion of 5-HT show full recovery of normal levels within 24 h (10). Mice may be intermediate between the profiles of young adult rats and hamsters in the foregoing studies (12). Recovery from brain 5-HT depletion thus shows some differences between species of rodents. In this report, we examine whether infant rats either sustain or recover from the effects of large doses of DFEN in utero. The study also examines the effects of DFEN given during pregnancy on weight gain of dams and birth weight of pups.

METHOD

Animals and Housing

Approximately timed pregnant Sprague-Dawley rats were either bred in our laboratory (Study A) or purchased from Harlan Industries (Indianapolis, IN) at about 1 week of gestation (Studies B and C) and housed individually in our vivarium with a 12 L:12 D cycle and ambient temperature of 23°C. During the last week of gestation and 3 weeks postnatally, they were placed in solid-bottomed maternity cages with pine shavings. Purina Rodent Chow pellets (#5001) and tapwater were available ad lib. Pups remained with their mothers throughout.

Administration of DFEN

On approximately day 14 of gestation, gravid rats were briefly (1–2 min) anesthetized by inhalation of methoxyflurane and an osmotic minipump [Alza Corp. (Palo Alto, CA) #20001, 7-day model] was implanted SC under the skin of the back. Pumps were preloaded with DFEN dissolved in water to give average daily dosages of either 6 or 12 mg/kg/day (based upon a constant 7-day output of 1 μl/h and using the mean group body weight on day 14). Control (no drug) rats were anesthetized but received no pump. Pumps were thus scheduled to expire on or just prior to the day of birth. These

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dosages of continuous DFEN administration do not produce the extreme peak concentrations observed after acute injection in rats (12,16) but are sufficient to produce obtunded weight gain in adult rats (3,9,13,15).

Somatic Measures

Dams were weighed daily after surgery until delivery and their weight gain determined. The number of pups delivered was counted within 24 h of birth (day 1) and they were weighed. A representative sample of pups was taken from each litter at this time for neurochemistry, and mothers were left with a maximum of 8 pups after day 1. Pups (one to four per litter) were again taken on days 6, 16, or 20 postnatally. Thus, litters were of decreasing size across days; each litter was culled in approximate proportion to the number remaining. Mothers were killed for neurochemistry 1-14 days after the last pups were removed.

Neurochemistry

Pups were removed from dams no more than 5 min prior to rapid decapitation. Their brains were removed and weighed. Brain tissue was homogenized immediately in 4-5 vol ice-cold perchloric acid-EDTA containing internal standard, centrifuged, and the supernatant assayed for 5-HT content by high-performance liquid chromatography with electrochemical detection. In Study C, brain tissue was quickly frozen at −70°C and the density of 5-HT uptake sites was subsequently determined by a membrane binding assay using [^{3}H]paroxetine (6). Briefly, Tris-homogenates (ca. 200 μg protein) were incubated (2 h, 23°C) with 0.5 nM [^{3}H]paroxetine (saturating) in the presence and absence of fluoxetine (10 μm). After rapid vacuum filtration on Whatman (Maidstone, UK) GF/B discs, membranes were washed with cold Tris (3 × 5 ml) and the membrane-bound radioactivity counted by liquid scintillation spectroscopy. Protein concentration of the homogenate was assayed by the BCA method (Pierce, Rockford, IL).

Statistics

All data from pups in a given litter/day were averaged to give a single datum from each litter on a particular day of age. Neurochemical data were expressed as % of the mean value in control rats assayed concurrently. All data were analyzed by one-way analysis of variance (ANOVA) or student's *t*-tests (SAS package, SAS, Cary, NC) with $\alpha = 0.05$.

Study A

In this study, six dams received DFEN (6 mg/kg/day) and six were controls. Dams were further along in pregnancy than we estimated so pups were born on days 4-6 of the 7-day DFEN regimen. Thus, mothers were lactating for a few days with DFEN. Pups were killed at 1, 6, or 16 days of age and whole brains were assayed for 5-HT content. Mothers were killed 4 weeks later (6.5 weeks after the end of the DFEN regimen) for 5-HT assay.

Study B

In this study, 23 dams were divided into four groups to receive either 0 (control), 6, or 12 mg DFEN/kg/day ($n = 6$), and an additional group that received no drug but was food restricted to four chow pellets (ca 20 g) per day during the last week of gestation. Mothers delivered 1-2 days after DFEN pumps expired. Pups were killed on days 1, 6, and 21 of age and mothers were killed on postpartum day 22. Whole-brain 5-HT levels were assayed.

Study C

In this study, nine dams received either 0, 6, or 12 mg DFEN/kg/day ($n = 3$). Pups were delivered within 1 day of expiration of pumps. Whole brains from 1-day-old pups in the same litter were pooled and used for paroxetine binding. Brains of pups sacrificed on day 21 and their mothers (day 22) were assayed for both 5-HT content (left prefrontal cortex) and paroxetine binding (right prefrontal cortex). (Samples were also taken from 6-day-old pups but were not assayed.)

TABLE 1

EFFECT OF DEXFENFLURAMINE DURING PREGNANCY ON WEIGHT GAIN OF MOTHER RATS AND ON VARIOUS PARAMETERS OF THEIR INFANTS/LITTERS

Treatment Group (*n*)	Gain (g/day) of Mothers	Litter Size	Weight (g) of Pups at Day of Age			
			1	6	16	21
Study A						
Control (6)	14.2 ± 1.3	10.7	6.5 ± .7	15.0 ± 2.2	40.5 ± 4.4	nd
DFEN 6 (6)	10.2 ± 1.6*	11.2	5.4 ± .4*	10.6 ± 2.3*	36.0 ± 3.9	nd
Study B						
Control (4)	10.3 ± .8	11.5	6.2 ± .2	nd	nd	48 ± 3
DFEN 6 (6)	6.3 ± 1.0*	8.6	5.6 ± .1	nd	nd	48 ± 3
DFEN 12 (5)	5.2 ± 1.1*	8.2	5.8 ± .2	nd	nd	38 ± 4
Restricted (3)	5.6 ± 1.1*	9.0	6.1 ± .2	nd	nd	49 ± 1
Study C						
Control (3)	14.9 ± 1.6	11.3	6.5 ± .2	14.6 ± 1.0	nd	49 ± 4
DFEN 6 (3)	13.8 ± 1.0	11.6	6.1 ± .2	13.9 ± 1.8	nd	51 ± 4
DFEN 12 (3)	9.6 ± .5*	9.3	6.2 ± .1	14.0 ± .5	nd	50 ± 1

Mean ± SE for *n*s indicated. Dose of DFEN (mg/kg/day) in **bold type**. nd, not measured.
*$p < 0.05$ treatment group differs from control.

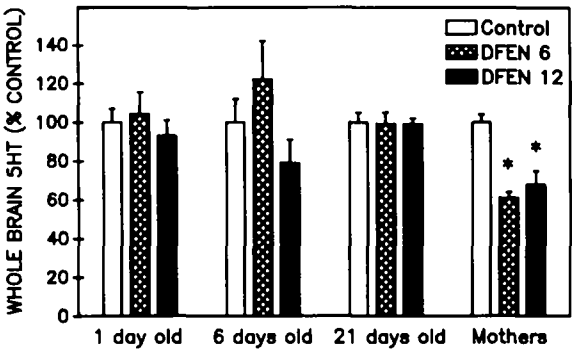

FIG. 1. Effect of treatment of pregnant rats with SC infusion of dexfenfluramine (6 or 12 mg/kg/day) during the last 7 days of gestation on whole-brain 5-HT content (expressed as mean ± SE of the untreated control mean) of pups on days 1, 6, or 21 of age. On the right are the corresponding levels in mothers, killed 1 day after the 21-day-old pups were removed. *$p < 0.05$ vs. controls.

RESULTS

Study A

Weight gain during the last days of pregnancy (Table 1) was reduced in DFEN-treated mothers. Deliveries occurred between 4 and 6 days after minipumps were implanted and so these means are averaged over 3–6 days for different mothers. The number of pups liveborn did not differ between control and DFEN groups. Early postnatal growth was significantly slower in pups of DFEN-treated mothers than in control pups (Table 1), but this difference was no longer significant by 16 days of age.

5HT concentration in the brains of 1-day-old pups of DFEN-treated mothers was 78 ± 13% of the control mean and this difference was just statistically significant ($p = 0.05$, t-test). Brain 5-HT levels on days 6 and 16 of age did not differ between DFEN and control litters. 5-HT levels in DFEN-treated mothers' brains (at 6.5 weeks) were 72 ± 6% of controls ($p < 0.01$).

Study B

The mean weight gain during the last week of pregnancy was significantly reduced in each of the treatment groups (Table 1) and, as was intended, the weight gains of the restricted- and high-dose DFEN groups were comparable. For reasons that are unknown, one to two rats in each treatment group failed to deliver pups (an autopsy suggested fetal resorption). The fact that the overall weight gain of dams in this study was lower than in Studies A or C suggests this batch of rats may have had a subclinical illness/infection. Excluding nonpregnant dams, the mean number of pups/litter did not differ between groups. The weights of pups did not differ significantly between groups (Table 1).

5-HT concentrations in pup brains (Fig. 1) were slightly, but not significantly, reduced by DFEN and food restriction. In contrast, 5-HT levels were substantially reduced in brains of dams treated with DFEN and killed 3 weeks later.

Study C

The mean weight gain during the last week of pregnancy was reduced by the high dose of DFEN (Table 1). Paroxetine binding to whole brain of pups did not differ between groups on either days 1 or 20. 5-HT levels also did not differ on day 20. In contrast, brains of mothers killed on day 21 showed profound depletions in 5-HT levels (to approximately 80 and 50% of control) and in paroxetine binding (to about 30 and 20% of control) in the 6- and 12-mg DFEN/kg/day groups, respectively ($p < 0.01$). Individual data for both these variables in mothers are shown in Fig. 2, indicating that paroxetine binding is reduced more than 5-HT content by prior DFEN treatment.

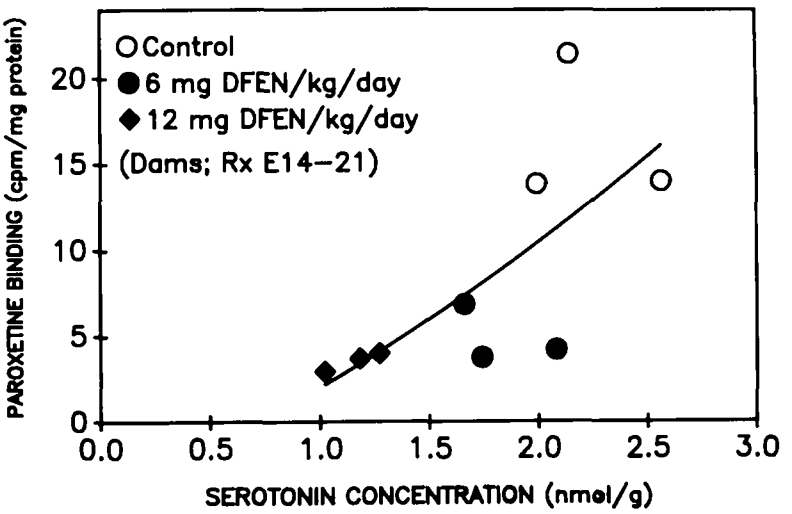

FIG. 2. 5-HT concentration (expressed as nmol/g wet tissue) and paroxetine binding (expressed as raw counts/min/μg protein in the assay homogenate) data from opposite halves of the frontal cortex of adult rats treated with dexfenfluramine (6 or 12 mg/kg/day or no drug control) during the last week of pregnancy and killed on postnatal day 21. Shown are scatterplot data (three rats/condition) and a quadratic best-fit line from the Sigmaplot (Jandel Scientific, Corte Madera, CA) program.

DISCUSSION

In the present studies, we show that administration of DFEN to rats in late pregnancy, at doses sufficiently high to produce long-lasting and substantial decrements in mothers' brain 5-HT indices, produced either no effects or only minor and transient (<6 day) depletions in these indices in pups. Two of the potential explanations for this are either that DFEN in maternal blood does not readily penetrate to the fetus or that infant brains are resistant to/recover very rapidly from 5-HT-depleting effects of DFEN. Our data cannot distinguish between these alternatives. However, since DFEN is quite lipid soluble, we have no reason to suppose it would not readily traverse the placenta. If this is correct, then the latter options that the immature brain is either immune to or recovers rapidly from DFEN appears viable. In this regard, the main procedural difference between Study A, in which a small depletion of 5-HT was observed on day 1, and Studies B and C, in which no such depletion was observed, is that DFEN treatment was for a shorter time prenatally and continued postnatally in Study A. Thus, administration of DFEN on or about days 14–16 of gestation (Studies B and C) may "protect" against depleting effects on or about days 18–20 of gestation. Alternatively, transmission of DFEN in mothers' milk on day 1 may be sufficient to cause 5-HT depletion in pups. Further studies will be needed to resolve these issues but, in either event, it is clear that the effects on brain 5-HT parameters are minimal and transient in infant compared with adult rats.

This conclusion is also supported by a recent abstract report (1) in which racemic fenfluramine was given by daily injection (5 mg/kg/day) to late-term mothers. The authors reported a 32–42% decrease in the [^{3}H]5-HT uptake into synaptosomes during the first week postnatally in fenfluramine-exposed pups relative to controls. This difference between groups was no longer evident at day 14 postnatally. The reason these authors found a greater initial depletion presumably reflects the differences from our study in the dependent variable (synaptosomal uptake), brain region (cortex vs. whole brain), agent/mode (*d,l*-fenfluramine as a daily bolus vs. DFEN as a continuous infusion), and possibly day(s) of treatment. Their injection regimen produces high peak levels of the agent (cf. 16), suggesting that it may be peak level rather than total exposure (i.e., area under curve) that determines any 5-HT-depleting action of DFEN. Adult hamsters (10) may recover 5-HT comparably to infant rats.

DFEN consistently reduced the weight gain of gravid rats late in pregnancy without affecting the number of pups born per litter or, in general, birth weight. The reduced pup weights in Study A may reflect postnatal effects of DFEN to reduce lactation rather than any gestational effect. The doses of DFEN in these studies exceed those used clinically by an order of magnitude and produce blood DFEN levels at least 10× those seen in humans. Further study will be needed to determine whether DFEN might be acceptable as an adjunct to dietary advice in pregnancies in which excessive weight gain poses substantial health hazards to either mother or fetus.

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REFERENCES

1. Akbari, H. M.; Kramer, H. K.; Azmitia, E. C. Prenatal cocaine and fenfluramine administration inhibits serotonin and dopamine fiber outgrowth. Soc. Neurosci. Abstr. 17:1182; 1991.
2. Appel, N. M.; Contrera, J. F.; DeSouza, E. B. Fenfluramine selectively and differentially decreases the density of serotonergic nerve terminals in rat brain: Evidence from immunocytochemical studies. J. Pharmacol. Exp. Ther. 249:928–943; 1989.
3. Carlton, J.; Rowland, N. E. Long term actions of *d*-fenfluramine in two rat models of obesity. I: Sustained reductions in body weight and adiposity without depletion of brain serotonin. Int. J. Obesity 13:825–847; 1989.
4. Duhault, J.; Roman, F.; Suzanna, O.; Molle, D.; Beregi, L. Fenfluramine and neuromediators. In: Garattini, S.; Samanin, R., eds. Anorectic agents: Mechanisms of action and tolerance. New York: Raven Press; 1981:113–123.
5. Guy-Grand, B.; Apfelbaum, M.; Crepaldi, G.; Gries, A.; Lefebvre, P.; Turner, P. International trial of long term dexfenfluramine in obesity. Lancet ii:1142–1145; 1989.
6. Habert, E.; Graham, D.; Tahraoui, L.; Claustre, Y.; Langer, S. Z. Characterization of [^{3}H]paroxetine binding to rat cortical membrane. Eur. J. Pharmacol. 118:107–114; 1985.
7. Kalia, M. Reversible, short-lasting, and dose-dependent effect of (+)-fenfluramine on neocortical serotonergic axons. Brain Res. 548:111–125; 1991.
8. Molliver, D. C.; Molliver, M. E. Anatomic evidence for a neurotoxic effect of (±)-fenfluramine upon serotonergic projections in the rat. Brain Res. 511:165–168; 1990.
9. Rowland, N. E. Effect of continuous infusions of dexfenfluramine on food intake, body weight and brain amines in rats. Life Sci. 39:2581–2586; 1986.
10. Rowland, N. E.; Carlton, J. Effects of fenfluramine on food intake, body weight, gastric emptying, and brain monoamines in Syrian hamsters. Brain Res. Bull. 17:575–581; 1986.
11. Rowland, N. E.; Carlton, J. Neurobiology of an anorectic drug: Fenfluramine. Prog. Neurobiol. 27:13–62; 1986.
12. Rowland, N. E.; Souquet, A. -M.; Edwards, D. J. Long-term actions of dexfenfluramine on food intake, body weight, and brain serotonin in rodents. In: Paoletti, R.; Vanhoutte, D. M.; Brunello, N.; Maggi, F. M., eds. Serotonin—from cell biology to pharmacology and therapeutics. Dordrecht: Kluwer Publishers; 1990:631–635.
13. Rozen, R.; Fumeron, F.; Betoulle, D.; Faigts, F.; Mandenoff, A.; Fricker, J.; Apfelbaum, M. Permanent administration of *d*-fenfluramine in rats: Paradoxical effects. Clin. Neuropharmacol. 11(suppl. 1):S105–S112; 1988.
14. Sotelo, C. Immunohistochemical study of short- and long-term effects of *dl*-fenfluramine on the serotonergic innervation of the rat hippocampal formation. Brain Res. 541:309–326; 1991.
15. Souquet, A. -M.; Rowland, N. E. Dexfenfluramine: Action with estradiol on food intake and body weight in ovariectomized rats. Am. J. Physiol. 258:R211–R215; 1990.
16. Zaczek, R.; Battaglia, G.; Culp, S.; Appel, N. M.; Contrera, F. J.; DeZousa, E. B. Effects of repeated fenfluramine administration on indices of monoamine function in rat brain: Pharmacokinetic, dose-response, regional specificity, and time course data. J. Pharmacol. Exp. Ther. 253:104–112; 1990.