ELSEVIER

European Journal of Pharmacology 369 (1999) R1–R3

ejp

Rapid communication

Effects of prenatal co-administration of phentermine and dexfenfluramine in rats

Jonathan Bratter, Ira H. Gessner, Neil E. Rowland *

Department of Psychology, University of Florida, Gainesville, FL 32611-2250, USA
Department of Pediatrics, University of Florida, Gainesville, FL 32610-0296, USA

Received 9 February 1999; accepted 12 February 1999

Abstract

Pregnant rats were infused with phentermine plus dexfenfluramine from days 3 through 17 of gestation. Control rats were either pair-fed or were fed ad libitum. There were no effects of prenatal drug treatment on number of offspring, their birth weights, or on their motor coordination assessed at 11 days of age. Mothers and pups were sacrificed 21 days postpartum. Drug-treated mothers, but not their pups, showed a reduced density of serotonergic axons in the hippocampus compared with controls. 25% of the pups from the prenatal drug group showed mitral valve thickening.

Reports of valvular heart abnormalities in obese patients taking the combination of phentermine and fenfluramine led to the voluntary removal of both racemic fenfluramine and its active enantiomer, dexfenfluramine, from the market. Abnormalities included mitral and aortic valve regurgitation, valvular thickening, and diastolic doming of the mitral valve leaflets (Connoly et al., 1997; Wadden et al., 1998). High doses of these agents also cause apparent atrophy of serotonin (5-HT) axons in the brain of animals (Zaczek et al., 1990). We now report the effects in rats of phentermine and dexfenfluramine given for 2 weeks during pregnancy on a brain 5-HT marker and on mitral valves in the offspring.

Twelve multiparous female Sprague–Dawley rats (Harlan, Indianapolis) were used. They were mated with males of the same stock, and daily vaginal smears were taken. The presence of sperm was taken as the first day of pregnancy and the female was then housed individually in a suspended metal cage with Purina Chow powder presented inside the cage in a glass jar. Tap water was present at all times.

Two or three days after transferring to these conditions, four of the rats were assigned to the phentermine/dexfenfluramine group and were implanted with a 14-day osmotic minipump (Alza, Palo Alto, model 2002). Surgery consisted of brief ($\sim$ 1 min) sedation with methoxyflurane, insertion of the pump subcutaneously in the lumbar region, and closure with a wound clip. The pump was filled with a solution (in water) to deliver 10 mg phentermine kg^{-1} day^{-1} (Sigma) and 3 mg dexfenfluramine kg^{-1} day^{-1} (Servier, France) for 14 days in a volume of 12 μl day^{-1}. Food intake was measured daily throughout the treatment. Another three rats were pair-fed controls and had become sperm positive one day after a drug-treated rat to which they were then yoked. The pair fed rats received a sham surgery (no implant) and were then given a ration of food equal to the amount consumed the previous day by its partner that was receiving phentermine/dexfenfluramine. The final group ($N = 5$) of rats were ad libitum fed controls.

After 14 days, or about 4 days prior to estimated parturition, the rats were transferred to individual polycarbonate cages with Sani-Chip (Teklad) bedding and Purina Chow pellets available ad libitum. Within 24 h of parturition, the pups were counted, weighed, and litters culled to a maximum of 9. On postnatal day 11, the pups were tested behaviorally for geotaxis and limb coordination. For the geotaxis task, the pups were placed head down on a 45° inclined mesh surface and the time taken to turn

* Corresponding author. Department of Psychology, University of Florida, PO Box 112250, Gainesville, FL 32611-2250, USA. Tel.: +1-352-392-0601 ext. 287; Fax: +1-352-392-7985; E-mail: rowland@psych.ufl.edu

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00100-4

Table 1
Various parameters in pups treated prenatally with phentermine + dexfenfluramine compared with either pair fed or ad libitum fed controls

Parameter	Phentermine/ dexfenfluramine ($N = 4$)	Pair fed control ($N = 3$)	Ad libitum fed control ($N = 5$)
Number of pups born	11.7 ± 1.2	11.7 ± 2.2	13.2 ± 1.3
Birth weight (g)	6.5 ± 0.3	7.4 ± 0.6	7.0 ± 0.3
Weight at 21 days (g)	47.9 ± 1.1[a]	58.4 ± 6.1	57.4 ± 2.2
Geotaxis latency (s)	2.5 ± 0.1	2.7 ± 0.2	2.5 ± 0.2
Limbs: latency to fall (s)	17.3 ± 0.9	10.4 ± 0.6	17.8 ± 2.9
Limbs: percent reaching end	24 ± 8	7 ± 6	36 ± 2

Shown are M ± S.E. of litter medians ($N = 7$–9 per litter).
[a] $p < 0.05$ differs from ad libitum fed control.

around and face up the incline was recorded. Limb coordination was assessed using a 15 × 2.5 cm ruler supported horizontally 18 cm above the ground. Pups were placed in the middle, and the latency to either fall off or reach the end was noted.

At 21 days postnatal, all animals were weighed and deeply anesthetized. Two pups from each litter and the mother were perfused through the heart with saline and paraformaldehyde for subsequent immunocytochemistry. The brains were post-fixed overnight, then sliced on a Vibratome into 100 μm coronal sections; these were incubated with a primary antibody to the 5-HT transporter protein and the antibody visualized by standard methods (Zhou et al., 1996). The hippocampus is particularly vulnerable to 5-HT loss (Zaczek et al., 1990) so we chose the dentate gyrus for analysis. The density of the 5-HT transporter-immunoreactive axons in this region was rated qualitatively on an ordinal scale (from 0 = absent to 5 = extremely dense) by two observers and the ratings averaged.

The remaining pups were not perfused; instead, the hearts were removed intact and placed in a jar of formaldehyde for at least 1 week prior to examination under the microscope. An incision was made into the right atrium and then through the tricuspid valve into the right ventricle. A second cut was made from the right ventricle through the pulmonic valve in order to expose the right ventricular outflow tract and the ventricular septum. The left ventricle was incised at its apex and a cut made from this point through the aortic valve exposing the left ventricular outflow tract and the mitral valve. The condition of the mitral valves was assessed by an experienced clinical cardiologist. Normal valves are transparent and elastic. Valves were considered to be abnormally thickened if they had a white, glossy appearance and thick or rigid consistency.

Phentermine/dexfenfluramine treatment produced substantial anorexia, especially for the first 3 days. The mean intake of the drug (and pair fed) groups was 7.3 g day^{-1} during this time. Thereafter, the intake rapidly rose to normal levels (20–30 g day^{-1}). These data are comparable to our previous findings in non-pregnant female rats (Roth and Rowland, 1998).

The mean number of pups per litter and median birth weight did not differ between groups (Table 1). At 21 days of age, however, pups from the phentermine/dexfenfluramine group weighed significantly less than those in the ad libitum fed group. Performance on the geotaxis and limb coordination tasks did not differ between groups (Table 1), and no external abnormalities were observed in any of the pups. However, 6 out of 24 hearts (25%) from drug-treated pups were rated as having thickening of the mitral valve. In contrast none of the ad libitum fed or pair fed pups had evidence of thickening. A χ^2 test indicated this represents a significant ($p < 0.01$) increase in the incidence as a function of phentermine/dexfenfluramine treatment. Further study will be required to characterize the functional aspects of this mitral valve thickening, whether it is reversible, as well as whether it is a product of the drug combination or instead can be produced by either agent alone.

As expected from our previous study using dexfenfluramine (Rowland and Robertson, 1992), mothers from the phentermine/dexfenfluramine group showed a marked loss of 5-HT transporter-immunoreactive axonal density compared with ad libitum fed and pair fed controls (mean ratings were 3.2 and 4.4, respectively; $p < 0.05$, Mann–Whitney). However, the 21-day old pups showed no group difference (mean ratings of 3.7 in drug vs. 3.2 in controls) indicating that either their 5-HT axonal densities were never affected or that they recovered rapidly. Finally, we caution that the clinical dosages of dexfenfluramine and phentermine (< 1 and < 0.2 mg kg^{-1} day^{-1}, respectively; e.g., Wadden et al., 1998), are substantially less than those used in the present study.

Acknowledgements

Dr. F. Zhou (Indiana Univ.) kindly provided the 5-HT transporter antibody.

References

Connoly, H.M., Crary, J.L., McGoon, M.D., Hensrud, D.D., Edwards, B.S., Edwards, W.D., Schaff, H.V., 1997. Valvular heart disease

associated with fenfluramine–phentermine. N. Engl. J. Med. 337, 581–588.

Roth, J.D., Rowland, N.E., 1998. Efficacy of administration of dexfenfluramine and phentermine, alone and in combination, on ingestive behavior and body weight in rats. Psychopharmacology 137, 99–106.

Rowland, N.E., Robertson, R.M., 1992. Administration of dexfenfluramine in pregnant rats: effect on brain serotonin parameters in offspring. Pharmacol. Biochem. Behav. 42, 855–858.

Wadden, T.A., Berkowitz, R.I., Silvestry, F., Vogt, R.A., St. John Sutton, M.G., Stunkard, A.J., Foster, G.D., Aber, J.L., 1998. The fen–phen finale: a study of weight loss and valvular heart disease. Obesity Res. 6, 278–284.

Zaczek, R., Battaglia, G., Culp, S., Appel, N., Contrera, J., DeSouza, E., 1990. Effects of repeated fenfluramine administration on indexes of monoamine function in rat brain: pharmacokinetic, dose–response, regional specificity and time-course data. J. Pharmacol. Exp. Ther. 253, 104–112.

Zhou, F.C., Xu, Y., Bledsoe, S., Lin, R., Kelley, M.R., 1996. Serotonin transporter antibodies: production, characterization, and localization in the brain. Mol. Brain Res. 43, 267–278.