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Short communication

The effect of D-fenfluramine on brain 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in male and female rats

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Abstract

Brain regional 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were determined in freely feeding male and female rats 7 days after giving a single dose of D-fenfluramine (3.8 mg/kg, p.o.) or vehicle. Males showed negligible effects except for a significant decrease of 5-HT in the rest of the cortex, whereas females showed significant decreases of 5-HT and 5-HIAA in the frontal cortex, the rest of the cortex, hippocampus and hypothalamus; 5-HT was also decreased in female midbrain. Females had substantially higher plasma and brain concentrations of fenfluramine and moderately but significantly lower concentrations of norfenfluramine than the males. Plasma fenfluramine + norfenfluramine concentrations of the females were significantly higher than those of the males. Corresponding brain values showed smaller but significant differences. Female brain and plasma areas under the curve for fenfluramine + norfenfluramine (0–24 h after administration of D-fenfluramine) were 20 and 35% higher than male values. However, results suggest that the sex difference in the effect of D-fenfluramine on brain 5-HT metabolism is not due to differences in the metabolism of the drug. © 1997 Elsevier Science B.V.

Keywords: D-Fenfluramine; 5-HT (5-hydroxytryptamine, serotonin); D-Norfenfluramine; Sex difference

1. Introduction

Drugs which affect behaviour and mood are largely studied using male animals but patients given such drugs for disorders of appetite and mood are predominantly female. Data on female animals is strikingly sparse (Blanchard et al., 1995). Work on D-fenfluramine which is used in the treatment of obesity (Davis and Faulds, 1996) conforms to this generalisation.

When 100 day old male and female rats were killed 2 h, 24 h and 7 days after a single dose of D-fenfluramine (3.8 mg/kg) p.o. (Oluyomi et al., 1994) hypothalamic 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic (5-HIAA) were moderately decreased. Percentage decreases became less marked in the males but not in the females as time after injection increased. Hypothalamic concentrations of fenfluramine and its metabolite norfenfluramine were similar in both sexes but the proportion of norfenfluramine was greater in the males. As D-norfenfluramine, assessed in terms of brain concentration is a slightly more potent 5-HT depleter than D-fenfluramine (Caccia et al., 1993) the greater effect of D-fenfluramine on 5-HT metabolism in the females was not explicable by differences of brain drug kinetics.

We have now determined the effect of a single dose of D-fenfluramine (p.o.) on 5-HT and 5-HIAA concentrations in the hypothalamus as before (Oluyomi et al., 1994) and also in other brain regions of male and female rats. Freely feeding animals were used instead of the food deprived animals used before to increase comparability with the conditions under which D-fenfluramine is given to humans. D-Fenfluramine and D-norfenfluramine were determined in brain as before and also in plasma.

2. Materials and methods

2.1. Animals

Male and female Sprague–Dawley rats (Charles River, UK) aged approximately 80 days on arrival were individually housed for 20 days before experimentation in a quiet

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room under a 12 h light–dark cycle (lights on 06.00 h) at 21 ± 1°C. Food (Charles River, UK, rat diet) and tap water were freely available.

2.2. Drug treatment

Rats were given either a single dose of D-fenfluramine (3.8 mg/kg) or 0.9% NaCl p.o. and killed by decapitation seven days later. The brains were removed immediately and dissected into regions on an ice-cold Petri dish as Glowinski and Iversen (1966) and stored at −72°C until analysis by high pressure liquid chromatography (HPLC). The cerebral cortex was further divided into the frontal cortex and the rest of the cortex.

2.3. Determination of 5-HT and 5-HIAA in brain regions

Concentrations of 5-HT and 5-HIAA, were estimated by HPLC with electrochemical detection as before (Datla and Curzon, 1996).

2.4. Determination of D-fenfluramine and D-norfenfluramine in brain and plasma

Rats of 100 days old were given a single dose of D-fenfluramine (3.8 mg/kg, p.o.) and killed 2, 8 and 24 h later. Brain and plasma were collected and fenfluramine and norfenfluramine determined as described by Richards et al. (1989).

2.5. Drugs

D-Fenfluramine hydrochloride (Servier, France) was dissolved in 0.9% NaCl before administration. Doses represent the weight of drug salt.

2.6. Statistics

The differences between groups were analyzed by Duncan's *t*-test after significant one-way analysis of variance (ANOVA) and effects of sex and drug treatment analysed

Table 1
Effect of D-fenfluramine (3.8 mg/kg p.o.) on 5-HT and 5-HIAA concentrations in brain regions

	5-HT		5-HIAA		5-HIAA/5-HT	
	Male	Female	Male	Female	Male	Female
Frontal cortex						
Control	0.43 ± 0.02	0.45 ± 0.02	0.23 ± 0.01	0.24 ± 0.01	0.54 ± 0.02	0.57 ± 0.03
Drug	0.40 ± 0.02	0.39 ± 0.02 [b]	0.23 ± 0.01	0.20 ± 0.01 [b,c]	0.54 ± 0.01	0.55 ± 0.02
Change (%)	−7	−14	−3	−17	0	−4
Rest of cortex						
Control	0.37 ± 0.02	0.34 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.67 ± 0.01	0.76 ± 0.03 [c]
Drug	0.32 ± 0.01 [b]	0.26 ± 0.01 [b,d]	0.24 ± 0.01	0.22 ± 0.01 [b]	0.74 ± 0.02	0.86 ± 0.02 [a,d]
Change (%)	−14	−24	−6	−17	+10	+13
Striatum						
Control	0.47 ± 0.03	0.45 ± 0.03	0.36 ± 0.01	0.38 ± 0.02	0.73 ± 0.02	0.85 ± 0.05
Drug	0.46 ± 0.02	0.41 ± 0.02	0.37 ± 0.01	0.35 ± 0.01	0.81 ± 0.03	0.86 ± 0.05
Change (%)	−2	−9	+4	−8	+11	+1
Hippocampus						
Control	0.33 ± 0.02	0.32 ± 0.01	0.27 ± 0.01	0.30 ± 0.01 [d]	0.82 ± 0.04	0.93 ± 0.04
Drug	0.30 ± 0.01	0.26 ± 0.01 [b,d]	0.25 ± 0.01	0.26 ± 0.01 [b]	0.84 ± 0.04	0.97 ± 0.03
Change (%)	−7	−18	−7	−15	+2	+4
Hypothalamus						
Control	0.71 ± 0.03	0.85 ± 0.04 [d]	0.39 ± 0.01	0.48 ± 0.02 [d]	0.54 ± 0.02	0.58 ± 0.02
Drug	0.75 ± 0.02	0.72 ± 0.03 [b]	0.42 ± 0.01	0.44 ± 0.01 [a]	0.56 ± 0.02	0.62 ± 0.02 [c]
Change (%)	+5	−16	+8	−8	+4	+7
Midbrain						
Control	0.68 ± 0.03	0.89 ± 0.04 [d]	0.54 ± 0.02	0.61 ± 0.02d	0.80 ± 0.03	0.72 ± 0.02
Drug	0.72 ± 0.02	0.71 ± 0.03 [b]	0.57 ± 0.02	0.57 ± 0.03	0.83 ± 0.03	0.81 ± 0.01
Change (%)	+6	−20	+7	−7	+4	+13
Brainstem						
Control	0.73 ± 0.04	0.78 ± 0.03	0.50 ± 0.02	0.55 ± 0.03	0.69 ± 0.01	0.69 ± 0.03
Drug	0.81 ± 0.03	0.71 ± 0.03 [c]	0.52 ± 0.03	0.55 ± 0.02	0.66 ± 0.02	0.74 ± 0.04
Change (%)	+11	−9	+4	+1	−4	+7

Values are in μg/g and expressed as means ± S.E.M. $n = 8–10$ per group. Differences between D-fenfluramine and 0.9% NaCl treated groups: [a] $P < 0.05$, [b] $P < 0.01$; differences between male and female groups: [c] $P < 0.05$, [d] $P < 0.01$ (Duncan's) test after 1-way ANOVA). Interactions of sex and drug treatments of 5-HT were significant in hypothalamus ($F_{1,36} = 9.33$; $P < 0.005$), midbrain ($F_{1,34} = 12.939$; $P < 0.001$) and brain stem ($F_{1,35} = 6.499$; $P < 0.02$). Interactions of sex and drug treatments on 5-HIAA were significant in hypothalamus ($F_{1,34} = 6.884$; $P < 0.02$) and midbrain ($F_{1,34} = 4.136$; $P < 0.05$) and approached significance in frontal cortex ($F_{1,34} = 3.618$; $P = 0.066$).

by two-way ANOVA. Areas under the curve for fenfluramine and norfenfluramine were calculated by the trapezoid method (Burden et al., 1981) and sex differences between groups were analysed using Student's *t*-test.

3. Results

3.1. Effect of D-fenfluramine on brain regional 5-HT metabolism

Table 1 shows the effect of D-fenfluramine on 5-HT and 5-HIAA concentrations and on 5-HIAA/5-HT ratios in brain regions of rats killed 7 days after drug administration. Female vehicle treated rats had significantly higher 5-HT and 5-HIAA concentrations than males in the hypothalamus and midbrain and significantly higher 5-HIAA concentrations in the hippocampus. 5-HIAA/5-HT ratios were higher in the vehicle treated females in terminal rich regions in general but significantly so only in the rest of the cortex. D-Fenfluramine only had a significant effect in male rats in the rest of the cortex where 5-HT decreased moderately. The females showed moderate but significant decreases of 5-HT and 5-HIAA in the frontal cortex, the rest of the cortex, hippocampus and hypothalamus, a significant decrease of 5-HT in the midbrain and a significant increase of 5-HIAA/5-HT ratio in the rest of cortex.

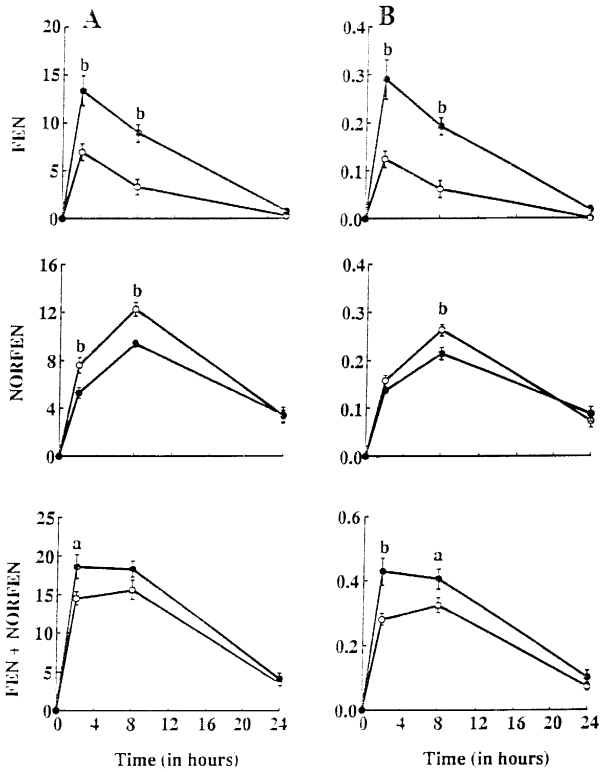

Fig. 1. Time–concentration curves of fenfluramine (FEN) and norfenfluramine (NORFEN) in brain (A) and plasma (B) in male (○) and female (●) rats after D-fenfluramine (3.8 mg/kg, p.o.). Values are in nmol/g wet tissue (brain) and nmol/ml (plasma) given as mean ± SEM of 6 rats per group. [a] $P < 0.05$, [b] $P < 0.01$ versus other sex. Duncan's test after significant one-way ANOVA. Areas under the curves (0–24 h) calculated by the trapezoid method (Burden et al., 1981) were as follows with brain and plasma values in nmol/h.g and nmol/h.ml, respectively (means ± SEM, 16–18 animals/group). Fenfluramine: brain; 65.9 ± 9.5 (male), 156.7 ± 12.1 (female), $P < 0.001$. Plasma: 1.14 ± 0.23 (male), 3.40 ± 0.28 (female), $P < 0.05$. Norfenfluramine: brain; 191.8 ± 7.9 (male), 151.7 ± 6.3 (female), $P < 0.001$. Plasma: 4.06 ± 0.14 (male), 3.59 ± 0.14 (female), $P < 0.05$. Fenfluramine + norfenfluramine: brain; 257.7 ± 15.0 (male), 308.4 ± 14.6 (female), ns. Plasma: 5.20 ± 0.25 (male), 7.03 ± 0.40 (female), $P < 0.001$. Student's *t*-test.

3.2. Fenfluramine and norfenfluramine concentrations in plasma and brain after giving D-fenfluramine

Plasma and brain concentrations of fenfluramine and norfenfluramine were maximal at 2 and 8 h, respectively, after drug administration and then fell sharply (Fig. 1). Fenfluramine values were significantly and substantially higher and norfenfluramine values significantly lower in the females than in the males. Concentrations of fenfluramine and norfenfluramine at both 2 and 8 h after D-fenfluramine administration were significantly higher in female than in male plasma and less markedly higher in female than in male brain with significance ($P < 0.05$) only at 2 h. Areas under the curve for fenfluramine were substantially and significantly greater for females than for males in both plasma and brain. Corresponding values for norfenfluramine were significantly lower in the females while values for fenfluramine + norfenfluramine were significantly higher for plasma (+35%) and significantly but less markedly (+20%) higher for brain.

4. Discussion

Indices of brain regional 5-HT metabolism tended to be greater in female than in male control rats, as indicated by significant differences of the concentrations of 5-HT and 5-HIAA (hypothalamus, midbrain) and of the 5-HIAA (hippocampus) and 5-HIAA/5-HT ratio (rest of cortex). Previous findings also revealed elevated regional 5-HT metabolism in females though results varied considerably between studies (Oluyomi et al., 1994 and references therein).

Only the females showed a decrease of hypothalamic 5-HT metabolism 7 days after a single dose of D-fenfluramine (3.8 mg/kg p.o.). The changes were smaller than before (Oluyomi et al., 1994), probably because brain fenfluramine and norfenfluramine concentration was also lower than found previously for the hypothalamus presumably due to slower drug absorption by the freely feeding rats than by the food-deprived animals used previously. They are unlikely to be due to differences between the whole brain and hypothalamic concentrations of the drug or its metabolite as these are evenly distributed in the brain (Invernizzi et al., 1991; Caccia et al., 1992).

The only significant effect of fenfluramine in males was a decrease of 5-HT in the rest of the cortex but in the females 5-HT and 5-HIAA were significantly decreased in 4/7 regions (frontal cortex, rest of the cortex, hippocampus, hypothalamus) and 5-HT also fell in the midbrain. Though regional differences were small the significant decreases in the cortex and hippocampus but not in the striatum and brainstem agree with the relative changes in these regions of male rats given D-fenfluramine or D-norfenfluramine at higher and/or repeated doses (Kleven and Seiden, 1989; Invernizzi et al., 1991; Caccia et al., 1992). The relative resistance of hypothalamic 5-HT to D-fenfluramine reported previously (Kleven and Seiden, 1989; Ricaurte et al., 1991; McCann et al., 1994) was not seen.

Central fenfluramine values were higher and norfenfluramine values lower in the females than in the males as before (Oluyomi et al., 1994). However, though hypothalamic fenfluramine + norfenfluramine concentrations were previously essentially identical in both sexes, the area under the curve for brain fenfluramine + norfenfluramine in the present study was 20% greater in the females than in the males. That this small difference explains the greater effect of the drug on 5-HT in the females is unlikely as the larger contribution of norfenfluramine to the central fenfluramine + norfenfluramine values of the males in both studies would have tended to decrease male rather than female brain 5-HT (Caccia et al., 1993).

The structurally related drug *p*-chloroamphetamine has been suggested to deplete brain 5-HT through the action of a metabolite of 5-HT formed after its peripheral release (Berger et al., 1990, 1992). A similar explanation of the sex difference in D-fenfluramine action is favoured by its higher levels in female plasma (Fig. 1) and by its greater ability than D-norfenfluramine to increase extracellular 5-HT (Garattini et al., 1979). However, according to this mechanism, brain 5-HT would be more decreased by D-fenfluramine if its metabolism to D-norfenfluramine was blocked. As this was not found (Caccia et al., 1993) and as tryptophan pretreatment increased the release of blood 5-HT by *p*-chloroamphetamine but opposed its depletory effect on brain 5-HT (Iyer et al., 1994) the present findings do not seem to depend on the mechanism of Berger et al.

Fenfluramine + norfenfluramine concentration in the brains of human subjects given the standard clinical dose of 30 mg D-fenfluramine/day for 10–30 days (Campbell et al., 1997) had a plateau value of 2 μM. As this is only about 11% of the peak value of 18.6 μM associated with decreases of brain regional 5-HT of up to 24% in female rats 7 days after a single dose of D-fenfluramine (Table 1) long term effects on brain 5-HT may have little clinical importance. Indeed, harmful central effects of D-fenfluramine were not detected in a study of more than 3000 patients (for review, see Laudignon and Rebuffe-Scrive, 1997). Nevertheless, as the great majority were female more work on sex differences in the effects of the drug on brain 5-HT would be of interest. In particular, it would be worthwhile to vary dosage and time after administration and also to determine the effect of chronic treatment and administration at different stages of the oestrus cycle.

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