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Decreased accumulation of brain 5-hydroxytryptophan after decarboxylase inhibition in rats treated with fenfluramine, norfenfluramine or *p*-chloroamphetamine

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Fenfluramine, norfenfluramine and *p*-chloroamphetamine all lead to rapid but long-lasting depletion of brain 5-hydroxytryptamine (5-HT) when injected into rats (Sanders-Bush et al 1975; Fuller et al 1978). These chemically similar agents are believed to affect 5-HT neurons through similar mechanisms and generally produce similar effects on 5-HT concentrations (see Fuller et al 1978, for references) and function (Trulson & Jacobs 1976; Curzon et al 1979; Fuller & Snoddy 1980; Fuller & Clemens 1981). Costa et al (1971) and Costa & Revuelta (1972a) reported *increased* turnover of tel-diencephalic 5-HT (increased specific activity of brain 5-HT after injection of radioactive tryptophan) shortly after treatment with fenfluramine or norfenfluramine, but *decreased* 5-HT turnover after *p*-chloroamphetamine (Costa & Revuelta 1972b).

These differences in effects of fenfluramine and norfenfluramine versus that of *p*-chloroamphetamine are surprising and are in contradiction to the similar decreases in turnover caused by fenfluramine and *p*-chloroamphetamine at longer times (Sanders-Bush 1973; Clineschmidt et al 1978; Steranka & Sanders-Bush 1979; Fuller et al 1978). Other studies of 5-HT turnover at early times after fenfluramine administration have not been reported, and the report by Costa et al (1971) that 5-HT turnover is enhanced by fenfluramine continues to be cited (Reuter 1975; Belin et al 1976: McKenzie 1981; Invernizzi et al 1982). Turnover methods involving 5-HT or 5-hydroxyindoleacetic acid accumulation or disappearance are not applicable since the steady state levels of these substances are changing at early times after fenfluramine administration. We therefore used the accumulation of 5-hydroxytryptophan (5-HTP) after inhibition of decarboxylase (Carlsson 1974) to evaluate 5-HT turnover after fenfluramine and included norfenfluramine and *p*-chloroamphetamine for comparison.

Male Wistar rats, 130–150 g from Harlan Industries, Cumberland, Indiana, were used. Fenfluramine hydrochloride (a gift from A. H. Robins Company, Richmond, Virginia), norfenfluramine hydrochloride (synthesized in the Lilly Research Laboratories), or *p*-chloroamphetamine hydrochloride (Regis Chemical Company, Morton Grove, Illinois) were injected intraperitoneally 4 h before rats were killed. NSD 1015 (*m*-hydroxybenzylhydrazine; Aldrich Chemical Company, Milwaukee, Wisconsin) was injected intraperitoneally 30 min before rats were killed. 5-HTP was measured by liquid chromatography with electrochemical detection. Rat brain areas (20–100 mg of tissue) were sonicated in 1 ml of 0·1 M trichloroacetic acid containing 40 ng ml^{-1} of α-methyldopa as an internal standard. The homogenate was centrifuged at 12 000 *g*, and the supernatant fluid was applied to a Sephadex SP column (5 × 20 mm bed in a Pasteur pipette) which adsorbs cations. The Sephadex column was washed with 1 ml of 0·01 M formic acid, then with 1 ml of 0·05 M sodium acetate, pH 5. The internal standard and 5-HTP were eluted with another 1 ml of 0·05 M sodium acetate, and the pH of the eluate was adjusted to 2 by the addition of 10 μl of 5 M trichloroacetic acid. A 50 μl portion of this eluate was injected onto a Bioanalytical Systems 5 μ C_{18} column under the following conditions. Mobile phase was 0·1 M monochloroacetic acid, 1 mM EDTA pH 2·6, 50 mg $litre^{-1}$ sodium octyl sulphate, 8% methanol; the flow rate was 1·5 ml min^{-1} at 41 °C. The detector was a glassy carbon electrode from Bioanalytical Systems, used at a working potential of 0·5 V.

Table 1 compares the effects of fenfluramine, norfenfluramine and *p*-chloroamphetamine. All three compounds caused large reductions in the accumulation of 5-HTP following decarboxylase inhibition, indicating reduced hydroxylation of tryptophan during this 30-min period. The similarity of findings with fenfluramine and norfenfluramine is expected, since fenfluramine is extensively metabolized to norfenfluramine, which accounts partly for the in-vivo effects that occur after fenfluramine (Clineschmidt et al 1978). The similarity between *p*-chloroamphetamine and fenfluramine is also expected because of the similar nature of the effects of these two compounds on brain 5-HT neurons (Fuller et al 1978). The findings agree well with the reported decreases in tryptophan hydroxylase activity assayed in-vitro after administration of fenfluramine (Sanders-Bush et al 1975: Fuller et al 1978) and *p*-chloroamphetamine (Sanders-Bush et al 1972, 1975).

In a second experiment, fenfluramine was injected at three different doses, and two additional brain regions were studied (Table 2). The magnitude of the decrease in 5-HTP accumulation was nearly the same at all doses of fenfluramine, indicating that essentially maximum reduction of 5-HT turnover was occurring. Similar

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Table 1. Influence of fenfluramine, norfenfluramine and *p*-chloroamphetamine on 5-HTP concentration in rats treated with NSD 1015. NSD 1015 was injected at 100 mg kg^{-1} 30 min before rats were killed and 4 h after the injection of fenfluramine or norfenfluramine (both at 0·1 mmol kg^{-1}) or *p*-chloroamphetamine (0·05 mmol kg^{-1}). Mean values ± standard errors for 5 rats per group are shown. Asterisks indicate significant difference from group with no pretreatment ($P < 0.05$).

Pretreatment	5-HTP in hypothalamus n mol g^{-1}
None	1·66 ± 0·10
Fenfluramine	0·64 ± 0·02*
Norfenfluramine	0·46 ± 0·01*
p-Chloroamphetamine	0·49 ± 0·02*

Table 2. Dose-dependence of the influence of fenfluramine on 5-HTP concentration in brain regions of rats treated with NSD 1015.

Dose of fenfluramine mg kg^{-1} (i.p.)	5-HTP, n mol g^{-1} Striatum	Hypothalamus	Brain stem
0	0·94 ± 0·03	1·76 ± 0·12	1·78 ± 0·09
10	0·53 ± 0·06*	0·72 ± 0·04*	0·79 ± 0·08*
20	0·50 ± 0·05*	0·63 ± 0·02*	0·68 ± 0·02*
40	0·46 ± 0·04*	0·57 ± 0·03*	0·76 ± 0·06*

NSD 1015 was injected at 100 mg kg^{-1} 30 min before rats were killed and 4 h after the injection of fenfluramine hydrochloride at the doses indicated. Mean values ± standard errors for 5 rats per group are shown. Asterisks indicate significant difference from zero dose group ($P < 0.05$).

effects were found in all three brain regions. The striatum was included because Knapp & Mandell (1976) had reported that striatal conversion of radioactive tryptophan to radioactive 5-HT was increased in-vitro in striatal synaptosomes isolated from rats given fenfluramine 2 or 4 h earlier. They pointed out the peculiarity of that finding in light of the simultaneous decrease in intrasynaptosomal (soluble) tryptophan hydroxylase activity. Our findings indicate that tryptophan hydroxylation is reduced in-vivo in the striatum just as in the other brain regions after fenfluramine injection. The decrease in tryptophan hydroxylase activity measured in vitro after administration of either fenfluramine or *p*-chloroamphetamine to rats occurs even at times as early as one hour (Sanders-Bush et al 1972; Fuller et al 1978; Neckers et al 1976), so a decrease in tryptophan hydroxylation in-vivo would be expected.

The doses of fenfluramine used ranged as high as that used by Knapp & Mandell (1976) (40 mg kg^{-1}) and both higher and lower than that used by Costa et al (1971) (90 μmol kg^{-1} or 27 mg kg^{-1} of the hydrochloride). The time at which we measured 5-HT turnover by accumulation of 5-HTP after decarboxylase inhibition is the same (4 h) as the time at which Costa & Revuelta (1972a) measured incorporation of radioactive tryptophan after norfenfluramine. Thus neither dose nor time differences could account for the discrepancy in the conclusions about turnover changes. The results of our studies then indicate that, as would be expected by the previously reported decrease in tryptophan hydroxylase activity measured in-vitro after administration of three similar drugs (fenfluramine, norfenfluramine and *p*-chloroamphetamine), the hydroxylation of tryptophan, hence synthesis of 5-HT, in rat brain in-vivo is reduced after treatment with these drugs.

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