COMMUNICATIONS

Elevation of 3, 4-dihydroxyphenylacetic acid concentrations in rat brain and stimulation of prolactin secretion by fenfluramine: evidence for antagonism at dopamine receptor sites

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Fenfluramine and amphetamine both elevate brain concentrations of homovanillic acid, a metabolite of dopamine (Jori, Dolfini & others, 1973), but recent evidence has indicated that the two drugs may affect dopamine neurons via different mechanisms. Garattini, Jori & others (1975) suggested that fenfluramine blocks dopamine receptors, whereas amphetamine stimulates the release of dopamine presynaptically, both effects leading to increased production of the extraneuronal dopamine metabolite, homovanillic acid. To test further the hypothesis that fenfluramine blocks dopamine receptors, we have measured concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), an intraneuronal metabolite of dopamine, in brain and of prolactin in serum.

Amphetamine lowers DOPAC concentrations (Roffler-Tarlov, Sharman & Tegerdine, 1971); in contrast, dopamine receptor antagonists elevate DOPAC concentrations (Gerardy & Dresse, 1974; Bürki, Ruch & Asper, 1975). Measuring DOPAC concentrations thus would be a simple means of discriminating between an amphetamine-like action and dopamine receptor blockade as mechanisms for the elevation of homovanillic acid produced by fenfluramine. Dopamine receptor antagonists stimulate prolactin secretion by blocking an inhibitory dopamine receptor (Meites & Clemens, 1972; Dickerman, Clark & others, 1972), whereas amphetamine does not affect prolactin concentrations acutely (J. A. Clemens, unpublished studies). Prolactin measurements would therefore be a second index of dopamine receptor blockade. Our findings support the hypothesis of Garattini & others (1975) that fenfluramine antagonizes dopamine receptors.

Male albino rats (Wistar strain, from Harlan Industries, Cumberland, Indiana), 130–150 g, were housed individually in hanging wire cages with free access to food and water. Fenfluramine hydrochloride and norfenfluramine hydrochloride were gifts from the A. H. Robins Company; *p*-chloroamphetamine hydrochloride was from the Regis Chemical Company. Drugs were injected intraperitoneally as aqueous solutions at 1 ml kg^{-1}. Rats were decapitated, whole brains rapidly removed, frozen on dry ice, and stored frozen before analysis. DOPAC and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in whole brain were measured spectrofluorometrically (Murphy, Robinson & Sharman, 1969; Miller, Cox & others, 1970). Prolactin concentrations in serum were measured by radioimmunoassay using the NIAMD kit and were expressed as ng of NIAMD rat prolactin RP-1 ml^{-1}.

Fig. 1a shows the effect of fenfluramine on DOPAC concentrations in rat brain. Within 30 min of fenfluramine injection, DOPAC concentrations were increased, and the increase persisted at 1 h. By 3 h they had decreased to below control values, and this decrease persisted for as long as 24 h. Fenfluramine is known to be metabolized by *N*-dealkylation to norfenfluramine (see Broekkamp, Weemaes & van Rossum, 1975). The biphasic effect of fenfluramine on DOPAC concentrations led us to consider the possibility that the drug itself might cause DOPAC elevation whereas its metabolic product norfenfluramine might be responsible for the subsequent lowering of DOPAC concentration. However, we found (Fig. 1b) that norfenfluramine produced a similar biphasic action on DOPAC concentrations. Since fenfluramine and nor-

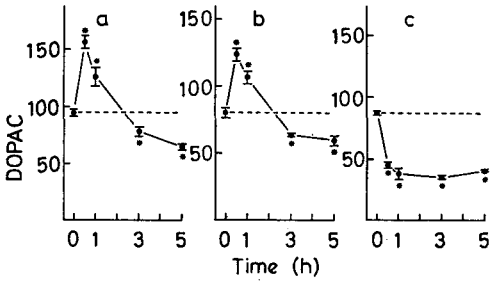

FIG. 1. DOPAC concentrations in brain (n mol g^{-1}) after intraperitoneal injection of a-fenfluramine, b-norfenfluramine, or c-*p*-chloroamphetamine into rats.

The hydrochloride salts of the drugs were injected at 15 mg kg^{-1} at zero time. Mean values ± standard errors for 5 rats per group are shown. * indicates values that differ significantly ($P<0.05$) from the corresponding zero time value.

* Correspondence.

Table 1. *Brain concentrations of 5-HIAA and DOPAC in rats at longer times after treatment with fenfluramine or p-chloroamphetamine.*

Treatment group	5-HIAA $\mu g\ g^{-1}$	DOPAC $ng\ g^{-1}$
Control	0·38 ± ·02	97 ± 6
24 h		
Fenfluramine	0·16 ± ·01**	74 ± 2**
p-Chloroamphetamine	0·10 ± ·01**	56 ± 2**
1 week		
Fenfluramine	0·31 ± ·02*	91 ± 7
p-Chloroamphetamine	0·15 ± ·02**	93 ± 7

* $P<0·05$. ** $P<0·01$. Fenfluramine hydrochloride and *p*-chloroamphetamine hydrochloride were injected intraperitoneally at 15 mg kg^{-1}. Mean values ± standard errors for 5 rats per group are shown.

Table 2. *Elevation of serum prolactin concentrations by fenfluramine and norfenfluramine in rats.*

Time after drug administration (h)	Fenfluramine	Norfenfluramine
	ng prolactin ml^{-1} serum	
0	40 ± 9	24 ± 2
0·5	90 ± 10*	55 ± 6*
1	59 ± 9	58 ± 5*
3	29 ± 9	34 ± 6
5	29 ± 2	33 ± 7

* Significantly higher than zero time value, $P<0·01$. Fenfluramine hydrochloride and norfenfluramine hydrochloride were injected intraperitoneally at 15 mg kg^{-1}. Mean values ± standard errors for 5 rats per group are shown.

fenfluramine deplete brain 5-hydroxytryptamine (5-HT) we tried another amphetamine derivative, *p*-chloroamphetamine, which also depletes 5-HT, to test the idea that the effects on DOPAC might be related in some way to interactions with 5-HT neurons. But *p*-chloroamphetamine produced only the amphetamine-like effect on DOPAC concentrations (Fig. 1c).

We compared fenfluramine and *p*-chloroamphetamine at longer times, since their effects on 5-HT neurons and on 5-HIAA concentrations persist for weeks after a single dose (Sanders-Bush, Bushing & Sulser, 1975). Table 1 shows that at 24 h after either drug, DOPAC and 5-HIAA concentrations were decreased; at one week, only 5-HIAA values remained decreased, DOPAC concentrations having returned to control values.

Table 2 shows the effects of fenfluramine and norfenfluramine on prolactin concentrations in rat serum. Within 30 min, prolactin values were increased by both drugs to more than twice the control and remained elevated at 1 h (though not significantly so with fenfluramine), but were not significantly changed at 3 and 5 h.

The findings that fenfluramine (and norfenfluramine) causes rapid increases in concentrations of brain DOPAC and serum prolactin support the hypothesis of Garattini & others (1975) that fenfluramine can antagonize dopamine receptors. The action of fenfluramine appears to be more complex than that of simple dopamine receptor antagonists, however, since at later times it lowers DOPAC concentrations (as do other amphetamines). The idea that fenfluramine blocks dopamine receptors is further supported by the finding of Berger, Brown & Krantz (1973) who observed that a 15 min pretreatment with fenfluramine blocked the hyperactivity and the stereotyped behaviour induced by amphetamine, effects thought to be mediated via dopaminergic mechanisms.

January 27, 1976

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