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Comparison of the effects of the stereoisomers of fenfluramine on the acetylcholine content of rat striatum, hippocampus and nucleus accumbens

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The (+)- and (−)- isomeric forms of fenfluramine were compared for their effects on rat brain area acetylcholine (ACh) content. The drugs showed similar patterns in increasing ACh content in the accumbens and hippocampus and in being ineffective in the brainstem. The actions differed in the striatum where the (+)-form markedly increased ACh content while the (−)-form produced no change. Both isomer-induced increases in ACh in the accumbens were prevented when 5-HT synthesis was blocked by *p*-chlorophenylalanine, thus denoting 5-hydroxytryptaminergic mediation of these effects. In striatum, the increase in ACh induced by (+)-fenfluramine was summated with the increase in ACh induced by dopamine receptor stimulation with apomorphine and was not prevented by dopamine receptor blockade with pimozide. On the other hand, apomorphine's effect was blocked by (−)-fenfluramine while pimozide pretreatment unmasked an increase in ACh induced by (−)-fenfluramine. The results favour the notion that there is a population of cholinergic neurons intrinsic to the striatum which is under inhibitory 5-HT regulation and independent of inhibitory dopamine regulation.

Fenfluramine is an anorectic drug the two optical isomers of which show differences in their biochemical and pharmacological characteristics. (+)-Fenfluramine is more active than the (−)-form both in decreasing food intake and in inhibiting, in vitro, 5-hydroxytryptamine (5-HT) uptake and release from rat brain synaptosomes (Garattini et al 1979). In vivo, both compounds lower brain 5-HT (Duhault & Verdavainne 1967). By contrast, the (−)-form, more than the (+)-form, acts on the dopaminergic system by increasing striatal homovanillic acid (Jori et al 1973) probably through an antagonistic effect as indicated by the antiapomorphine activity of the drug (Jori et al 1974).

(+)-Fenfluramine, but not the (−)-form, increased the level of acetylcholine (ACh) in the striatum (Consolo et al 1979a). This increase has been found to be due to a selective action of (+)-fenfluramine as a releaser of 5-HT from nerve terminals (Ladinsky et al 1978; Consolo et al 1979b). Besides such regulation by 5-hydroxytryptaminergic neurons, found by Butcher & Cho (1976), Euvrard et al (1977), Samanin et al (1978) and Ladinsky et al (1978), it is well known that cholinergic neurons intrinsic to the striatum are also under the inhibitory influence of dopaminergic neurons terminating in this area. We therefore investigated whether the antidopaminergic property of (−)-fenfluramine is implicated in the unexpected inactivity of the drug on striatal ACh despite its 5-hydroxytryptaminergic agonistic activity. Normally, DA receptor blockade leads to a decrease in striatal ACh content (Consolo et al 1975). It was also of interest to clarify whether these isomers shared common actions in other brain regions where there is little evidence for the presence of a dopaminergic-cholinergic link.

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METHODS

Female CD-COBS rats (Charles River, Italia), 210–220 g, were housed in groups of six in makrolon cages under standard conditions of temperature, humidity and light cycles for at least 4 days before experiments. The animals were given a standard diet of laboratory chow (Altromen-MT) and free access to water.

The rats were killed by near-freezing and the brain areas were removed under n-pentane at −5°C as described earlier (Consolo et al 1977). ACh was determined by the radioenzymatic method of Saelens et al (1970) with some modifications (Ladinsky et al 1976).

The isomers of fenfluramine HCl were dissolved in saline and administered intraperitoneally. The rats were killed 30 min later for biochemical assay. *p*-Chlorophenylalanine (PCPA) was suspended in 0·5% carboxymethylcellulose and given in 3 consecutive daily oral doses of 100 mg kg^{-1}, 72, 48 and 24 h before the experiment. Pimozide was dissolved

in 200 mM tartaric acid and administered i.p. at a dose of 1 mg kg^{-1} for 240 min. Apomorphine HCl was dissolved in saline and administered i.p. at a doses of 0·7 or 1·5 mg kg^{-1} for 25 min. Control animals received the appropriate volumes of vehicle.

The data were analysed by Student's *t*-test (Table 1) and by ANOVA (2 × 2) factorial analysis, Tukey's test and Tukey's test for unconfounded means.

RESULTS

The isomers of fenfluramine similarly increased the levels of ACh in the hippocampus and n. accumbens but had no effect in the brainstem. The drug actions differed in the striatum where the (+)-form markedly increased ACh content at 30 min after an i.p. injection of 7·5 mg kg^{-1}, whereas the (−)-form produced no change at double the dose (Table 1).

Table 1. Effects of the (+)-and (−)-forms of fenfluramine on the ACh concns in rat brain areas. (+)-Fenfluramine was administered i.p. at 7·5 mg kg^{-1} and (−)-fenfluramine at 15 mg kg^{-1}. The animals were killed 30 min later.

	ACh nmol g^{-1} wet wt		
Brain area	Saline	(+)-fenfluramine	(−)-fenfluramine
Hippocampus	16·5 ± 0·5	23·9 ± 0·7**	19·9 ± 0·5**
n. Accumbens	34·1 ± 1·3	43·0 ± 1·5**	42·1 ± 1·7**
Striatum	31·5 ± 0·7	48·0 ± 1·9**	29·7 ± 1·0
Brainstem	22·3 ± 0·5	23·8 ± 0·5	22·8 ± 0·8

** $P<0·01$ vs the saline treated group.
Figures are the means ± s.e.m. (n = 9–15).

Both isomer-induced increases in ACh in the n. accumbens were prevented when 5-HT synthesis was blocked by PCPA (Table 2), denoting a 5-hydroxytryptaminergic action of these drugs.

In striatum, the increase in ACh induced by (+)-fenfluramine was summated with the increase induced by a supramaximal dose of apomorphine (Consolo et al 1978), a direct-acting dopaminergic agonist (Table 3). On the contrary, (−)-fenfluramine

Table 2. Effect of PCPA on the concns of ACh in n. accumbens of rats treated with (+)- and (−)-fenfluramine.

	ACh nmol g^{-1} wet wt				
Drug in C and D	A Vehicle	B PCPA	C Drug	D Drug + PCPA	Interaction
(+)-Fenfluramine	33·7 ± 2·0	37·7 ± 1·9	42·0 ± 1·6*	36·4 ± 1·9	F 1,24 = 6·7 $P<0·05$
(−)-Fenfluramine	30·7 ± 2·8	34·4 ± 1·1	40·7 ± 1·9**	33·3 ± 1·3	F 1,28 = 8·6 $P<0·01$

* = $P<0·05$; ** = $P<0·01$ vs the vehicle treated group.
Vehicle: 0·5% CMC.
See Table 1 for legend.

Table 3. Effect of apomorphine on the concns of ACh in the striatum of rats treated with (+)- and (−)-fenfluramine (doses as in Table 1). Apomorphine was administered at a dose of 1·5 mg kg^{-1} in the (+)-fenfluramine experiment and at 0·7 mg kg^{-1} in the (−)-fenfluramine experiment. The rats were killed 35 min after fenfluramine and 25 min after apomorphine.

	ACh nmol g^{-1} wet wt				
Drug in C and D	A Saline	B Apomorphine	C Drug	D Drug + apom.	Interaction
(+)-Fenfluramine	33·2 ± 1·0(9)	49·1 ± 2·5**(9)	47·1 ± 1·5**(9)	64·3 ± 1·4**(9)	n.s.
(−)-Fenfluramine	33·3 ± 1·8	51·3 ± 2·8**	30·2 ± 1·1	37·6 ± 1·4	F 1,28 = 7·9 $P<0·01$

** $P<0·01$ vs saline groups.

completely prevented the increase in ACh produced by apomorphine. Pretreatment with pimozide, a powerful dopamine receptor blocker, by itself markedly decreased the level of striatal ACh (Table 4). Effective blockade of striatal dopamine receptors in this manner did not prevent the increase in ACh produced by (+)-fenfluramine and unmasked an increase in ACh produced by the (−)-form of the drug.

Table 4. Effect of pimozide on the concns of ACh in the striatum of rats treated with (+)- and (−)-fenfluramine (doses as in Table 1). Pimozide was administered i.p. at a dose of 1 mg kg^{-1} and the animals were killed 240 min later.

	ACh nmol g^{-1} wet wt				
Drug in C and D	A Vehicle	B Pimozide	C Drug	D Drug + pimo.	Interaction
(+)-Fenfluramine	30·4 ± 1·1 (9)	17·0 ± 0·9** (9)	47·0 ± 4·4** (9)	27·7 ± 2·3 (9)	n.s.
(−)-Fenfluramine	31·1 ± 1·4	16·2 ± 0·8**	25·2 ± 1·7**	23·8 ± 1·2**	F 1,40 = 25·3 $P<0·01$

** $P<0·01$ vs vehicle.
Vehicle: 200 mM tartaric acid.

DISCUSSION

(−)-Fenfluramine behaves similarly to (+)-fenfluramine on cholinergic neurons of brain areas except in the striatum, where it produced no change in ACh content. But it did block apomorphine's action on ACh content, denoting, together with other biochemical and behavioral data (Jori et al 1973, 1974), that the drug may possess dopaminergic antagonistic activity. In theory, (−)-fenfluramine may decrease ACh through its antidopaminergic action and increase ACh as a 5-HT releaser. The latter effect is demonstrated by the experiment with pimozide which unmasked an increase. It is therefore conceivable that (−)-fenfluramine's inactivity is a result of these concomitant opposing effects. Alterna-

tively, it can also be argued that the antidopaminergic action of (−)-fenfluramine in the striatum prevails over its 5-hydroxytryptaminergic action but that there is no decrease in the level because ACh depletion is of a kind that can be compensated for by de novo synthesis.

The summation of the effect of (+)-fenfluramine on striatal ACh with supramaximal doses of the dopaminergic agonist, apomorphine, and the antagonist, pimozide, supports the notion first proposed by Guyenet et al (1977) that there is a population of cholinergic neurons intrinsic to the striatum which is under inhibitory 5-HT regulation and independent of inhibitory dopamine regulation.

The increase in ACh content in the n. accumbens by both isomers of fenfluramine, and their blockade by PCPA, implicates 5-hydroxytryptaminergic modulation of these drug actions on cholinergic neurons in this area. The dopaminergic role of (−)-fenfluramine was not expressed in the n.accumbens, an area rich in both dopamine and ACh, perhaps because a link between these two neurotransmitters is missing (Consolo et al 1977).

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