J. Pharm. Pharmac., 1971, 23, 600–605 *Received February* 17, 1971

Some 5-hydroxytryptamine-like actions of fenfluramine: a comparison with (+)-amphetamine and diethylpropion

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The 5-hydroxytryptamine (5-HT)-like effects of fenfluramine have been investigated in mice in two different experiments. In a behavioural test in mice pretreated with tranylcypromine, fenfluramine caused stereotyped changes which were similar to those produced by 5-hydroxytryptophan (5-HTP) and were antagonized by methysergide or pretreatment with *p*-chlorophenylalanine (PCPA). Like 5-HTP, fenfluramine reduced the conditioned response in a one-trial conditioning test, an effect antagonized by methysergide or by PCPA pretreatment. The reduction in the conditioned response caused by a maximal electroconvulsion was also antagonized by PCPA, an effect prevented by 5-HTP. Equivalent anorectic doses of (+)-amphetamine and diethylpropion caused a small increase in stereotyped behaviour, but this was not modified by methysergide; both anorectic drugs were inactive in the one-trial conditioning test. It seems probable that the observed actions of fenfluramine are caused indirectly through the release of endogenous brain 5-HT.

Recently Jespersen & Scheel-Krüger (1970) reported that dogs treated with fenfluramine showed hypothermia and behavioural signs similar to those observed after an injection of 5-hydroxytryptophan (5-HTP) and that these effects were prevented by the 5-hydroxytryptamine (5-HT) antagonist, methysergide. These observations are consistent with earlier reports (Duhault & Verdavainne, 1967; Opitz, 1967) indicating that fenfluramine causes the release and subsequent depletion of brain 5-HT in rats. We have investigated the behavioural effects of fenfluramine in mice using two experimental situations to determine 5-HT-like activity. For comparison, two other anorectic agents, (+)-amphetamine and diethylpropion, were also examined.

METHODS

Behavioural effects

Groups of ten male mice (Tuck TFW strain), 18–24 g, were treated orally with 10 mg/kg of the monoamine oxidase inhibitor, tranylcypromine. Four h later the animals were given fenfluramine (8 or 32 mg/kg), (+)-amphetamine (2 or 8 mg/kg), diethylpropion (8 or 32 mg/kg), 5-HTP (10 or 40 mg/kg) or saline (10 ml/kg) by the intraperitoneal route. After 15 min, note was made of the number of animals which responded with head twitching, head waving, forelimb movement and backward locomotion, each feature being assigned an arbitrary score value of one. Further groups of mice were pretreated similarly with tranylcypromine but, in addition to the intraperitoneal dose of the anorectic agents, 5-HTP or saline, the animals received concomitantly a subcutaneous dose of the 5-HT-antagonist, methysergide, 20 mg/kg.

All the drug and dosage combinations were randomized (random permutations—Fisher & Yates, 1957) and the behavioural signs scored blindly. The mean score values were then calculated and compared for statistical significance using Student's *t*-test.

A further group of mice was given two oral doses of 200 mg/kg of the tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine (PCPA) 48 and 24 h before tranylcypromine which was then followed 4 h later by an intraperitoneal dose of 32 mg/kg of fenfluramine.

One-trial conditioning test

Groups of ten male mice were placed individually into the well-lit side of a two-compartment box, which had a communicating hole to a larger dark area fitted with a grid floor. On entering the dark compartment, escape was prevented by a slide door, and the animals were given a conditioning foot-shock of 2 mA, 350 V for 5 s. When returned to the test situation 24 h later the time taken by the conditioned mice to enter the dark compartment (step-through latency, STL) was measured and compared with that of non-shocked (unconditioned) control mice. To evaluate the effects of drug treatment on conditioning, STL was measured in mice given intraperitoneal doses of fenfluramine (16 or 32 mg/kg), (+)-amphetamine (4 or 8 mg/kg), diethylpropion (16 or 32 mg/kg) or 5-HTP (20 or 40 mg/kg) 30 min before the foot-shock. Other groups of mice were pretreated similarly with intraperitoneal doses of fenfluramine or 5-HTP but, in addition, 10 or 20 mg/kg methysergide was given concomitantly subcutaneously.

In another experiment using groups of 16 mice, the effect of fenfluramine on STL was compared with that of a maximal electroconvulsion (MEC) induced through pinna electrodes (20 mA, 0·2 s, 50 Hz sinusoidal current) immediately after exposure to the foot-shock. In a further experiment, the effect of fenfluramine on STL was measured in animals given either saline or PCPA (200 mg/kg) by mouth 48, 24 and 2 h before the conditioning trial.

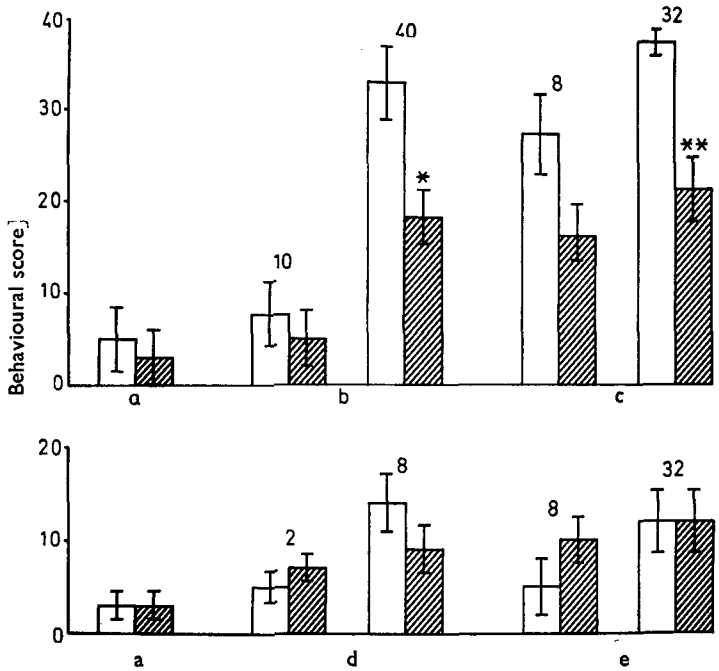
Tests for statistical significance of the differences between groups were made using the Mann-Whitney U-test (Siegel, 1956).

RESULTS

Behavioural effects

Control mice, pretreated with tranylcypromine, and injected with saline, showed only occasional head twitching, head waving and forelimb movement, with group scores of 3 to 5 compared with a possible maximum score of 40.

The effects of giving mice (pretreated with tranylcypromine to inhibit monoamine oxidase) 5-HTP, fenfluramine, diethylpropion or (+)-amphetamine are shown in Fig. 1. The low dose of 5-HTP (10 mg/kg) was without behavioural effect but 40 mg/kg caused a highly significant ($P < 0{\cdot}001$) increase in the behavioural score to 32. Fenfluramine produced the same behavioural syndrome as the high dose of 5-HTP: 8 and 32 mg/kg of fenfluramine gave scores of 27 and 37 which were significantly different ($P = 0{\cdot}01$–$0{\cdot}001$ and $P < 0{\cdot}001$ respectively) from that of the saline-treated control animals. Methysergide (20 mg/kg) caused no change in the behaviour of the control animals but gave a significant reduction (43%, $P = 0{\cdot}02$–$0{\cdot}01$ and 44%, $P < 0{\cdot}001$) in the behavioural scores elicited by both 5-HTP (40 mg/kg) and



FIG, 1. Behavioural effects of a, saline, b, 5-HTP, c, fenfluramine, d, (+)-amphetamine, and e, diethylpropion in tranylcypromine-pretreated mice. Open columns represent behavioural scores produced by intraperitoneal doses (mg/kg) of the drugs alone; hatched columns show the effects of the drugs administered concomitantly with methysergide, 20 mg/kg subcutaneously. Bars give the standard error of the mean behavioural scores. Asterisks indicate a statistically significant antagonism by methysergide (Student's t-test, $*P = 0{\cdot}02$–$0{\cdot}01$, $**P < 0{\cdot}001$).

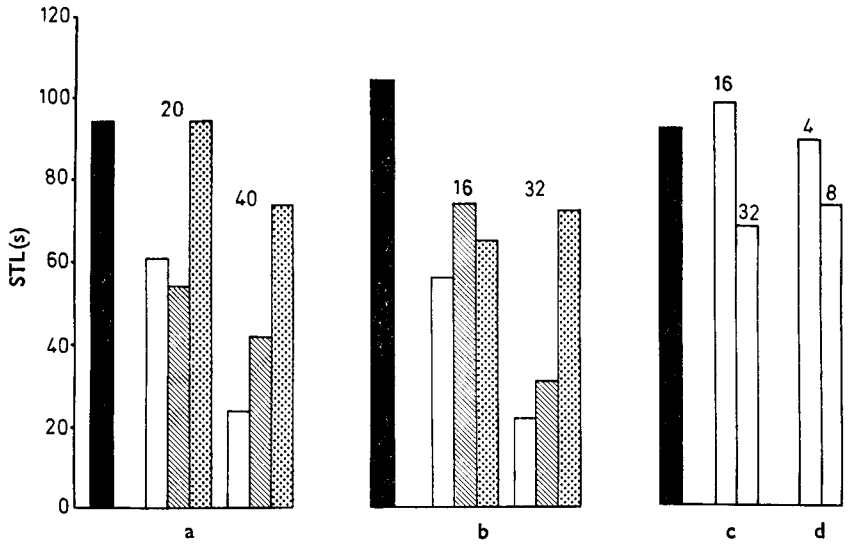
fenfluramine (32 mg/kg). Pretreatment with two oral doses of 200 mg/kg of PCPA caused a significant reduction ($P < 0{\cdot}001$) of 64% in the behavioural score elicited by the high dose of 32 mg/kg of fenfluramine.

The highest doses of (+)-amphetamine (8 mg/kg) or diethylpropion (32 mg/kg) also caused a significant increase in behavioural responses ($P = 0{\cdot}01$–$0{\cdot}001$ and $P = 0{\cdot}05$–$0{\cdot}02$ respectively), but both drugs were much less active than fenfluramine and 5-HTP, with maximum scores of only 14 and 12. Methysergide (20 mg/kg) failed to modify the behavioural effects caused by (+)-amphetamine or diethylpropion.

Single trial conditioning experiments

In eight experiments, the mean step-through latency of unconditioned (non-shocked) saline treated mice was 12·8 s. After a conditioning foot-shock the STL was prolonged, the mean values ranging from 77 to 104 s (Figs 2 and 3).

5-HTP gave a dose-related reduction in STL (Fig. 2). With the highest dose of 5-HTP (40 mg/kg) the reduction in STL was highly significant ($U = 0$, $P < 0{\cdot}001$) the mean value of 24 s approaching that of the unconditioned control animals. Methysergide (10 and 20 mg/kg) antagonized the reduction in STL caused by 5-HTP but this effect was statistically significant ($U = 15$, $P = 0{\cdot}01$–$0{\cdot}001$) only for the high dose of methysergide given with 40 mg/kg of 5-HTP.



FIG, 2. The effect on step-through latency of intraperitoneal doses of a, 5-HTP, b, fenfluramine, c, diethylpropion and d, (+)-amphetamine administered alone or concomitantly with methysergide given subcutaneously. Solid columns indicate the results obtained in control saline-treated animals; the other columns show the effects of the drugs alone (open columns) and combined with methysergide, 10 mg/kg (hatched columns) or 20 mg/kg (stippled columns).

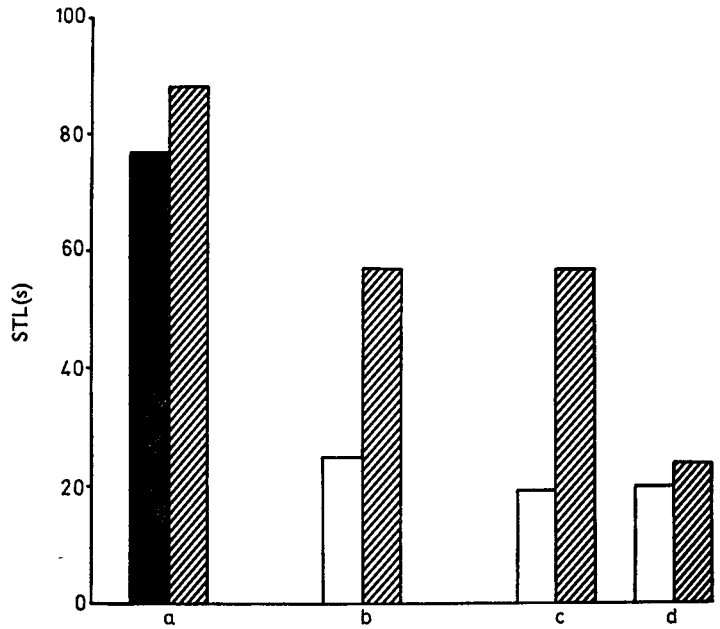


FIG. 3. The effects of oral pretreatment with *p*-chlorophenylalanine (PCPA), 3 × 200 mg/kg, on the reduction of step-through latency caused by a, saline, b, fenfluramine (32 mg/kg), c, maximal electro-convulsion (MEC) or d, MEC plus prior treatment with 5-HTP (20 mg/kg). Solid column indicates the results obtained in control saline-treated animals; open columns show the effects of the treatments alone and hatched columns the effects of the treatments after PCPA.

Fenfluramine, like 5-HTP, also gave a dose-related reduction in STL (Fig. 2) which was statistically significant ($U = 1$, $P < 0{\cdot}001$) only with the high dose of 32 mg/kg, which reduced the mean STL to 22 s. Methysergide (20 and 40 mg/kg) antagonized this reduction in STL caused by fenfluramine and this antagonism was again statistically significant ($U = 21$, $P = 0{\cdot}05$–$0{\cdot}025$) only with the high dose combination of the two drugs. Pretreatment with three oral doses of 200 mg/kg of PCPA also significantly antagonized ($U = 24$, $P = 0{\cdot}05$–$0{\cdot}025$) the reduction in STL caused by 32 mg/kg of fenfluramine (Fig. 3).

In contrast to 5-HTP and fenfluramine, diethylpropion (16 and 32 mg/kg) and (+)-amphetamine (4 and 8 mg/kg) did not produce a statistically significant reduction in STL (Fig. 2).

Like 5-HTP and fenfluramine, a maximal electroconvulsion (MEC) elicited immediately after conditioning, also reduced STL to the level found in unconditioned control animals (Fig. 3). Pretreatment with PCPA significantly antagonized MEC ($U = 19{\cdot}5$, $P < 0{\cdot}001$), an effect prevented by the prior intraperitoneal administration of 20 mg/kg of 5-HTP ($U = 20{\cdot}5$, $P < 0{\cdot}001$).

DISCUSSION

Experiments with tranylcypromine-pretreated mice showed that a high dose of the 5-HT precursor, 5-HTP, caused characteristic stereotyped behaviour which was antagonized by the 5-HT blocking agent, methysergide. In agreement with the work of Jespersen & Scheel-Krüger (1970) in dogs, we also found that fenfluramine caused 5-HTP-like behavioural changes which, similarly, were blocked by methysergide and, moreover, were prevented by the prior administration of the tryptophan hydroxylase inhibitor PCPA (Koe & Weissman, 1966; Jéquier, Lorenburg & Sjoerdsma, 1967). In contrast to these findings, although diethylpropion and (+)-amphetamine in doses equianorectic with fenfluramine caused a significant change in behaviour, their effect was small and not modified by methysergide, indicating that they are unlikely to be exerting a major action on 5-HT mechanisms.

In the one-trial conditioning experiments, STL was prolonged 24 h after a conditioning foot-shock, and this prolongation was antagonized by MEC elicited immediately after conditioning. This result confirms the work of Essman (1968) who showed that consolidation of memory could be disrupted by an electroconvulsion, an effect which was associated with an increase in the brain concentrations of 5-HT. Our findings are consistent with the conclusion that prolongation of STL can be modified by an action on 5-HT mechanisms, as the prior administration of 5-HTP prevented the increase in STL following conditioning and this action was antagonized by methysergide. Moreover, our experiments have also shown that reduction of STL by MEC is antagonized by prior treatment with PCPA and this effect, in turn, is prevented by the concomitant administration of 5-HTP.

In agreement with the results of the behavioural experiments, the effect of fenfluramine in the one-trial conditioning tests mimicked that of 5-HTP and also that of MEC. The reduction in STL caused by fenfluramine was antagonized by methysergide and also by pretreatment with PCPA. In the doses used, neither (+)-amphetamine nor diethylpropion significantly affected STL, indicating a difference in the actions of these anorectic drugs.

The effects of fenfluramine on behaviour and on STL, their similarity with the effects of 5-HTP and their modification by both methysergide and PCPA can all be

explained by an increase in the activity of a 5-HT-sensitive system. Although brain concentrations of 5-HT were not measured in our experiments, it seems probable that the action of fenfluramine may be exerted indirectly through the release of endogenous brain 5-HT. Duhault & Verdavainne (1967) showed that fenfluramine in oral doses of 10 to 30 mg/kg caused depletion of brain 5-HT and 5-hydroxyindoleacetic acid, maximal reductions appearing approximately 4 h after dosing. In our experiments, observations were made 30 min and 1 h after intraperitoneal and oral treatment respectively, when elevated brain concentrations of 5-HT could be anticipated.

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